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10th European Cytogenetics Conference 2015

4–7 July 2015, Strasbourg – France

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EDITORIAL

This Supplement of Chromosome Research contains abstracts of the invited lectures and submitted oral and poster presentations at the 10th European Cytogenetics Conference held on 4–7 July 2015 in Strasbourg.

It was 150 years ago, in 1865, that Mendel first presented his paper on Experiments on Plant Hybridization at the Natural History Society in Brno (now in Czech Republic); the paper was published the following year. Gregor Johann Mendel was a German-speaking Augustinian friar who carried out his now famous experiments on pea plants in the experimental garden at his monastery. He studied seven characteristics of pea plants: plant height, pod shape and colour, seed shape and colour, and flower position and colour. He coined the terms 'recessive' and 'dominant' in reference to certain traits and he demonstrated the actions of invisible 'factors'—now called genes—that provided for visible traits in predictable ways. He kept meticulous records of his observations and based on these he formulated the laws of segregation and of independent assortment, which later came to be known as Mendel's laws of inheritance. However, it was more than three decades later, at the turn of the 20th century, that the profound significance of Mendel's work was recognized. His work was rediscovered and his experimental findings were verified by a number of scientists, thus ushering in the era of modern genetics. At this Conference we celebrate the 150-year anniversary of Mendel's laws. Two exceptional and well-known speakers, Trude Schwarzacher and Marcus Pembrey, will show us the relevance of Mendel's work to the genetics of today.

In the Opening lecture Terry Hassold will give us new insights into the age-old problem of aneuploidy in humans. An important topic at this conference is the impact of next generation sequencing (NGS) and whole genome sequencing. In recent years the massive sequencing has changed the practice of clinical genetics and has implications for cytogenetics. In the session "Impact of Whole Genome Sequencing on Future Diagnostics", Joris Veltman will talk about NGS and cytogenetics and Claudia Haferlach will address the impact of NGS on the genetic diagnosis of malignant haemopathies. The sessions on Chromosome Rearrangements include among others a lecture by Sandra Rodriguez Perales on engineering of human tumour-associated chromosomal translocations by CRISPR-Cas9 system.

Another highlight of the programme is the session on Prenatal Diagnosis in which Charles Strom will talk about the positive predictive value of non-invasive prenatal screening (NIPS), and Kamlesh Madan will speak about the impact of prenatal technologies on sex ratio.

Besides these highlights, there are two interesting topics in the session on Clinical Cytogenetics, one is on the CNVs on the X chromosome by Damien Sanlanville and the other is an intriguing question "Can Down syndrome be treated?" posed by Renaud Touraine. In the session on Animal and Plant

Cytogenetics, Jaroslav Dolezol will discuss polyploidy and interspecific hybridization in plants, and Elena Giulotto will talk about the functional organization of satellite-less equid centromeres.

Finally, there are a number of interesting topics in sessions on Tumour Cytogenetics, Fragile sites and Chromosome Instability, Segmental Duplications and CNVs and Accreditation and Quality control. The programme ends with the Keynote Lecture on the 22q11.2 rearrangement disorders by Bernice Morrow.

I would like to draw your attention to the many interesting submitted oral communications and posters, the abstracts of which also appear in this supplement.

As always, it is our intention that this meeting should provide the participants with an opportunity for not only contributing their knowledge and experience, but also for interacting with each other. Strasbourg, being one of Europe's most attractive cities is the ideal place for this; the entire town centre has been listed as UNESCO World Heritage. We hope that the participants will take the opportunity to visit the centre to discover its history, its cuisine and its unique cultural atmosphere.

Finally, on behalf of the ECA and the Scientific Programme Committee, I wish all participants a stimulating, useful and an enjoyable meeting.

José M. García-Sagredo
President

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10TH EUROPEAN CYTOGENETICS CONFERENCE PROGRAM

SATURDAY, 4 JULY 2015

14:00–15:00	Room 12	Permanent Working Group–Clinical and Molecular Approaches to Cytogenetic Syndromes
15:00–16:00	Room 12	Permanent Working Group–Molecular Cytogenetics and Array-CGH
16:00–17:00	Room 12	Permanent Working Group–Marker Chromosomes
14:00–15:00	Room Boston	Permanent Working Group–Cytogenetics of Haematological Malignancies
15:00–16:00	Room Boston	Permanent Working Group–Prenatal Diagnosis
16:00–17:00	Room Boston	Permanent Working Group–Animal, Plant and Comparative Cytogenetics
14:00–15:00	Room Dresden	CEQAS Participants' Meeting
15:00–16:00	Room Dresden	Permanent Working Group–Quality Issues and Training in Cytogenetics
16:00–17:00	Room Dresden	International Breakpoint Mapping Consortium, Ancillary Meeting
14:00–15:00	Room Leicester	Permanent Working Group–Cancer Cytogenetics, Solid Tumor Studies
15:00–16:00	Room Leicester	Permanent Working Group–Cytogenetics, Toxicology and Mutagenesis
16:00–17:00	Room Leicester	Permanent Working Group–Meiotic Studies
17:00–17:50		Industry-sponsored Satellite Symposium
18:00–19:00		**Opening Lecture** *Chairs: José M. Garcia-Sagredo, Damien Sanlaville* Aneuploidy in humans: new insights into an age-old problem **Terry Hassold** (USA)

SUNDAY, 5 JULY 2015

08:30–10:30	**Plenary Session: Clinical Cytogenetics** *Chairs: Elisabeth Syk Lundberg, Kamlesh Madan*
08:30–09:05	CNV on the X Chromosome. Clinical interpretation and pitfalls **Damien Sanlaville** (F)
09:05–09:40	Can Down syndrome be treated? **Renaud Touraine** (F)
09:40–10:30	*Selected Oral Presentations* Detecting small Copy Number Variations, smaller than 400 kb, improves the diagnostic yield of CMA in intellectual disability **Marie Faoucher** (F) The power of social media for karyotype-phenotype analysis of rare chromosome disorders **Conny van Ravenswaaij-Arts** (NL) Reorganisation of chromosome 9 pericentromeric segmental duplications in inv(9) carriers and in evolution **Stefan Müller** (D)
10:30–11:00	Coffee Break
11:00–12:00	**Plenary Session: 150 Years Since Mendel's Laws (Brno, Natural History Society 1865, published 1866)** *Chairs: Pat Heslop-Harrison, Mariano Rocchi*
11:00–11:30	Mendel, his time, and the rediscovery of his laws **Trude Schwarzacher** (GB)
11:30–12:00	From genetic to epigenetic inheritance

	Marcus Pembrey (GB)
12:00–14:40	Poster Session
12:40–13:30	Industry-sponsored Satellite Symposium
13:40–14:30	Industry-sponsored Satellite Symposium
14:40–15:45	**Concurrent Session: Chromosomal Rearrangements I**
	Chairs: Orsetta Zuffardi, Damien Sanlaville
14:40–15:05	Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system
	Sandra Rodriguez Perales (E)
15:05–15:30	Genome rearrangements in hereditary disorders
	Karl Heinimann (CH)
15:30–15:45	*Selected Oral Presentation*
	Unraveling the internal structure of neocentromeres in ring chromosomes harboring genomic amplification in soft tissue tumors
	Gemma Macchia (I)
14:40–15:45	**Concurrent Session: Segmental Duplications and Copy Number Variants I**
	Chairs: Joris Vermeesch, Serge Romana
14:40–15:05	Salivary amylase CNV and obesity
	Philippe Froguel (GB)
15:05–15:30	Polymorphism of the pseudoautosomal region I
	Martin A. Mensah (D)
15:30–15:45	*Selected Oral Presentation*
	Unbalanced de novo chromosome translocations and inversions: a two-step origin?
	Maria Clara Bonaglia (I)
15:45–16:15	Coffee Break
16:15–17:20	**Concurrent Session: Fragile Sites and Chromosome Instability**
	Chairs: Lidia Larizza, Jean-Michel Dupont
16:15–16:40	Fragile X Syndrome: an overview
	Jean-Louis Mandel (F)
16:40–17:05	CINister problems, modeling aneuploidy in the mouse
	Floris Foijer (NL)
17:05–17:20	*Selected Oral Presentation*
	The International Breakpoint Mapping Consortium (IBMC). Systematic mapping of chromosomal breakpoints in the context of phenotypes and nuclear genome organization
	Niels Tommerup (DK)
16:15–17:20	**Concurrent Session: Segmental Duplications and Copy Number Variants II**
	Chairs: Nicole de Leeuw, Juan Cigudosa
16:15–16:40	The growing importance of CNVs: new insights for detection and clinical interpretation
	Sebastien Jacquemont (CDN)
16:40–17:05	Copy number variation as a key to unlocking the enigma that is schizophrenia
	Aiden Corvin (IRL)
17:05–17:20	*Selected Oral Presentation*
	Direct visualisation of complex structural and copy-number variation by molecular combing and multicolour-FISH
	Fengtang Yang (GB)
17:20–18:30	Poster Session
17:30–18:20	Industry-sponsored Satellite Symposium
18:30	E.C.A. General Assembly

MONDAY, 6 JULY 2015

08:30–10:30	**Plenary Session: Tumor cytogenetics I**
	Chairs: Felix Mitelman, Claudia Haferlach
08:30–09:00	Molecular breakdown of AML with EVI1
	Ruud Delwel (NL)
09:00–09:30	Myelodysplastic syndromes: from 5q- to somatic mutation of RNA splicing machinery
	Mario Cazzola (I)
09:30–10:30	*Selected Oral Presentations*
	Comparison of microarray-based genomic profiling and karyotyping in myelodysplastic syndromes (a HOVON89 study)
	Marian Stevens-Kroef (NL)
	Epigenetic regulation of UNCX, a novel homeobox transcription factor gene activated in acute myeloid leukemia
	Giulia Daniele (I)
	Rapid and comprehensive routine diagnostic approach for prognostic genetic markers in multiple myeloma
	Elodie David (CH)
10:30–11:00	Coffee Break
11:00–12:15	**Concurrent Session: Chromosomal Rearrangements II**
	Chairs: Nicole de Leeuw, Franck Pellestor
11:00–11:30	Retrotransposable elements, unbalanced translocations and chimerism
	Joris Vermeesch (B)
11:30–12:00	SD, CNV and complex regions of the human genome
	Francesca Antonacci (I)
12:00–12:15	*Selected Oral Presentation*
	Meiotic recombination and complex chromosome rearrangements: how whole-genome sequencing helps to resolve them
	Vincent Gatinois (F)
11:00–12:15	**Concurrent Session: Animal and Plant Cytogenetics**
	Chairs: Pat Heslop Harrison, Valerie Fillon
11:00–11:30	Chromosome-centric approaches unravel the impact of polyploidization and interspecific hybridization
	Jaroslav Dolezel (CZ)
11:30–12:00	Functional organization of satellite-less equid centromeres
	Elena Giulotto (I)
12:00–12:15	*Selected Oral Presentation*
	Comparative chromosome painting in lacertid lizards: highly conserved karyotypes but independent origins of sex chromosomes
	Veronica Rojo (E)
12:15–14:30	Poster Session
12:40–13:30	Industry-sponsored Satellite Symposium
13:40–14:30	Industry-sponsored Satellite Symposium
14:30–15:45	**Plenary Session: Impact of Whole Genome Sequencing on Future Diagnostics**
	Chairs: Eva Klopocki, Juan Cigudosa
14:30–15:00	NGS and cytogenetics
	Joris Veltman (NL)
15:00–15:30	Genetic diagnosis in malignant hemopathies
	Claudia Haferlach (D)

15:30–15:45	*Selected Oral Presentation* Exome sequencing and SNP arrays as a tool to describe genetic diversity of premalignant and malignant cells: lesson from monoclonal gammopathies **Aneta Mikulasova** (CZ)
15:45–16:15	Coffee Break
16:15–17:30	**Concurrent Session: Tumor Cytogenetics II** *Chairs: Roberta Vanni, Thierry Lavabre-Bertrand*
16:15–16:45	Constitutional and somatic rearrangement of chromosome 21 in ALL **Christine J. Harrison** (GB)
16:45–17:15	Developmental disease and cancer: Biological and Clinical Overlaps **Alfonso Bellacosa** (USA)
17:15–17:30	*Selected Oral Presentation* Assessment of SNP genomic microarray analysis as an alternative to FISH analysis for the detection of copy number changes in pediatric solid tumors **Mary Shago** (CDN)
16:15–17:30	**Concurrent Session: Accreditation and Quality Control** *Chairs: Harald Rieder, Martine Doco-Fency*
16:15–16:40	European Standards for Clinical Laboratory Geneticists **Thomas Liehr** (D)
16:40–17:00	Changes in ISO 15189: practical implications **Konstantin Miller** (D)
17:00–17:30	Mandatory 15189 ISO accreditation for French medical laboratories: how to best reconcile regulatory and normative requirements for cytogenetics? **Philippe Lochu** (F)
17:30–18:30	Poster Session
17:30–18:20	Industry-sponsored Satellite Symposium

TUESDAY, 7 JULY 2015

09:00–10:30	**Plenary Session: Prenatal Diagnosis** *Chairs: Konstantin Miller, Sevilhan Artan*
09:00–09:30	The Positive Predictive Value of Noninvasive Prenatal Screening (NIPS) **Charles Strom** (USA)
09:30–10:00	Prenatal technologies and sex ratio **Kamlesh Madan** (NL)
10:00–10:30	*Selected Oral Presentations* Noninvasive prenatal testing: more caution is needed in high risk pregnancies **Beatrice Oneda** (CH) Digital PCR in an efficient alternative to massively parallel sequencing as a non–invasie, prenatal test for trisomy 21: the results of a pilot study **Laila Allach El Khattabi** (F)
10:30–10:50	Coffee Break
10:50–11:40	Industry-sponsored Satellite Symposium
11:50–12:20	**Keynote Lecture** *Chairs: Mariano Rocchi, José M. Garcia-Sagredo* The chromosome 22q11.2 rearrangement disorders **Bernice E. Morrow** (USA)
12:20	**Closing Ceremony** *Chairs: José M. Garcia-Sagredo, Damien Sanlaville*

Invited Lecture Abstracts

L1

Aneuploidy in humans: new insights into an age-old problem

Terry Hassold
School of Molecular Biosciences, Washington State University, Pullman WA–USA

Aneuploidy is the most common genetic complication of pregnancy, with approximately 0.2–0.3 % of newborn infants being trisomic. However, this represents just the tip of a large iceberg, because most aneuploid conceptions die in utero. Indeed, studies of preimplantation embryos suggest that a large proportion, if not a majority, of fertilized human eggs have extra or missing chromosomes. Because the vast majority of errors result from the fertilization of a chromosomally abnormal egg by a normal sperm, attention has focused on why human female meiosis is so error-prone.

In this presentation, we will summarize recent studies indicating that there are multiple routes to female-derived aneuploidy; e.g., studies of model organisms indicate the contribution of errors occurring during the long meiotic arrest stage or as part of the meiotic cell cycle checkpoint machinery. We will also discuss our own work, which has focused on analyzing human meiosis "as it happens" in fetal oocytes and in spermatocytes. These studies demonstrate remarkable differences between human males and females in the way in which chromosomes find and synapse with one another, in the packaging of chromatin, and in the control of the meiotic recombination pathway. Further, they suggest that errors in fetal oogenesis are relatively common and are important contributors to male:female differences in meiotic nondisjunction, as well as to the maternal age-related increase in aneuploidy levels in our species.

L2

CNV on the X chromosome. Clinical interpretation and pitfalls.

Damien Sanlaville
Genetics Department, Hospices Civils de Lyon, UCBL1, Lyon-France

Intellectual disability (ID) and multiple congenital anomalies (MCA) have an estimated prevalence of 3 %–5 % in resource-rich countries. These conditions make significant demands on healthcare expenditure. Standard karyotyping can identify about 10 % of chromosomal abnormalities in patients with ID/MCA. Genome-wide array comparative genomic hybridization (array-CGH or SNP array) has become the first-line diagnostic tool in the clinical investigation of patients presenting with ID/MCA. This approach detects pathogenic genomic imbalances with a mean diagnostic yield of 12.2 % in these patients (Miller et al. AJHG 2010).

The X chromosome is often implicated in intellectual disability. More than 100 loci on the X chromosome are associated with intellectual disability and copy number variations of the X chromosome are frequently detected in routine diagnostics.

In this session, we will focus on the difficulties of interpreting X chromosome CNV detected by array-CGH/SNP array:

1) Potential sex mismatch may make results for the X chromosome difficult to interpret. Moreover, abnormal log ratios differ between males and females. Some examples are presented.

2) Females have two X chromosomes. One of these chromosomes is inactivated. This inactivation is usually random, but this is not always the case. We will discuss the utility of X inactivation studies in females with X-chromosome CNV.

3) Chromosome inactivation does not involve the entire X chromosome. Some regions of the chromosome, such as the pseudo-autosomal regions, escape inactivation. Careful analysis of the log ratio is required for these regions.

4) The interpretation of X-chromosome CNV may differ between male and female patients. For example, X chromosome duplication in males results in X functional disomy.

5) Several recurrent CNVs have been identified by array-CGH/SNP array. We present the most frequent of these CNVs, together with their associated clinical phenotypes.

6) We discuss some difficult-to-interpret recurrent CNVs found on the X chromosome, such as CNV at the STS locus. Indeed, the discovery of numerous copy number variants of benign or unknown clinical significance has rendered interpretation problematic. For example, the submicroscopic duplication of Xp22.31 has been

reported as a possible cause of intellectual disability and/or developmental delay or as a benign variant.

7) We present several cases with incidental findings and for which genetic counseling is difficult. Indeed, most X-chromosome CNVs are inherited from the mother and carrier status might be revealed.

Finally, we present our experience with a custom X array containing more than 100,000 X-chromosome probes, making it possible to detect CNV over less than 1 kb, and we discuss the utility of such arrays for diagnosis.

L3

Can Down Syndrome be treated?

Renaud Touraine, Marie Ducrocq, Bénédicte de Fréminville
Service de Génétique, Centre de Référence des Anomalies du Développement, CHU de Saint Etienne, France

Down Syndrome (DS or Trisomy 21) is still the most frequently recognized cause of intellectual deficiency with an incidence at birth of 1/1500-1/2000 in most European countries. A further decrease is likely to come with the use of non-invasive prenatal diagnosis as a replacement for current prenatal screening strategies. Eventually, giving birth to a child whatever his chromosomal status is parents' choice, and life expectancy of people with DS is more than 55 years, making DS not a "disappearing" birth defect

Persons with DS often have mild intellectual deficiency (ID), less frequently severe ID. They benefit from early intervention and continuous educative and re-educative supports. Self-determination is also a major source of improvement. Therefore a majority of persons with DS are able to attain convenient autonomy to live in our societies, as long as some support is maintained. These limited results are not sufficient indeed and a better achievement for a larger number of those persons is on hold.

Up to 2014, no drug or supplement mixture has proven any efficacy on improving cognitive performance in persons with DS. This year and the very next may be the beginning of effective therapies.

Doing therapeutic trials to increase cognitive functions in people with ID raised several difficulties.

The very first question is the likelihood of effect on an adult brain: is it too late to expect any effect after birth or infancy? We can consider that the nature of brain defect in DS is not only developmental but also functional, making room for possible cognitive improvement even in older children or adults.

It is also very difficult to evaluate amelioration in cognitive functions since there are no gold standard tests because no trial in any intellectual disorder has been successful so far. Along these lines, what is the meaning in real life of a statistically significant modification of few points or seconds in a neuropsychological test?

In addition, cognitive functions are a "work in progress" that depends on environmental experiences and positive effects may require adequate stimulating environment and a longer period of time than the usual duration of clinical trials.

Nevertheless better knowledge of brain functioning and chromosome 21 genes content have allowed several teams to identify potential therapeutic approaches in DS. These different approaches are not exclusive to each other and may not be specific to DS since some deficient pathways may be affected in other causes of ID as well.

The two most promising molecules currently in trial in persons with DS are EGCG and RG1662.

EGCG (epigallocatechinegallate) is an inhibitor of DYR1A whose coding gene is on the chromosome 21. *DYRK1A* overexpression has clearly a role in brain dysfunction in DS persons and DS animal models. In these animal models, EGCG administration reduced the negative impact of the presence of 3 copies of *Dyrk1a*. EGCG is a polyphenol extracted from green tea leafs. The first trial of EGCG therapy versus placebo in adults with DS has been conducted in Barcelona, Spain (Prof de la Torre) and its results are expected soon.

RG1662 is a selective $GABA_A$ alpha5 negative allosteric modulator developed by ROCHE™. In simple words, it can be viewed as an "anti-diazepam" drug. RG1662 re-establishes a balance between excitatory and inhibitory systems in brain (related to Glutamate and GABA respectively). A phase II double-blind multicentric trial is currently in progress in adults and adolescents with DS (CLEMATIS®).

The hope of reducing the burden of DS has never been so well founded, even if it will not "treat" DS but its most handicapping consequence i.e. intellectual deficiency.

L4

Mendel, his time, and the rediscovery of his laws.

Trude Schwarzacher
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'Few publications have so enduringly and variously influenced science as had the short monograph [Versuche über Pflanzenhybriden, 1865] by the Augustinian monk of Brünn [now Brno], Pater Gregor Mendel. Forgotten for decades, within a few years after its rediscovery it gave a mighty impetus to the doctrine of heredity; and as Mendelism, his teaching had now become the central theme of biological research as well as the foundation of manifold practical application'—this is how Hugo Iltis (1966) starts the preface of his book 'The Life of Mendel'.

I will be looking at the scientific landscape of the time of Mendel's discovery in the mid 19th century, as well as the rediscovery 50 years later and describe the key findings. These involved his famous studies with peas, introducing the terms dominant and recessive, but Mendel also worked with bees and mice and the plant *Hieraceum*. But his paper was seen as essentially about hybridization rather than inheritance and had little impact and was cited only three times over the next 35 years. At that time **blended inheritance** was the accepted theory of inheritance where traits from each parent are averaged together—not so different from what we now know to be the case for multiple genes and quantitative traits—while Mendelian **discontinuous inheritance** applies to single genes.

By the turn of the 19th century, there were rapid advances in cytology, and especially in the knowledge of the nucleus. Cell division is always preceded by nuclear division (mitosis) and chromosomes were stained and named (Waldeyer 1888). Boveri, Weismann and Flemming showed their integrity and continuity and proved that the number of chromosomes is fixed for each species. Thus, it was assumed that 'heredity factors' are contained in the chromosomes and that each sexually reproduced organism has received a complete set of paternal and maternal chromosomes. Finally, Mendel's laws were rediscovered independently of each other by the Dutch Hugo de Vries and the German Carl Correns, and to some extend the Austrian Erich von Tschermak. Following their publications (in 1900 within 2 months), Mendel's results were replicated and genetic linkage formally described.

I will be presenting Mendel's discoveries in the context of data from our own lab, studying chromosomes, inheritance, combination and recombination of genes, and in particular repetitive DNA elements in plants and animals. We now know about mechanisms of Mendelian inheritance of genes and alleles, directly giving quantitative traits and linkage, and genetic maps. But we also understand and can ascribe mechanisms for non-Mendelian inheritance and many aspects of LaMarkian inheritance: linkage drag, and the ever-increasing fields of epigenetics, genomic interactions and chromatin cytogenetics that are largely dependent on the repetitive landscape of chromosomes.

More details will be available at our website, www.molcyt.com.

L5

From Genetic to Epigenetic Inheritance.

Marcus Pembrey
Genetics and Epigenetics in Health and Disease Section, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK
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By the time Mendel's Laws were 'rediscovered' and integrated into the chromosomal theory of inheritance, August Weismann's idea of a barrier between the soma and germ plasm (proposed in the 1890's) had taken hold. This, and the difficulty of demonstrating Lamarckian inheritance of acquired characteristics using inbred animals, helped define the favoured view of biological inheritance in the 20th century. Inheritance was either transmission of DNA and its sequence variations or social transmission through imitation and learning. By the 1960's somatic nuclear transplantation experiments indicated that all cells had a full complement of DNA and therefore some form of epigenetic regulation of gene expression during development had

to exist. Modification of DNA by methylation of cytosines was shown in 1975 and it soon became clear that methylation patterns influenced gene expression and were stably transmitted though somatic cell division to create a cellular memory. Studies of early mouse development in the 1980s, whilst challenging Weismann, also showed genome wide erasure of DNA methylation in the preimplantation embryo. However imprinted genes escape this early methylation erasure. Discovered in the 1980's and 1990's, mammalian genomic imprinting was the starting point for a reconsideration of the idea that sperm might carry information about the ancestral environment—with transgenerational epigenetic inheritance as a candidate mechanism. The focus was on male-line transgenerational effects because female-line studies are confounded by transplacental signals, etc. There were animal experiments at this time showing male-line transgenerational effects of drugs and hormones, but no epigenetic studies. Human male-line studies really took off around 2000 with the three-generation historic analysis of food supply in childhood and descendant longevity by Lars Olov Bygren and colleagues using his Overkalix cohorts in Northern Sweden. It was also about this time that environmental epigenomics became established as an idea. I will describe my collaboration with Bygren and my work with Professor Jean Golding to demonstrate transgenerational responses to early-life parental smoke exposure using the Avon Longitudinal Study of Parents and Children (ALSPAC). This characterisation of human transgenerational responses does suggest some form of non-genetic, non-social transmission is occurring between generations [see Pembrey et al. *J Med Genet* 2014;51:563 and Golding et al. *Am J Hum Biol* 2014;26:731]. There are three main messages to take from these initial (male-line) transgenerational responses to early-life parental or ancestral food supply or tobacco exposures: (i) they are not usually Lamarckian inheritance of acquired characteristics; (ii) there are exposure-sensitive periods during development; and (iii) both the transmission and offspring outcomes are often sex-specific. It looks as if transgenerational responses contribute to developmental variation in the population. Support for this conclusion comes from rodent experiments over the last decade. They have started to provide direct evidence of transgenerational epigenetic inheritance particularly in terms of changes in sperm microRNAs or DNA methylation.

L6

Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR-Cas9 system.

Sandra Rodriguez Perales
Molecular Cytogenetics Group, Centro Nacional Investigaciones Oncológicas (CNIO) C/ Melchor Fernández Almagro, Madrid-Spain

Cancer is a multistep process that involves multiple changes in its genome, including genetic specific point mutations and other large genome rearrangements. A frequent and key genomic event in cancer initiation and progression are chromosome translocations, an exchange of large DNA fragments between two non-homologous chromosomes that in many cases leads to the creation of new fusion genes. Those chromosomal translocations are generated through the illegitimate joining of two non-homologous chromosomes affected by double-strand breaks (DSB). Despite their importance, it is difficult to generate models (either murine or human cellular models) to study the complex scenario of cancer genetics; but the cancer-modelling field is now experiencing a conversion with the recent emergence of the RNA-programmable CRISPR-Cas9 system, a flexible methodology to produce essentially any desired modification in the genome.

We have developed a new strategy for generating cancer-related human chromosomal translocations based on the CRISPR system. Using this approach, we have induced chromosomal translocations resembling those described in acute myeloid leukaemia (AML) and Ewing's sarcoma (ES) with unprecedented high frequencies in human cell lines and primary human stem cells. Cytogenetic and molecular analysis of the

engineered cells revealed the reliability and accurately of the CRISPR system approach, providing a powerful tool for cancer studies. Furthermore, we have also used the CRISPR system to induce other cooperative hits found in Ewing sarcoma patients. Using this advance, it is now possible to easily and accurately generate cell and animal models harbouring the same alterations that define tumour cells in human patients, allowing researchers to experimentally recapitulate the genomic alterations needed to transform a healthy cell/organism or tissue. We have demonstrated that the CRISPR system has been prove as a robust technology

that made possible to generate animal and human cell models that recapitulate tumour specific cooperative alterations rapidly and at low cost, providing a new way to interrogate the development and progression of cancers. These advances will in time lead to the development of better therapeutic approaches to the treatment of cancer, thanks to the use of more accurate and faithful models.

L7

Genome rearrangements in hereditary disorders.

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Several lines of evidence suggest that genomic rearrangements (GRs) in humans occur in a non-random manner and correlate with local sequence composition. Whereas most of the pertinent data on the processes leading to this type of genetic instability have come from studies on somatic tissues, little is known about GRs in the germ line of patients with hereditary disorders. In this study we used 112 pathogenic germ line GRs to investigate how sequence architecture impacts on the type and frequency of GRs in genes predisposing to hereditary colorectal cancer, namely APC, MLH1, MSH2, MSH6 and PMS2. Of these, we fine-mapped 17 GRs (from a discovery set of 31 GRs) at 1 bp resolution using custom-designed arrays and chromosome walking, whereas the breakpoint (BP) positions for the remainder (n=95) were retrieved from public sources. To analyse in a hypothesis-free manner the breakpoint-flanking sequences we used available and custom algorithms to identify 4-mer sequence motifs for homology-driven (hGR) and non-homology-driven rearrangements (nhGR) as well as complexity analysis for higher order features of genome architecture. Subsequently, all statistically significant 4-mer sequences were cross-referenced with motifs reported in the context of genome reorganisation to look for mechanisms underlying hGR and nhGR formation. In hGR BPs the most common motifs consisted of mutational “super hotspots” (40 %) and DNA polymerase arrest / slippage sites (25 %) compared to nhGR BPs, where DNA topoisomerase sites (27 %) and “super hotspots” (19 %) represented the most frequent recombination-associated motifs. Finally, assuming the sequence-related vulnerability of DNA to mutation(s) we wondered if the DNA motif-based logic derived from germ line GR sites could be extrapolated to a genomic scale. We therefore partitioned the human genome HG18 into 5 kb windows and calculated the ratio of nhGR to hGR motifs, referred to as recombination type ratio (RTR). As we could show for all CRC genes investigated as well as for 16 additional, clinically important gene loci with adequate numbers (≥15) of precisely mapped germ line GRs, an RTR cut-off of 6.6 correctly associated the predominant type of recombination for the majority (>85 %) of pathogenic rearrangements. Hence, our study lends further support to the sequence-directed nature of human mutations and provides a new approach to study the mechanisms underlying GRs on a genomic scale within and, possibly, between species.

L8

Salivary amylase CNV and obesity

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Frequent Nucleotide Polymorphisms (SNP) only explain a small fraction of obesity heritability. Beyong point mutations, it has been proposed that Structure Variants may contribute to complex traits. We previously evidenced a potent gene dosage effect at chromosome 16p11 where large Copy Number Variant (CNV) cause highly severe obesity (when DNA is deleted) or medical leanness (when DNA is duplicated). Further, we developed system biology approaches in human, combining transcriptomics in adipose tissue and GWAS analysis in sib-pairs discordant for obesity, and we identified a gene dosage effect of a multi-allelic CNV linked to obesity on chromosome 1 at amylase genes regions. This complex region is known to witness human evolution and natural selection associated with agriculture development and starch consumption. Using qPCR and novel digital PCR analysis we showed that high number of copies of salivary amylase (AMY1) strongly protects against obesity, in particular in high starch diet populations. Our recent data also involves polymorphic pancreatic amylase (AMY2) as a putative player. Both plasma enzyme activities strongly

associates with adiposity and metabolic phenotypes. Metabonomics analysis in human and animal studies incriminates the gut microbiome as a link between diet, amylase CNVs and obesity. Salivary amylase is a potent marker of response to stress and environment induced stress is a well known risk factor for obesity and metabolic diseases.

L9

Polymorphism of the pseudoautosomal region I.

Martin A. Mensah, Hestand MS, Larmuseau MHD, Isrie M, Vanderheyden N, et al. (2014)
Pseudoautosomal Region 1 Length Polymorphism in the Human Population. PLoS Genet 10(11): e1004578. doi:10.1371/journal.pgen.1004578. doi: 10.1371/journal.pgen.1004578
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The human sex chromosomes differ in sequence, except for the pseudoautosomal regions (PAR) at the terminus of the short and the long arms, denoted as PAR1 and PAR2. The boundary between PAR1 and the unique X and Y sequences was established during the divergence of the great apes. During a copy number variation screen, we noted a paternally inherited chromosome X duplication in 15 independent families. Subsequent genomic analysis including BAC mediated targeted paired-end and single molecule sequencing demonstrated that an insertional translocation of X chromosomal sequence into the Y chromosome generates an extended PAR. The insertion is generated by non-allelic homologous recombination between a 548 bp LTR6B repeat within the Y chromosome PAR1 and a second LTR6B repeat located 105 kb from the PAR boundary on the X chromosome. The identification of the reciprocal deletion on the X chromosome in one family and the occurrence of the variant in different chromosome Y haplogroups demonstrate this is a recurrent genomic rearrangement in the human population. This finding represents a novel mechanism shaping sex chromosomal evolution.

L11

CINister problems, modeling aneuploidy in the mouse

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Two out of three tumors display abnormal chromosome content, a state defined as aneuploid. Paradoxically, aneuploidy inhibits cell proliferation of non-transformed cells, suggesting that cancer cells have acquired mutations that help them cope with the disadvantages of aneuploidy. Together, these features make aneuploidy a promising target for cancer therapy. To better understand the responses to aneuploidy in vivo, we developed conditional knockout mouse models, in which we can provoke aneuploidy in tissues of choice, at time points of choice. Our models have allowed us to induce highly aneuploid cancers in various tissues, including liver and T-cells in a highly reproducible fashion. This allowed us to extract common features of aneuploid cancer cells in vivo, and allows us to further investigate how aneuploid cancer cells cope with the detrimental consequences of aneuploidy. In my presentation, I will discuss how different tissues show sometimes quite opposing responses to aneuploidy, suggesting lineage-specific aneuploidy-coping strategies. Understanding these tissue-specific responses will be crucial for the future development of aneuploidy targeting cancer therapies. Furthermore, I will discuss how we use our single cell-sequencing platform to quantify karyotype heterogeneity in aneuploid mouse tumors and human tumors. This data is is revealing that human aneuploid tumors, similar to our aneuploid mouse tumors, exhibit frequent missegregation events, especially when put under selective pressure, which might impact therapeutic response.

L12

The growing importance of CNVs: new insights for detection and clinical interpretation.

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Copy Number Variants (CNVs) have been recognized as major contributors to neurodevelopemental disorders (NDs) but the effects of these variants on cognition and

behavior and how they lead to neuropsychiatric disorders remain largely unknown. Because some CNVs (such as those at the 16p11.2 locus) occur recurrently at a specific locus as either a loss or reciprocal gain of genomic copies, they allow beyond a simple case–control study, to investigate relationships between gene dosage and clinical as well as intermediate traits. Our group has studied the 16p11.2 genomic region and characterized the effect of gene dosage on cognitive, behavioral and structural neuroimaging phenotypes as well as medical comorbidities (obesity in particular). We are extending this systematic study to all major recurrent genomic variants associated with autism and schizophrenia to understand how multiple genomic loci may converge on key mechanisms leading to these 2 neuropsychiatric disorders.

L13

Copy number variation as a key to unlocking the enigma that is schizophrenia

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Schizophrenia is a complex, heritable brain disorder defined by core symptoms affecting perception of reality, thinking, behavior and motivation. Schizophrenia is challenging in that it forces people to confront their perception of the world and is associated with significant morbidity and mortality. Current therapies are partially effective and a cohesive understanding of pathophysiology is lacking, even whether one or more disease mechanisms are involved.

Recent progress is illuminating a genetic architecture of schizophrenia that involves a substantial contribution from many common risk variants of small effect, but also rare mutations (e.g. copy number variants (CNVs)) of large effect. This work, in particular the findings from CNV studies is making us re-think how we conceptualize schizophrenia, and its relationships with other psychiatric and brain disorders. The data suggests convergence on molecular risk mechanisms implicating synaptic plasticity, immune function and chromatin remodeling. At a molecular level, these mechanisms appear to overlap across a spectrum of neurodevelopmental disorders.

The current state of play in our understanding of the genetic architecture of schizophrenia and how this may impact clinical care will be reviewed. In particular, this presentation will provide an update on the latest findings from CNV discovery, how these findings overlap across clinical boundaries and their likely diagnostic relevance. CNVs can arise through a number of different mechanisms and detecting risk variants may require different research approaches. As illustration, we recently identified a novel risk duplication at chr20p12.2 identified in the Irish and UK populations that is likely to have been inherited as a single ancestral event. Finally, we consider where the field is moving and how genomics may reshape how we diagnose and treat schizophrenia in the future.

L14

Molecular breakdown of AML with EVI1.

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Acute myeloid leukemia (AML) is the most common form of acute leukemia. Understanding of the molecular- and biological abnormalities is essential for the development of hypothesis driven therapy of AML. Based on large scale gene expression and gene methylation profile studies (Cancer Cell, 2010) we identified AML subtypes with unique genetic and epigenetic aberrations and study the biological consequences of these abnormalities. In particular, we discovered a unique mechanism of transformation of AMLs with either an inv(3)(q21q26.2) or a translocation t(3;3)(q21;q26.2) [inv(3)/t(3;3], malignancies associated with a dismal clinical outcome (Cell, 2014). Applying functional genomics (4C-seq, ChIP-Seq) and genome-engineering (TALENS and CRISPR/CAS9), we demonstrated reposition of a distal *GATA2* enhancer from chromosome 3q21 to the leukemia disease gene *EVI1,* located at 3q26. This unique translocation of the GATA2 enhancer causes two simultaneous events, i.e. ectopic activation of *EVI1* combined with *GATA2* mono-allelic expression. Functional haploinsufficiency arising from inactivating mutations in *GATA2* zinc finger DNA binding domains or in *GATA2* promoter sequences has been well established as an underlying cause of syndromes characterized by predisposition to

myeloid leukemia. Thus, our data show that structural rearrangements involving single chromosomal repositioning of enhancers can cause deregulation of two unrelated distal genes, with cancer as the outcome. Importantly, we found that the rearranged enhancer near *EVI1* had turned into a BET-inhibitor hypersensitive "super-enhancer". Inv(3)/t(3;3) AML cells appear to be highly sensitive to these BET-inhibitors, which cause strong downregulation of EVI1 expression accompanied by a cell cycle block and induction of differentiation and apoptosis. Our studies emphasize that molecular understanding of defective transcriptional control may provide leads for hypothesis driven targeting of cancer.

L15

Myelodysplastic syndromes: from 5q- to somatic mutation of RNA splicing machinery

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Myelodysplastic syndromes are clonal hematopoietic stem cell disorders characterized by cytopenia, myelodysplasia, ineffective hematopoiesis, and increased risk of progression to acute myeloid leukemia (AML).[1] The vast majority of myeloid precursors are clonally derived in MDS.[1,2] During the natural course of the disease, the acquisition of additional driver mutations leads to formation of subclones of hematopoietic cells with further impaired differentiation and/or maturation capacity, and eventually to development of AML.

Recurrent chromosomal abnormalities are detected in about half of patients with MDS,[3] and the most common aberrations include del(5q), trisomy 8, del(20q), and monosomy 7.[3] While most of these abnormalities are secondary genetic events, the del(5q) of the 5q- syndrome represents a founding event, involving haploinsufficiency for *RPS14* and miR-145.[4–6]

Our understanding of the molecular basis of MDS has improved dramatically in the last 5 years.[7–12] Driver mutant genes include those of RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2*), DNA methylation (*TET2*, *DNMT3A*, and *IDH1/2*), chromatin modification (*ASXL1* and *EZH2*), transcription regulation (*RUNX1*), DNA repair (*TP53*), signal transduction (*CBL*, *NRAS*, and *KRAS*), and cohesin complex (*STAG2*). Only 6 genes (*TET2*, *SF3B1*, *ASXL1*, *SRSF2*, *DNMT3A*, and *RUNX1*) are consistently mutated in 10 % or more MDS patients, whereas a long tail of ~50 genes are mutated less frequently. Recent studies indicate that the development of MDS reflects the occurrence of somatic mutations in hematopoietic stem cells with aging.[13,14]

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L16

Retrotransposable elements, unbalanced translocations and chimerism

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The human embryo has been shown to be a cradle of chromosomal rearrangements, with multiple cells carrying segmental deletions and duplications. To proof that those rearrangements can result in chromosomal disorders, we reasoned that de novo unbalanced translocations originating postzygotically could exist out of compound maternal/paternal derivative chromosomes. We analyzed 12 de novo unbalanced translocations and mapped the breakpoints in nine. We demonstrated the existence of compound heterozygotes and estimate that up to 30 % originate postzygotically. In contrast to balanced translocations where large-scale analysis of balanced chromosomal translocation breakpoints has shown nonhomologous end joining and microhomology-mediated repair to be the main drivers of interchromosomal structural aberrations, we identify nonallelic homologous recombination (NAHR) between (retro)transposable elements and especially long interspersed elements (LINEs) as the main mutational mechanism. This finding shows yet another involvement of (retro)transposons in genomic rerangements and exposes a profoundly different mutational mechanism compared with balanced chromosomal translocations.

L17

SDs, CNVs and complex regions of the human genome

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There is a complex relationship between the evolution of segmental duplications (SDs) and rearrangements associated with human disease. The repeat architecture of the human genome predisposes certain regions to non-allelic homologous recombination (NAHR) resulting in copy number variants (CNVs). Over the last years, several studies have identified hotspots of recurrent CNVs associated with intellectual disability, autism, epilepsy, and schizophrenia. Most rearrangements are large and flanked by high homologous SDs. We sought to better understand the mechanisms leading to genomic instability of previously known disease associated loci by characterizing their SD architecture in humans and non-human primates. We discovered and characterized alternate structural configurations of these regions in humans that often arose as a result of human-specific expansions of SDs in conjunction with independent evolutionary inversion events. Our findings emphasize the importance of correctly defining alternative human genomic configurations in order to assess variable risk of subsequent pathogenic rearrangements. Molecular cytogenetics, genomic approaches, and sequencing of long molecules from single haplotypes remain the only way to correctly resolve these complex architectures of the human genome.

L18

Chromosome-centric approaches unravel the impact of polyploidization and interspecific hybridization

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The process of doubling somatic chromosome number, or polyploidization, played a major role in plant genome evolution and speciation. Based on their mode of formation, polyploid species are classified into auto-polyploids, which originate from single species, and allopolyploids, which arise by hybridization between distinct species. Polyploidization is critical for the newly established hybrids to be fertile and propagate sexually. Apart from creating reproductive isolation, increasing the number of chromosome sets results in the increased number of genes, providing substrate for the evolution of complex regulatory and metabolic pathways. Recent

allopolyploids represent suitable models to study genome changes induced by interspecific hybridization and polyploidization, and the impact of these processes on genome organization, evolution and function. One of such species is hexaploid bread wheat, a crop of global importance. However, until recently, wheat was not considered a suitable model due to huge and complex hexaploid genome comprising three homoeologous chromosome sets. The situation changed with the advent of high-throughput next generation sequencing technologies. After coupling with flow cytometric chromosome sorting, which makes it possible to dissect nuclear genomes to single chromosomes, a powerful toolbox became available to overcome difficulties encountered when analyzing (allo)polyploid genomes due to their enormous sequence redundancy. This approach permits identification of genic sequences separately on each chromosome and perform comparative gene analysis of subgenomes and putative parental species. It also facilitates the analysis of transcriptional activity of homoeologous genes during plant growth and development. Although the chromosome-centric approaches have been employed most extensively in wheat and other cultivated and wild species of the tribe Triticeae, they can be applied in any species from which mitotic chromosomes can be isolated by flow-sorting.
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L19

Functional organization of satellite-less equid centromeres

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The centromere is the site of kinetochore assembly required for the correct chromosome segregation during cell division. In centromeric chromatin, despite the evolutionary conservation of proteins, DNA sequences are highly variable, even among related species. This paradox is now explained by the well established knowledge that the centromeric function is epigenetically specified. This important chromatin domain has so far escaped comprehensive molecular analysis due to its typical association with highly repetitive DNA (satellite DNA). Although satellite DNA is a common feature of mammalian centromeres, we proved that, in *Equus* species (horses, asses and zebras), several centromeres are completely satellite-free [1, 2], thus representing a unique model for studying the organization, function and evolution of mammalian centromeres.

The simple sequence organization of the equid satellite-less centromeres is related to the fact that they were generated recently during evolution through a centromere repositioning process (shifting of the centromere function without chromosome rearrangement) [3]. We suggest that the DNA component of these evolutionarily young centromeres did not have enough time to acquire the typical complexity of the majority of the mature mammalian centromeres described so far.

The functional organization of the single-copy centromere of horse chromosome 11 was also analysed in different individuals by ChIP-on-chip with an antibody against CENP-A, the centromeric histone-H3 variant [4]. Surprisingly, inter-individual positional variation of CENP-A domains was observed; these were scattered in a ~500 kb region, giving rise to "epialleles". These results proved that the centromeric domains are autonomous relative to the underlying DNA sequence and are characterized by positional instability.

We recently investigated the molecular organization of the donkey centromeres by ChIP-seq and by immune-FISH on chromatin fibres: 16 satellite-less centromeres were identified and the underlying DNA sequence was *de novo* assembled. The results of a comparative analysis of centromeric and non-centromeric chromatin will be presented and the possible role of DNA breakage and methylation in the establishment and function of the centromere will be discussed.

Taken together, our discoveries demonstrate that, in the genus *Equus*, centromeres are extraordinarily plastic and represent an important driving force in genome evolution. Our results also indicate that the availability of a number of satellite-less centromeres now opens the way to detailed molecular studies.

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L20

NGS and cytogenetics

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Our genomes are full of variation, including the larger structural variations that are the traditional focus in cytogenetics. Genome sequencing technologies are now allowing us for the first to obtain an integrated view of all this variation present in a genome. This results in a more complete picture of genome variation and an integration of the fields of "DNA diagnostics" and "cytogenetics" into "genome diagnostics". In addition, the widespread automation of next generation sequencing technologies has an impact on the workload in a genome diagnostics laboratory, where a switch is required from the "wet lab" to the "dry lab", the bioinformatics interpretation of large amounts of genomic variation. In this presentation, I will discuss our switch from exome to genome sequencing in research on severe intellectual disability. I will demonstrate how this has resulted in an enormous improvement in the detection of structural variation, also in comparison to high resolution genomic microarrays. Also, I will discuss how a focus on copy number variations may be of enormous value in further understanding the functional and clinical relevance of genomic variation in the non-coding part of our genome, the largely unexplored part of our genome.

L21

Genetic diagnosis in malignant hemopathies

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Genetic diagnostics in hematological malignancies today encompasses several cytogenetic techniques such as chromosome banding analysis, fluorescence in situ hybridization (FISH) on interphase nuclei and on metaphases including 24-color FISH and array based comparative genomic hybridization as well as a variety of moleculargenetic techniques such as RT-PCR, quantitative RT-PCR, fragment analysis, melting curve analysis and sequencing. As high through put sequencing technologies are available now whole exome and whole genome sequencing can be performed in larger patient cohorts. Thus, data on molecular mutations and their diagnostic and prognostic impact has increased tremendously in several hematological malignancies.

Today the karyotype as assessed by chromosome banding analysis and molecular mutations play an important role in the diagnosis, classification, prognostication and treatment decisions in several hematological neoplasms. The WHO Classification of tumours of haematopoietic and lymphoid tissues 2008 requires the karyotype for the diagnosis and classification of several entities either as the sole feature as in certain AML subtypes or more frequent in combination with cytomorphology such as in MDS and MPN. The impact of the karyotype on prognosis in hematological malignancies has best been shown in MDS and AML for which detailed scoring systems have been developed. More recently also molecular mutations have been implemented in prognostic scoring systems in AML and MDS.

Treatment decisions are dependent on genetic information in a large variety of hematological malignancies. For example the detection of the Philadelphia translocation leading to a *BCR-ABL1*-rearrangement provides the basis for a treatment with tyrosine kinase inhibitors. In AML and ALL the genetic profile determines whether or not an allogeneic stem cell transplantation is the treatment of choice.

From the diagnostic perspective a highly sophisticated characterization of the karyotype/genotype of every individual patient has to be aimed at. However, the value of diagnostics is dependent of the therapeutic options that can change patients prognosis. Thus, the more targeted treatments become available the higher the requirements for comprehensive diagnostics gets.

The question for tomorrow is whether the "chromosomal karyotype" will be substituted by a "molecular karyotype". In AML powerful prognostic models have been developed which are solely based on markers detectable by molecular genetic techniques. Further high through put sequencing techniques challenge the need for cytogenetics. Whole genome sequencing is able—as is chromosome banding analysis—to provide

information on the whole genome and can—as chromosome banding analysis does—detect balanced rearrangements leading to fusion genes as well as unbalanced rearrangements leading to copy number changes. Compared to chromosome banding analysis the resolution is considerable higher and also molecular mutations are detectable. Thus this techniques has the ability to provide the most important information required in a diagnostic setting. As this technique does not provide data on the single cell level clonal evolution can only be calculated and indirectly assumed and not separated from independent clones. However, although technical feasible in the laboratory, bioinformatics necessary to use the sequencing data in a routine setting is still in its infancy. Further, it is still an open question how genetic data should be handled that provides information beyond the primary diagnostic question.

The strategies to incorporate the "old" and "new" techniques in the future diagnostic work-flow in hematological malignancies will be discussed.

L22

Constitutional and somatic rearrangements of chromosome 21 in acute lymphoblastic leukaemia

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Chromosome 21, although the smallest chromosome in the karyotype, is frequently involved in cancer. It plays a major role in acute lymphoblastic leukaemia (ALL), manifesting as a range of chromosomal abnormalities. It often occurs as a numerical chromosomal change, either as the sole gain, giving rise to acquired trisomy 21 or, more frequently, as the gain of two or more copies within a high hyperdiploid karyotype (51–65 chromosomes with non-random chromosomal gains). Patients with high hyperdiploidy account for approximately 30 % of childhood ALL and have an excellent outcome. In contrast, patients in the rare numerical cytogenetic subgroup, near haploidy (25–30 chromosomes)/low hypodiploidy (31–39 chromosomes) account for less than 1 % patients and have a dismal outcome. In this subtype, chromosome 21 is always retained as two copies onto the haploid chromosome set. Interestingly individuals with Down Syndrome, with constitutional gain of chromosome 21, have a highly increased risk of developing acute leukaemia. Important structural changes of chromosome 21 occur in childhood ALL, for example the translocation, t(12;21)(p13;q22), which gives rise to the *ETV6-RUNX1* fusion, accounts for ~25 % of childhood ALL associated with an extremely good prognosis.

More recently we have described an intriguing rearrangement of chromosome 21, specifically occurring in childhood ALL. It was found whilst screening for the presence of the *ETV6-RUNX1* fusion by FISH. Approximately 2 % childhood ALL patients, in absence of the fusion, showed multiple copies of the *RUNX1* gene in their interphase cells, which were found to be tandemly duplicated along the long arm of a grossly chromosome 21 in metaphase. The patients were older (median age 9 years) with a high risk of relapse on standard therapy. Intensive treatment was shown to dramatically improve outcome. The abnormal chromosome 21 was highly heterogeneous between patients as confirmed by SNP arrays, with recurrent amplification of megabase regions of chromosome 21. This abnormality was termed iAMP21 (intrachromosomal amplification of chromosome 21). We used genomic, cytogenetic and transcriptional analysis, coupled with novel bioinformatic approaches, to reconstruct the evolution of iAMP21 ALL. We find that individuals born with the rare constitutional Robertsonian translocation between chromosomes 15 and 21, rob(15;21)(q10;q10)c, have ~2700-fold increased risk of developing iAMP21 ALL compared to the general population. In such cases, amplification was initiated by chromothripsis, simultaneously involving both sister chromatids of the Robertsonian chromosome, a novel mechanism for cancer predisposition. In sporadic iAMP21, breakage-fusion-bridge cycles are typically the initiating event, often followed by chromothripsis. In both sporadic and rob(15;21)c-associated iAMP21, the final stages frequently involve large-scale duplications of the abnormal chromosome. The end-product is a derivative of chromosome 21 or the rob(15;21)c chromosome with gene dosage optimised for leukemic potential, showing constrained copy number levels over multiple linked genes. Thus, dicentric chromosomes may be an important precipitant of chromothripsis, as rob(15;21)c is constitutionally dicentric and breakage-fusion-bridge cycles generate dicentric chromosomes somatically.

Furthermore, these findings illustrate that several cancer-specific mutational processes, applied sequentially, can co-ordinate to fashion copy number profiles over large genomic scales, incrementally refining the fitness benefits of aggregated gene dosage changes. These changes are major drivers of cancer, engineered from a finite, but increasingly well annotated, repertoire of mutational mechanisms, which can potentially generate correlated copy number alterations across hundreds of linked genes, as exemplified by iAMP21 ALL.

L23

Developmental disease and cancer: Biological and Clinical Overlaps

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Numerous parallelisms exist between development and cancer. In this presentation, I will review some of the founding ideas linking development and cancer, and highlight clinical conditions exhibiting features of both developmental derangement and cancer predisposition, including cohesinopathies, rasopathies, phakomatoses, Proteus Syndrome and other overgrowth disorders, recessive chromosome breakage syndromes, and dominant hereditary cancer syndromes. I suggest that these disorders encompass a continuous spectrum spanning clinical genetics and clinical oncology, and derive some general implications that might be useful in the future for the treatment of these diseases. As an additional example, I will discuss the role of active DNA demethylation in development and cancer.

L24

European Standards for Clinical Laboratory Geneticists

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There is an increasing need for recognition of also a laboratory speciality in clinical genetics, in line with last year's recognition of clinical/medical genetics as an EU recognized speciality under the UEMS umbrella. The process of achieving such a clinical speciality was long and cumbersome, mainly because approval had to be given by an EU recognition committee, which required almost unanimous agreement between all 27 EU countries. To ease the bureaucracy in such certification processes, the EU has proposed to establish a professional qualifications directive scheme. Hopefully this proposal, where also the ESHG has given substantial input to the process, will be passed within the next years. In order to have a Clinical Laboratory Geneticists (CLG) curriculum ready if such a proposal is passed, an ESHG ad hoc committee has been very active since 2011 under the ESHG umbrella and its work is being continued accordingly. A proposal for a core curriculum (see https://www.eshg.org/clg.0.html) has been finalized in 2012, and has been approved by the ESHG Executive Committee, the Board and received support of a qualified of the corresponding representatives of European national societies. In 2012/2013 150 individuals in 43 countries helped aligning the requirements of the CLG as an EU-recognized specialist profession with those of their national guidelines of education. Information has been returned by colleagues from 41 countries. Thus, in 2014/15 the first round of application for European CLG title was opened to individuals holding a national CLG title from the following countries: Austria, Belgium, Czech Rep, Finland, Germany, Hungary, Italy, Lithuania, Macedonia, Netherlands, Poland, Portugal, Slovenia, Sweden and Switzerland. >250 applications were received and were evaluated. Applicants from other countries could not apply for the European CLG title in 2014/15, but we hope to find ways to open this process also for them in due course. Members of the European Board of Medical Genetics, Clinical Laboratory Geneticist Professional Branch (CLG) are: Thomas Liehr (Germany—chair), Isabel Carreira (Portugal—co-chair), Dilek Aktas (Turkey), Egbert Bakker (The Netherlands), Ana Carrio (Spain), Domenico Coviello (Italy), Lina Florentin (Greece), Martina Rincic (Croatia) and Hans Scheffer (The Netherlands).

L25

Changes in ISO 15189: practical implications.

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A new version of the European standard: Medical laboratories—Particular requirements for quality and competence (EN ISO 15189:2007) was published in 2012 and a corrected version followed in 2014. The changes and newly introduced chapters should be taken into consideration by the laboratories when they apply for accreditation. The changes concern for example:

- document control: the identification of changes to documents,
  page number to total number of pages
- reference to any work referred to other laboratories,
- programme to introduce new staff,
- periodical review of training and competence of staff,
- feedback on staff suggestions,
- immediate corrective or preventive action in case of non-conformities
  indicated by external reviews,
- adverse incidents reporting,
- recorded reviews of verifications and validations,
- automatic selection and reporting of results,
- validation of the laboratory information management system.

A major element of the new ISO standard is the introduction of risk management as a planned process linked to preventive and corrective actions. In risk management of medical laboratories, the impact of work processes and failures on examination results is evaluated because it affects patient safety. This requires the identification of risks and their impact by analysing non-conformities, errors, incidents or complaints, or the application of a failure modes and effect analysis. A second step is the development of risk handling strategies that aim at risk reduction, risk elimination, or acceptance of controlled risk. At the end of this process, monitoring for risk control is established. Planned risk management together with anticipatory preventive and reactive corrective actions are the basis of continuous improvement in the laboratory.

L26

Mandatory 15189 ISO accreditation for French medical laboratories: how to best reconcile regulatory and normative requirements for cytogenetics?

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The French law n°2013-442 of 30 May 2013 has completely reformed the French medical biology, affirming its status as a medical speciality. The patient is at the centre of the medical practice. The law has also made the accreditation mandatory, according to the standard NF EN ISO 15189 and by following a calendar established from 2013 to 2020. In 2020, all French medical laboratories, public or private, must be accredited for the totality of their activity. The next administrative deadline will be on 31st of October 2016. All French laboratories will have to give proof to the ARS (Regional Health Agencies) that they are accredited for 50 % of their activities with at least one examination accredited per field.

In addition to the mandatory accreditation of the medical laboratories, the practice of constitutional and molecular cytogenetics in France is highly regulated. In order to practice this discipline i) the biologists must obtain approval from the Agency of Biomedicine, and ii) the establishment must obtain an administrative approval issued by the ARS to practice these examinations.

Today, cytogenetic laboratories must prioritize regulatory requirements and standards which apply to them and that will be assessed.

In France, the French Committee for Accreditation (Cofrac), the national accreditation body designated by decree in 2008, is the sole authority able to issue accreditation. The Healthcare section is in charge of the accreditation of medical laboratories. The assessment is based on skill assessment conducted by peers. To date, only 7 technical assessors are qualified to assess this discipline.

To help medical laboratories in the accreditation process, the Cofrac provides them technical guides including recommendations (SH GTA 01, SH GTA 04…) as well as standards (SH REF 02, SH REF

04 …) which encompass regulatory requirements in addition to the requirements of the standard NF EN ISO 15189.

To date, more than 70 laboratories perform cytogenetic tests (prenatal diagnosis and genetic activities …) and are mainly located in hospitals (>65 %).

On April 1st 2015, 14 LBM were accredited for performing cytogenetic examinations (4 for somatic cytogenetic, 7 for constitutional cytogenetic and 3 for both disciplines).

By April 30th 2015, all medical laboratories performing genetic examinations must submit the accreditation request to the Cofrac (Decree N°2015-205 of 23 February 2015) for at least one constitutional genetic examination and / or one somatic genetic examination. This examination will be either a conventional or molecular cytogenetic examination or a molecular genetic examination.

L27

The Positive Predictive Value of Noninvasive Prenatal Screening (NIPS)

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Recently tremendous progress has been made in analyzing free floating fetal DNA ffDNA in the maternal circulation for the presence of aneuploidies. Several validation trials has described sensitivities and specificities >99 %. Since Positive Predictive Value (PPV) for any ties is proportional not just to the specificity by also to the prevalence of the disorder, a specificity of 99 % will lead to a positive predictive values of under 50 % for a disorder such as Down Syndrome in a high risk population with a 1 % prevalence. We analyzed data from 161 consecutive prenatal karyotypes where the indication for testing was an high risk NIPS result. The results of this study were positive predictive values of 87 % for Trisomy 21, 64 % for Trisomy 18, 48 % for Trisomy 13 and 46 % for sex chromosome Aneuploidies.

When our data is combined with other reports, in 1,353 samples the PPV's for Trisomy 21, 18, 13 and Sex chromosome aneuploidies are: 92, 73, 37, and 46 % respectively.

In an attempt to improve upon these PPV's, we developed a Laboratory Developed NIPS using massively parallel whole genome sequencing, advanced automation, GC correction, proprietary bio-statistical and bioinformatics analysis . We were able to achieve a 100 % sensitivity and specificity on a sample set of more than 3000 samples and 100 trisomic samples. True Trisomy samples had Z score>10 when fetal fractions were >5 % and all unaffected pregnancies demonstrated Z scores<4. We will be contacting each physician following a positive result and will report the preliminary PPV's of our test.

L28

Prenatal Technologies and Sex ratio

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It is known that the sex ratio is skewed in favour of males in countries in South and East Asia, in particular in China and India. The fact that this is also the case in some countries in Central Asia and East Europe is less well known. In all these countries the skewing of sex ratio is unequivocally linked to the availability of prenatal technologies. The preconditions and causes of sex selection will be discussed. The facts and figures from the Census of India and the history of prenatal sex selection in India will be presented. The shortage of women of a marriageable age is predicted to peak in the middle of this century and the problem is expected to continue into the next century. The other consequences of shortage of women will be briefly discussed. Wealth and education do not appear to be the solution, on the contrary. Information on what is being done and what could be done will be presented.

L29

The chromosome 22q11.2 rearrangement disorders

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The chromosome 22q11.2 region is particularly susceptible to meiotic chromosome rearrangements leading to

birth defect syndromes. DiGeorge syndrome/velo-cardio-facial syndrome is the most well-known of the 22q11.2 syndromes and is frequently referred to as 22q11.2 deletion syndrome (22q11DS). The majority of affected individuals have developmental delay, behavioral disorders as well as physical malformations such as craniofacial anomalies and congenital heart disease. Our lab has been interested in two aspects of this syndrome. Firstly, we have been trying to understand the reason why the microdeletion occurs in the first place, and secondly, why phenotypes are so variable in affected individuals.

Over 90 % of 22q11DS patients have a similar sized 3 Mb deletion that usually occurs as a *de novo* event, which is slightly more frequent in female rather than male meiosis. The deletion results from non-allelic homologous recombination events (NAHR) between low copy repeats termed, LCR22. There are four LCR22s in the 3 Mb region (LCR22A, -B, -C, -D) and they are of different sizes. The typical 3 Mb deletion is flanked by LCR22A and LCR22D, which are over 250 kb in size. Each LCR22 is composed of modules of pseudogenes that have been duplicated during primate evolution. Because of their recent evolution, individual modules are 99 % identical in sequence. This high sequence homology makes them prone to NAHR and gene conversion events, making detailed analysis challenging. The current goal is to understand whether there are particular hotspots within the LCR22s that might be responsible for chromosome rearrangements. If so, it would suggest that individuals with this hotspot might be prone to 22q11.2 rearrangements. To identify chromosome breakpoints, we are performing whole genome sequencing of trio families (proband-mother-father). This is being followed by analysis of the haplotypes of LCR22s.

One important question has been whether there are varied rearrangements on 22q11.2 mediated by the other LCR22s. Relevant to this, syndromic individuals have been identified with proximal, LCR22A-B or LCR22A-C deletions as well as distal, LCR22B-D or LCR22C-D deletions. Finally, patients have been identified with reciprocal duplications. Most of these individuals have been discovered from clinical microarray testing because they had undiagnosed conditions. The fact that 22q11.2 rearrangements are so common, underscores the mutagenic properties of the LCR22s and their importance in causing human disease. It also suggests that enough individuals with less well-known rearrangements can now be identified so as to have the statistical power to make genotype-phenotype correlations.

The existence of different chromosome 22q11.2 rearrangements may explain some of the phenotypic variability connected to this interval, but clearly not all, since most patients have the same sized 3 Mb deletion. As part of an International 22q11.2 Consortium, we are using Affymetrix 6.0 SNP arrays for genome-wide association studies and whole exome sequencing to uncover genes that may explain heterogeneity in disease severity for those with the typical 3 Mb deletion. Once genes are identified, they can be tested for functional consequences in animal models.

Oral Abstracts

1. Clinical Cytogenetics

O1

Detecting small Copy Number Variations, smaller than 400 kb, improves the diagnostic yield of CMA in intellectual disability.

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Cytogenetic microarray (CMA) allows the elucidation of 15 to 20 % of remaining unexplained cases of intellectual disability. A threshold of 400 kb is proposed to consider Copy Number Variants (CNV). Array use in diagnostic setting could detect smaller CNV mostly polymorphic generating numerous confirmations. Here, we propose to evaluate the diagnostic interest of small CNV (inferior to 400 kb) in intellectual disability (ID). This collaborative study groups an Illumina SNP-array

platform and an Agilent CGH-array platform for a total of 3330 patients explored for syndromic or isolated intellectual disability from 2011 to 2014.

Over 26,866 CNV detected, 22,683 (84.4 %) were smaller than 400 kb. Among the 1440 CNVs considered as relevant, 850 (40.9 %) were smaller than 400 kb increasing by 70 % the amount of necessary confirmation by another technique. We concluded that 44 of 605 (7.3 %) of these CNVs (smaller than 400 kb) were pathogenic. 36 were deletions which involved pathogenic gene in ID and 8 were duplications. It is noteworthy that, except a MECP2 duplication, all small duplications were either intragenic or probably truncating an ID gene possibly leading to haploinsufficiency.

To conclude, analyzing CNV smaller than 400 kb significantly increases the number of verification but enhances the diagnostic yield of 1.3 % in patients with ID, which is equivalent to 44 patients in our cohort. However, we propose not to consider small non-truncating duplications. While massively parallel sequencing techniques are becoming routine diagnosis, it seems preferable to detect as many pathogenic CNVs as possible using CMA.

O2

The power of social media for karyotype-phenotype analysis of rare chromosome disorders

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This presentation is the direct result of a study that was performed on request of a Facebook Group of parents of children with a chromosome 6 disorder. The successful use of social media in this study demonstrates how eager *parents* are to give and receive information about the condition of their child.

In our study we focused on isolated 6q deletions. These can be divided into proximal (6q11-q16), intermediate (6q15-q25), and distal (6q25-qter). We were able to collect data through an extensive parent questionnaire, medical records and detailed breakpoint characterization, of 15, 21 and 27 patients for these three groups, respectively. Proximal deletions result in a variable degree of cognitive impairment, minor facial dysmorphisms, hypermobility, hypotonia, congenital heart defects, hernias, and limb abnormalities. The main features seen in intermediate deletions are pre- en postnatal growth retardation, developmental delay, hypotonia, Prader-Willi-like features, facial dysmorphisms, limb and cranial abnormalities, congenital heart defects, and abnormalities of respiratory control. Distal deletions are predominantly characterized by intellectual disability, hypotonia, microcephaly, corpus callosum agenesis, seizures, and ear, eye, cardiac, genital and limb anomalies. Other clinical findings may include vertebral and rib anomalies, cleft palate, hydronephrosis, and diaphragmatic hernia. A more detailed analysis of the data allowed the confirmation or identification of candidate genes for specific features, e.g. in congenital heart defects.

We also received preliminary information on 6q duplications (14), 6p deletions (25) and 6p duplications (8), demonstrating how effective our approach is. This prompted us to initiate the development of an interactive online database system based on the intelligent collection, combining and presentation of information for chromosome 6, in collaboration with the Chromosome 6 Facebook Group, Unique, ECARUCA and Cartagenia. Eventually, the same approach can be applied to all chromosome disorders.

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O3

Reorganisation of chromosome 9 pericentromeric segmental duplications in inv(9) carriers and in evolution

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The evolutionary history of human chromosome 9 is complex, and includes structural rearrangements such as inversions and neocentromere formation, accompanied by expansion and reorganisation of large blocks of segmental duplications and of pericentromeric constitutive heterochromatin. The complexity of these

evolutionary changes is mirrored by the fact that human chromosome 9 is heteromorphic, with numerous structural heterochromatic and euchromatic variants described, including copy number variant segmental duplications.

Here we report on a BAC-FISH study in 11 chromosome 9 pericentric inversion carriers, including patients with clinical manifestations and their healthy relatives, with the aim to more precisely delineate the breakpoints and the CNV pattern in these cases. We used a set of seven BACs from pericentromeric segmental duplication and flanking single copy regions, which were combined with alphaSat, betaSat and SatIII probes. Our results demonstrate at least three distinct inversion patterns with breakpoints localized either at the heterochromatin boundary, or within segmental duplications, accompanied with local duplications or triplications. The patterns observed here could only partly be reconciled with previously published inversion patterns, suggesting an even more complex inversion scenario in the human population than previously anticipated.

To better understand the organisation of human chromosome 9 pericentromeric CNV in an evolutionary framework, we further included chimpanzee, bonobo, gorilla, orangutan, a gibbon and three Old World monkey species in our study. To this end, we confirmed the recent explosive evolutionary copy number expansion of chromosome 9 segmental duplications in the last common ancestor of human/African apes, and in particular in the human/chimpanzee ancestor. Further, our results indicate that the evolutionary forces that shaped the human p-arm CNVs may at least to some extent be different from those on the q-arm side, possibly reflecting their evolutionary origins in different genomic neighbourhoods.

2. Tumour Cytogenetics

O4

Comparison of microarray-based genomic profiling and karyotyping in myelodysplastic syndromes (a HOVON89 study)

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In myelodysplastic syndromes (MDS) karyotyping is generally considered as the gold standard. However, karyotyping is limited by its low resolution (5–10 Mb in size). Microarray-based genomic profiling analyses allow a genome-wide detection of copy number alterations (CNAs), down to 100 kb in size, and regions of copy neutral loss of heterozygosity (CNLOH).

We have compared karyotyping with microarray-based genomic profiling with respect to the detection yield for genetic abnormalities in bone marrow samples from lower risk MDS patients in a prospective phase II randomized multicenter study (HOVON89). Data regarding cytogenetics and microarray were obtained in a fully blinded manner for 91 MDS patients. For microarray-based genomic profiling we used CytoScan HD Array (Affymetrix) platform.

Thirty-two MDS patients had an abnormal karyotype, while 49 patients showed an abnormal array profile. Of interest, in 16 of these last patients no cytogenetic abnormalities were detected. The abnormalities that were observed by microarray only were <5 Mb CNAs or regions of CNLOH involving regions and genes recurrently affected in hematological malignancies. Almost all CNAs identified by karyotyping (except 2 subclones) were also observed by microarray-based genomic

profiling. As expected balanced translocations were not identified by microarray-based genomic profiling.

We demonstrate that microarray-based genomic profiling allows the identification of almost all copy number abnormalities also observed by karyotyping. In addition, we show that microarray-based genomic profiling allows the detection of potential prognostic relevant abnormalities which would not be detected by karyotyping. The clinical value of these novel CNAs and CNLOH will be evaluated within the ongoing prospective HOVON89 study.

O5

Assessment of SNP genomic microarray analysis as an alternative to FISH analysis for the detection of copy number changes in pediatric solid tumours

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Medulloblastoma, astrocytoma, and neuroblastoma are amongst the most common solid tumours in children. Recurrent gains or losses of distinct chromosomal regions are prognostic in these three tumours. In medulloblastoma, amplification of CMYC or NMYC is associated with a poor prognosis, and loss of chromosome 6 with a good prognosis. A 7q34 duplication resulting in BRAF-KIAA1549 fusion is associated with good outcome in low grade astrocytomas. In neuroblastoma, amplification of NMYC and loss of 1p36 are associated with a poor prognosis. Currently, we test these loci by FISH analysis, followed by karyotyping if sufficient material remains.

We have validated the use of the genomic microarray platform Affymetrix Cytoscan HD and Chromosome Analysis Suite software as an alternative to FISH analysis for the assessment of copy number changes in these tumours. DNA was extracted from frozen samples of 4 neuroblastoma, 6 medulloblastoma, and 5 astrocytoma for which FISH (+/- karyotype) information was available.

There was 100 % correlation of SNP array data with FISH/G-banding data in detecting the major clonal unbalanced chromosome abnormalities. SNP array data also refined G-band results, detected regions of LOH, and allowed for copy number assessment of additional prognostic whole chromosomes or segmental regions, not currently tested by FISH. SNP array could not detect cytogenetic changes present in minor subclones. The combined use of the allele track and the copy number track provided accurate identification of aneuploidies, but SNP array analysis had difficulty normalizing copy number for tetraploid tumours. Amplifications of MYCN in neuroblastoma and CMYC in medulloblastoma were adequately detected, although this was dependent upon tumour sampling. SNP array analysis offers advantages and technical challenges as a diagnostic tool for pediatric solid tumours.

O6

Epigenetic regulation of UNCX, a novel homeobox transcription factor gene activated in acute myeloid leukemia

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Homeobox (HB) genes encode for transcription factors showing a crucial role in normal hematopoiesis and in

leukemogenesis. Interestingly, the deregulation of HB genes, often embedded within low-methylated regions (≤10 %) called "canyons", was described in murine hematopoietic stem cells (HSCs) as a consequence of altered methylation at canyons edges (borders) due to Dnmt3a inactivation. Among them there is Uncx. UNCX (7p22.3), encoding for a transcription factor involved in somitogenesis and neurogenesis, is specifically expressed in eye, brain and kidney. We identified a M5 AML patient with a t(7;10)(p22;p14) translocation as the sole cytogenetic anomaly and showing ectopic expression of UNCX. Since UNCX was never reported in association with cancer but only with common myeloid cell proliferation and regulation of cell differentiation, we decided to investigate its contribution to leukemogenesis. We observed UNCX ectopic expression in 32.3 % (20/62) and in 8 % (6/75) of acute myeloid leukemia (AML) patients and cell lines, respectively. Notably, retroviral-mediated UNCX transfer in CD34+ HSCs induced a slow-down in their proliferation and differentiation and transduced cells showed a lower growth rate but a higher percentage of CD34+ stem cells in liquid culture than controls. Additionally, UNCX infected cells displayed a decrease of MAP2K1 proliferation marker but increase of KLF4, HOXA10, and CCNA1, associated with impaired differentiation and pluripotency. Similarly, UNCX-positive patients revealed alteration of gene pathways involved in proliferation, cell cycle control and hematopoiesis. Moreover, UNCX activation was accompanied by methylation changes at both its canyon borders, clearly indicating an epigenetic regulation of this gene. Taken together, our results indicate a novel putative leukemogenic role of UNCX, whose activation may be ascribed to epigenetic regulators other than DNMT3A, differently from what previously described in murine HSCs.

O7

Rapid and comprehensive routine diagnostic approach for prognostic genetic markers in multiple myeloma

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Multiple myeloma (MM) is a plasma cell (PC) malignancy characterized by clonal expansion of terminally differentiated B cells in the bone marrow (BM). It is the second most common malignant blood disorder in the Caucasian population and associated with complex genetic changes predicting patients' outcome. Evaluation of genetic lesions is an important tool for risk assessment and treatment monitoring.

Due to resolution and low proliferation activity of PC, the detection of chromosomal aberrations by conventional karyotyping has proven to have too strong limitations. Whilst molecular cytogenetic approaches such as SNP-array and iFISH performed on PC enriched BM samples have shown that genomic alterations are present in more than 90 % of the MM patients. Recent studies demonstrated that disease evolution is associated with an accumulation of specific genome imbalances with important prognostic values. Furthermore, one of the main genetic events in MM is the translocation of the immunoglobulin heavy chain gene complex (IGH) with different chromosomes partners with the gene CCND1 at 11q13 being the most frequently observed.

Due to the often small quantities of material available after PC enrichment, the assessment of the full MM genome for prognostic markers including balanced translocations of the IGH gene can be challenging in a routine diagnostic setting. To overcome this, we developed a sensitive and reliable analytical protocol allowing whole genome screening with low cell quantities (minimum 5000 cells) using a combination of SNP-array and a tricolour customized iFISH. This approach interrogates genomic imbalances, CNLOH as well as IGH rearrangements with CCDN1 or other partner and allows rapid turnaround time of analysis. Workflow, performance and analytical results will be presented and discussed.

3. Prenatal Diagnosis

O8

Digital PCR is an efficient alternative to massively parallel sequencing as a non-invasive, prenatal test for trisomy 21: the results of a pilot study

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The analysis of circulating cell-free DNA has prompted considerable progress in the field of non-invasive prenatal testing for fetal aneuploidy. Molecular counting can be achieved with both massively parallel sequencing and digital PCR. However, the large number of PCR reactions needed to meet statistical requirements has prevented digital PCR from being applied in a clinical setting. Here, we designed an octoplex droplet digital PCR (ddPCR) approach, which allows increasing the number of available targets and thus overcomes statistical obstacles.

After technical optimization, we performed a pilot study on samples of plasma DNA from 300 pregnant women for whom a fetal karyotype was available. Molecular counting of circulating cell-free DNA was performed using a mix of hydrolysis probes targeting chromosome 21 and a reference chromosome. The results of our validation experiments showed that ddPCR detected trisomy 21 even when only 5 % of the DNA was trisomic. In a validation study of plasma samples from pregnant women, ddPCR discriminated clearly between the trisomy 21 group and the euploidy group. The shape of the receiver operating characteristic curve indicated that our screening test is highly efficient.

Our results demonstrate that ddPCR is a promising technique for non-invasive prenatal testing of trisomy 21. This approach is technically simple, relatively cheap, easy to implement in a diagnostic setting and compatible with ethical concerns regarding access to nucleotide sequence information. These advantages make it a potential technique of choice for population-wide screening for trisomy 21 in pregnant women, as well as for other aneuploidies.

O9

Noninvasive prenatal testing: more caution is needed in high risk pregnancies

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Purpose: Non-invasive prenatal testing (NIPT) is increasingly being used in prenatal diagnosis. However, published data on confirmatory cytogenetic testing after positive NIPT in order to collect more information on the positive predictive value for noninvasive testing is still scarce.

Methods: Concordance of results among cases with NIPT referred for cytogenetic prenatal and/or postnatal studies by karyotyping, fluorescence in situ hybridization and molecular karyotyping was evaluated in 47 consecutive cases.

Results: True positive and false positive NIPT results were obtained in 56.4 % and in 41 % of the cases, respectively. Inconclusive results followed by cytogenetically pathologic results were encountered in three cases. Likely false negative NIPT results were observed in two cases, one of which ended with the birth of a severely affected girl. The birth of severely affected children occurred in three more cases, in which only NIPT, which resulted normal, was offered, despite ultrasound abnormalities.

Conclusion: The positive predictive value of abnormal NIPT should not be overestimated and not be confused with the overall specificity value. In addition, more caution is needed in offering NIPT in high risk pregnancies with ultrasound anomalies.

4. Animal and Plant Cytogenetics

010

Comparative chromosome painting in lacertid lizards: highly conserved karyotypes but independent origins of sex chromosomes

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In this work we applied for the first time high-resolution molecular cytogenetic techniques (chromosome painting and comparative genomic hybridisation) to carry out comparative analyses of sex chromosomes among five lacertid species, namely, Iberolacerta monticola, I. galani, I. bonnali, Lacerta schreiberi, and Timon lepidus. In addition, flow-sorted chromosome paints from I. monticola were used in genome-wide comparisons with the chromosomal complements of L. schreiberi and T. lepidus, in order to detect chromosomal rearrangement and homologies among the species of the three different genera.

Chromosome painting with the W of I. monticola (IMOW) showed that the euchromatic region of the female-specific sex chromosome appears to be conserved in the other two species of Iberolacerta. In I. bonnali, a screening with the remaining flow-sorted fractions of I. monticola indicates that its biarmed W chromosome most likely has undergone a centric fusion with either chromosome 15 or 16. IMOW and comparative genomic hybridisations led to the unequivocal identification of the W chromosome both in L. schreiberi and T. lepidus, and indicate a massive accumulation of female-specific repetitive sequences characteristic of each lineage, leading to the formation of highly heterochromatic sex chromosomes.

Our preliminary data from chromosome painting using the Z chromosome suggest at least two independent origins of sex chromosomes in these lacertid lizards. The study of chromosome synteny with the remaining IMO chromosome fractions revealed a high degree of karyotype conservation. The microchromosomes of T. lepidus and L. schreiberi are painted by the IMO fraction containing chromosomes 11 and 12, thus indicating that a translocation of microchromosomes to macrochromosomes generated this difference in karyotype structure of I. monticola.

5. Molecular Mechanisms of Chromosome Rearrangements

O11

Unraveling the internal structure of neocentromeres in ring chromosomes harboring genomic amplification in soft tissue tumors

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Neocentromeres have been reported as frequently arising on ring/rod-shaped chromosomes, particularly in sarcomas. It has been reported that the CENP-A centromeric protein is rapidly recruited at double-strand break (DSB) by the DNA repair machinery. This finding suggested a potential link between the process of DSB repair and neocentromere seeding. We studied four cancer cell lines (three well-differentiated liposarcomas and a lung-sarcomatoid carcinoma) carrying neocentromeres on ring/rod-shaped chromosomes, to investigate this phenomenon. SNP array, FISH, and anti-CENP-A ChIP-seq experiments were performed to characterize the marker chromosomes and the neocentromeric domains. Whole genome next-generation sequencing was also carried out to finely define the inner structure of the marker chromosomes. Our results revealed that neocentromeres were seeded on a "patchwork" of short-sized (<100 Kb) amplified fragments, some from different chromosomes, underlining the epigenetic nature of this phenomenon and suggesting that the massive recruitment of CENPA to repair DSB might trigger the neocentromere formation. We are currently evaluating the chromatin status of the neocentromeric regions by performing targeted bisulphite sequencing and H3K4me1-3/H3K4me27 ChIP-seq assays. Furthermore, we are performing RNA-Seq experiments to identify transcripts likely involved in the neocentromeres seeding and maintenance. Moreover, two of our liposarcoma cell lines derive from the same primary tumour; this will allow us to investigate the evolution of marker chromosomes and neocentromere from the original tumour. Finally, several cases of soft tissue

tumours carrying neocentromeres will be analysed to gain a broader view of this phenomenon in primary malignant sarcomas. The expected results will unveil the existing link between DSB repair and neocentromere seeding, and add new insight into the mechanisms behind the genesis of ring/rod-shaped marker chromosomes.

O12

Meiotic recombination and complex chromosome rearrangements: how whole-genome sequencing helps to resolve them.

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The presence of at least 3 chromosome breakpoints defines Complex Chromosome Rearrangements (CCRs). They can mix translocations, inversions, deletions and/or duplications. Complexity of CCRs can be very high and the related meiotic disturbances result in important chromosomal imbalances in gametes. So, genetic counseling is tricky for carriers of a balanced CCR.

We report 2 families with heavy miscarriage histories. For them, standard karyotypes revealed CCR. Surprisingly, we observed that the degree of complexity of CCRs changed through generations. Using whole genome sequencing we determined the real number of breakpoints and specified the position of each of them. In the first family, the discovery of a multiple malformation syndrome on ultrasound examination indicated a prenatal chromosome analysis. The fetal karyotype showed an unbalanced CCR. Subsequent chromosome analysis of the mother revealed a much more complex but apparently balanced CCR. For the second family, a simple reciprocal translocation was inherited through several generations. For one of the daughters, the rearrangement was more complex involving a third chromosome. Fine molecular analysis allowed ascertaining the real nature of this CCR.

For both families, a re-building of the initial rearrangement occurred through meiotic recombination, giving rise to simpler or more complicated CCR. Combination of next-generation DNA sequencing and other standard approaches such as karyotyping and FISH analysis shed light on the complexity of these CCRs. This new approach has improved the quality of genetic counseling.

O13

Unbalanced de novo chromosome translocations and inversions: a two-step origin?

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The origin of de novo unbalanced translocations and unbalanced rearrangements mimicking a derivative from a pericentric inversion (herein called unbalanced inversions), all presenting simultaneous deletion and duplication of two terminal chromosomal regions, is still enigmatic although their frequency, at least for de novo unbalanced translocations, has been estimated to be 22 % at amniocentesis and 30 % in subjects with developmental disorders.

We have analyzed 35 de novo unbalanced rearrangements, of which one in mosaic, including simple translocations (n=25), inv-dup-del translocations (n=6), and unbalanced inversions (n=4), in order to highlight their parental origin and mechanisms of formation. In most cases, we could characterize the rearrangement at the molecular level and determine the parental origin of both deleted and duplicated segments. Both imbalances were of maternal origin in 14 subjects, paternal in 8, and bi-parental in 2. In 6 additional cases, parental origin was informative for only one of the imbalances (4 paternal and 2 maternal) while in the remaining 5 no information was available.

Our findings suggest that partial rescue of an abnormal chromosome complement consisting in either (i) a rearranged chromosome, such as a dicentric "mirror" chromosome, or (ii) a supernumerary chromosome, presumably already present in the zygote, is the most likely mechanism leading to this type of rearrangement. This rescue may also generate alternative products in different cells of the early embryo, leading to mosaic lines of which the most viable one(s) can be detected in post-natal tissues. The occurrence of two unbalanced translocations with the two imbalances having bi-parental origin supports the proposed two-step mechanism. In addition, the preferential maternal origin of both imbalances might indicate that the final rearrangement results, at least in some cases, from an original non-disjunction event although the expected association with increased maternal age

014

Direct visualisation of complex structural and copy-number variation by molecular combing and multicolour-FISH

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Molecular Combing is an advanced DNA-stretching technology that allows molecules of 200–600 kb long to be uniformly combed onto a glass surface. As the stretching is constant along the length of a DNA molecule, when combined with multicolour fluorescent in-situ hybridisation (M-FISH), Molecular Combing holds the promise for unambiguous characterisation of genomic structure and variation by direct visualisation. Apart from defining order, orientation, and copy number, this technique also allows the quantification of length, gap and overlap sizes. However, to visualize large and complex structural variations and to reduce potential artefacts associated with broken fibres, much longer molecules (~1000 kb) and references marking the two ends of variant regions are often required. Here, by modifying the Molecular Combing protocol, we improved the retention rate of longer DNA molecules on combed coverslips. We also introduced dinitrophenol, fluorescein and Cy5 haptens, and optimised primary and secondary antibody-combinations for use in M-FISH detection, in addition to the widely used biotin and digoxigenin two-colour combination. We have successfully applied this modified fibre-FISH technique to assembling the pig Y-chromosome sequence and characterising a series of complex structural and copy-number variation involving large multiallelic gene families or repetitive sequences identified by array-based assays and next–generation sequencing, including the well-studied amylase genes. While confirming the copy-number changes predicted by alternative methods for the majority of genomic regions being investigated, we uncovered structural details that would be impossible to elucidate using array-CGH or short-read sequencing. This approach also allows pairs of complex haplotypes to be decoded unambiguously. Our results demonstrate that Molecular Combing, when combined with M-FISH, is a definitive approach for the validation and genotyping of complex structural and copy-number variation, as well as for assembling regions enriched in repeats such as mammalian Y chromosomes.

015

The International Breakpoint Mapping Consortium (IBMC). Systematic mapping of chromosomal breakpoints in the context of phenotypes and nuclear genome organization

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We still lack genotype-phenotype-information for ~80–90 % of our protein-coding genes, and for almost all non-coding RNA (ncRNA) genes and regulatory elements. Even with exome and full genome sequencing, it will take decades and tremendous resources to saturate the exome, trancriptome and regulome with mutations/variants that can be linked to abnormal and normal phenotypes. Furthermore, NGS-based proximity mapping has revealed a nuclear organization of the genome, with ~2–3,000 topologically associating domains (TAD), which can be further organized into loops. To facilitate the functional characterization of TADs, TAD-boundaries and loops, we have initiated a systematic mapping of constitutional "balanced" chromosomal rearrangements (BCR) that must affect this nuclear organization. The first unbiased reexamination of prenatally diagnosed de novo BCRs (pBCRdn) show that breakpoints affects genomic compartments according to their size, e.g. protein coding/non-coding genes; regulatory domains associated with long-range position effects (LRPE); unannotated gaps; and TAD and TAD-boundaries. We also show that the unbiased morbidity risk of pBCRdn are 2–3 fold higher than previously estimated (~20 %), due to later onset neurodevelopmental disorders. This morbidity can in some cases be linked to truncation of known disease genes and LRPE-regions, but we also highlight the existing gap in genotype-phenotype knowledge. To address this gap, we have established IBMC, presently involving >150 partners from >50 countries and six continents, with the aim to mate-pair map ~10,000 chromosomal breakpoints. This will target an estimated ~2,700 protein coding genes, ~1,500 ncRNA genes, most of the LRPE-regions that specify the vertebrate body plan, link phenotypes with disruption/rearrangement of nuclear domains, and identify human models for their functional characterization. The project will include participants from undeveloped, developing and developed countries, and hence facilitate global mobility and technology tranfer.

6. Impact of Next Generation Sequencing on Clinical Diagnosis

016

Exome sequencing and SNP arrays as a tool to describe genetic diversity of premalignant and malignant cells: lesson from monoclonal gammopathies

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Whole exome sequencing (WES) and SNP arrays have opened a new landscape to study comprehensive tumour genetic architecture and its evolution during tumour associated processes at the genome-wide level. Transformation from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma (MM) can be used as a unique model for cancer development studies as an analysis of pure tumour population in clearly clinically distinguishable states. In our study, we optimized WES and SNP array protocols from whole-genome amplified DNA to describe genetic diversity of premalignant and malignant cells at the genome-wide level.

Overall, 33 and 69 MGUS patients were included in a WES and SNP array study, respectively. For WES, NEBNext kit and SureSelect Human All Exon V5 (Agilent) were used, samples were sequenced by HiSeq2000 (Illumina). Copy number alterations (CNAs) were tested by SurePrint G3 CGH+SNP, 4 × 180K (Agilent). Results were compared to 463 and 91 MM patients analysed by WES and SNP arrays, respectively.

CNAs and somatic gene mutations (SNVs) were detected in 68 % (47/69) and 100 % (33/33) of MGUS patients in comparison to 100 % (91/91, $p<10^{-4}$) and 100 % (463/463) of MM patients, respectively. However, overall number of both CNAs and SNVs per patient was significantly lower in MGUS (CNAs: median 2, range 0-15; SNVs: median 89, range 9–315) than in MM (CNAs: median 16, range 2–49, $p<10^{-18}$; SNVs median 123, range 1–897, $p<10^{-4}$). We proved that complex genetic instability is formed before tumour clinical manifestation at the gene level followed by the chromosome level. Then, the number of random genetic hits increases to form a landscape for significant oncogenic hits driving the premalignancy transition to a clinically manifested tumour disease. Data in detail are presented.

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Poster Abstracts

1. Clinical Cytogenetics

1.P1

45,X/46,X,idic(Y) in azoospermia: clinical, hormonal and molecular findings in an adult man of very short stature

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Background: In the majority of cases, 45,X/46,X,idic(Y) results in normal appearance of the male genitalia in the neonatal period; followed later on by infertility. The postnatally developing phenotype is quite variable and unpredictable, ranging from otherwise normal sterile male to genital ambiguity and variable Turner Syndrome (TS) features. We report on a male with this rare cause of infertility and discuss the inherent consequences regarding assisted reproductive technology and tumor surveillance for gonadoblastoma.

Clinical report: A 40 year old man sought the advice of a urologist for infertility. Anamnesis was unremarkable and precluded external detrimental influences. Clinical examination showed markedly short stature (150 cm), small testicles, normal penis size and normal pubic hair. Semen analysis demonstrated complete azoospermia and low gonadotrophins and testosterone levels.

Lab investigation and results: Standard lymphocyte culture from the peripheral blood revealed two cell lines: a majority of 45,X cells and an abnormal Y chromosome in the remaining cells. FISH investigation confirmed a similar distribution with 2 centromeres, 2 SRY, and 2 SHOX signals in the rearranged Y. Multiplex PCR analysis demonstrated microdeletion in AZFb and AZFc loci.

Discussion: Isodicentric Y chromosomes are the most commonly reported Y chromosome aberrations, mainly as mosaics with a 45,X cell line, but their overall prevalence is lower than 0.5 % in chromosomally induced infertility. This Y aberration removes spermatogenesis loci resulting most likely in a Sertoli-only syndrome. Timely diagnosis seems important because of a small but significant risk for developing gonadoblastoma. In addition, variable features of TS may be observed. Therefore, in similar cases diagnosed prenatally (even with apparently normal neonatal appearance) regular follow-up is a responsible task for timely assisting development and education in a condition with unpredictable outcome.

1.P2

Unusually mild course in trisomy 13 mosaicism with multiple hemangiomas

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Background. Trisomy 13 mosaicism is rare, occurring in only 5 % of all trisomy 13 cases. Phenotype varies from

very severe to milder forms depending on the proportion of cytogenetically normal and trisomic cells. Several major congenital anomalies are usually associated with trisomy 13, including capillary hemangiomas. We report on an 18 month old girl with unexpectedly mild clinical developmental delay presenting with several hemangiomas of different shapes and sizes, but no other malformations.

Case report.

2nd child of a 44-year old mother; combined test results in favour of normal fetal development. Dystrophic birth at 28 weeks of gestation, 534 g/33 cm/22 cm (all<Pc3). No cardiopulmonary problems, good muscle tonus, mobility, absolute strength of the extremities and reflexes within normal range of age. Port wine stains in the frontal region; in addition a 4 cm2 large hemangioma planotuberosum on the forearm. MRI of the brain and eye examination were normal. Due to unspecific dysmorphic features genetic investigation was performed: molecular karyotyping (CytoScan HD, Affymetrix) revealed mosaicism 47,XX,+13/46,XX with 24 % of cells being trisomic at the time of investigation, cytogenetically confirmed with a comparable proportion of mosaicism. Development has been quite promising so far, with weight and length gain along the 3rd percentile. Clinical diagnosis of trisomy 13 is usually straightforward due to specific facial features and malformation pattern, including cardiac and CNS midline defects, hexadactyly, cleft lip/palate and severe growth and mental retardation. Our patient is interesting for several reasons: lack of any major malformation considered typical for Patau syndrome, the remarkably mild cognitive delay, postnatal low-normal growth and weight, and a pattern of different hemangiomas. To our knowledge only one has been reported with similar skin anomalies without any other organic malformations.

1.P3

Microdeletion of 15q24 detected by Array-CGH technique, in an Iranian patient with intellectual disability, autism, seizures, and dysmorphism

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15q24 microdeletion syndrome is a rare syndrome with a frequency of about 0.3 % in patients with intellectual disability and about 0.1–0.2 % in patients with autistic symptom disorder. This syndrome was described for the first time in 2007 by Sharp et al. The reported clinical features included growth retardation, intellectual disability, unusual facial characteristics, skeletal and genital anomalies, hypotonia and behavioral problems. Since then 19 individuals have been reported with deletions of different sizes ranging between 1.7 and 6.1 Mbp, detected by array CGH technique. These clinical manifestations are heterogeneous between patients.

We report a 17-year-old boy with multiple clinical features including intellectual disability, autism, unusual craniofacial characteristics and seizures. The high resolution karyotyping was normal 46, XY. The MLPA result for all the subtelomeric regions was also normal. However, array CGH technique using CytoChip international standard cytogenetic array 4× 44 k (v2.0) platform with a resolution of 150–200 kb, revealed a 2.5 Mb deletion of 15q24.1 q25.1.

A genotype phenotype study and comparison with other similar cases will be presented.

1.P4

Small Deletion of 143 Kb Encompassing Exon 2 of the AUTS2: Rise of a New Microdeletion Syndrome?

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Chromosome microarray analysis is a powerful diagnostic tool and is being used as a first-line approach to detect chromosome imbalances associated with intellectual disability, dysmorphic features and congenital anomalies. This test enables the identification of new

copy number variants (CNVs) and their association with new microdeletion/microduplication syndromes in patients previously without diagnosis.

Here we report the case of a 17 year-old female with severe intellectual disability, absence of speech, microcephaly and congenital abnormalities with a previous normal karyotype performed at a younger age.

Affymetrix CytoScan HD chromosome microarray analysis was performed detecting a 143 Kb deletion at the 7q11.22 breakpoint, encompassing exon 2 of AUTS2 gene: arr[hg19] 7q11.22(69238957-69381975)×1.

The AUTS2 gene has been recently implicated in neurodevelopment and is a candidate gene for numerous neurological disorders. Common clinical features described in patients with deletions in AUTS2 gene include intellectual disability, speech delay and microcephaly, among others. Thus, the CNV identified in our patient explains the phenotype observed.

We compare our patient with other similar reported cases, adding additional value to the phenotype-genotype correlation of deletions in this region. The growing collection of new cases with similar phenotypes, and the observation of this deletion occurring frequently de novo, indicates this CNV as a possible new single gene microdeletion syndrome.

1.P5

Association of structural and numerical anomalies of chromosome 22 in a patient with syndromic intellectual disability.

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Array comparative genomic hybridization CGH is now widely adopted as a first-tier clinical diagnostic test for patients with developmental delay/intellectual disability DD/ID, autism spectrum disorders, and multiple congenital anomalies. Nevertheless, classic karyotyping still has its impact in diagnosing genetic diseases, particularly mosaic cases.

We report on a 30 year old patient with syndromic intellectual disability, a 22q13.2 microdeletion and mosaic trisomy 22. The patient had the following clinical features: intrauterine growth retardation, mild hypotonia, cryptorchidism, phimosis, facial asymmetry, enophthalmus, mild prognathism, bifid uvula, hypoplastic upper limb phalanges, DD including speech delay, and ID. Whole genome oligonucleotide microarray CGH 105 K (Agilent Technologies) showed a de novo 1 Mb interstitial deletion in 22q13.2, confirmed by fluorescence in situ hybridization in all cells examined. Moreover, 18 % cells had three chromosome 22 signals in addition to the 22q13.2 deletion. G-banded karyotype was done and revealed a trisomy 22 in mosaic.

Almost all 22q13 deletions published so far have been terminal deletions with variable sizes (2.7–6.9 Mb). Very few cases of smaller, interstitial 22q13.2 deletions were reported. In its mosaic form, trisomy 22 is compatible with life, and there are about 20 reports in the literature. It has a variable clinical presentation: growth restriction, dysmorphic features, cardiovascular abnormalities, hemihyperplasia, genitourinary tract anomalies and ID. Neurodevelopmental outcome ranges from normal to severe DD.

Our case points out the role of conventional cytogenetic tools in mosaic cases that could be missed by microarray technology. With the advances of CGH, new microdeletion and microduplication syndromes are being reported. Data is lacking about the interactions of more than one genetic anomaly detected on a same patient, making the genotype/phenotype correlation quite difficult.

1.P6

Mixoploidy combined with aneuploidy in a patient with severe multiple congenital abnormalities and mental retardation

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We report a 10-year-old female patient born to healthy, non-consanguineous parents, presenting with metopic ridge, palatoschisis, dysmorphic features, high-arched eyebrows, multiple congenital contractures, broad

thorax with widely separated nipples, thoracic scoliosis, short stature, intellectual disability, high pitched voice, nasal speech, hypotonia and failure to thrive. Metabolic investigation shortly after birth was normal. Karyotyping revealed a 45,X karyotype in peripheral blood, consistent with Turner syndrome.

As the girl's phenotype could not be explained by this Turner karyotype, further genetic analyses were performed. ASXL1 mutation analysis for Bohring-Opitz trigonocephaly was normal. Subtelomeric MLPA and genomic array using DNA from peripheral blood confirmed the 45,X karyotype. Additionally, array showed a paternally inherited, small interstitial loss on chromosome 4q31.22 (unknown clinical significance).

As still no satisfactory explanation for the girl's phenotype was found, a mosaic genetic aberration was suspected and genomic array was repeated using DNA from a skin biopsy. An abnormal profile was observed, suggesting the presence of a triploid cell line with loss of one X chromosome besides the 45,X cell line, which was confirmed using karyotyping on skin fibroblasts. Retrospectively, the dysmorphic features and congenital anomalies resemble earlier descriptions of triploid/diploid mixoploidy syndrome, however in combination with the Turner phenotype these were difficult to recognize.

In conclusion, the patient's phenotype can be explained by a tissue specific mosaic abnormal karyotype with both an abnormal triploid and an abnormal diploid cell line being present (mixoploidy). Mixoploidy in combination with an aneuploidy, as observed in this patient, is extremely rare. This observation underscores the importance of re-examination and second tissue analysis of patients with multiple congenital anomalies when microarray copy number analysis appears normal.

1.P7

Copy number variations (CNVs) in patients with congenital heart diseases and 22q11.2 DS clinical suspicion

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The 22q11.2 Deletion Syndrome (22q11.2 DS) has a prevalence of 1:4000 births and is characterized by multiple anomalies, including several types of congenital heart diseases. The phenotypic spectrum is variable and overlaps with other clinical conditions. Consequently, clinical diagnosis is complicated and several patients with clinical features do not present the 22q11.2 deletion. A cohort of 78 patients with clinical suspicion of 22q11.2 DS and congenital heart disease, without the deletion, previously tested by FISH and/or MLPA, were investigated by whole-genome array Genomic Hybridization (aGH) (CytoScan HD chip, Affymetrix®). A total of 66 copy number variations (CNVs)≥300 Kb were observed, corresponding to 32 deletions and 34 duplications. Of these, 50 CNVs involved genes and are potential loci related with the phenotype of these individuals. Nine patients showed CNVs already described in patients with multiple congenital anomalies, such as deletions at 4q35.1-q35.2 (4.5 Mb), 5p15.1-p15.33 (17.4 Mb), 8p23.1 (3.8 Mb), 10q22.3-q23.2 (7.8 Mb), 16p11.2 (611 kb), 17q12 (1.8 Mb) and 22q13.33 (1.1 Mb); and duplications at 3p26.3 (455 kb and 482 kb) and 3q26.2 (2 Mb). These CNVs are potentially pathogenic and dosage effects of genes such as IRX4, BMPR1A, GATA4 and SOX7 can be involved in cardiac development. These results confirms the wide phenotypic spectrum and the overlap with different conditions of patients with 22q11.2 DS suspicious. Further studies may contribute for the identification of other CNVs, not mapped at 22q11.2 region, in patients with clinical suspicion of 22q11.2 DS and congenital heart diseases.

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1.P8

Whole-genome screening in a set of Czech patients with mental retardation and developmental abnormalities using array-CGH technique: our experience from 2007 to 2014

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Introduction: Mental retardation (MR) represents a health condition defined as a heterogeneous manifestation of CNS dysfunction characterized by significantly subnormal intellectual functions. It affects 1–3 % of Western population and about 0.4 % people suffer from its severe form. MR may be a result of various genetic events or environmental factors affecting prenatal and postnatal development as well. Despite a rapid progression in diagnostic methods and analytical approaches up to 50 % MR cases do not have a known etiology.

Material and methods: In the course of our 7-year experience with chromosomal microarray analysis in individuals with MR and associated phenotypic abnormalities we have examined 171 Czech patients at the Department of Medical Genetics (University Hospital Brno) using three different oligonucleotide array-based platforms by Agilent Technologies and Oxford Gene Technologies.

Results: Overall we found 65 clinically significant CNAs (38 %) and 29 were confirmed as pathogenic (17 %). Seven CNAs were evaluated with undetermined significance (VOUS), seven CNAs were found to be likely benign and 22 CNAs remain to be confirmed and evaluated. Totally we detected 43 deletions and 23 duplications (from 0.67 kb to 31 Mb) and 5 cases of segmental or whole-chromosome UPD/LOH. Conclusion: Array-CGH technique represents an effective tool in the diagnostic algorithm for MR cases in our laboratory. From this year, patients with negative array-CGH results will be involved in a pilot study using NGS technology to analyze mutation status of MR-associated genes. Based on recent studies, the combination of array-CGH and exome sequencing technologies leads to the increase of the diagnostic yield in the cases of idiopathic MR and may help to elucidate their causes.

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1.P9

Copy-neutral loss of heterozygosity pattern in systemic lupus erythematosus

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Cytogenetic approaches have been applied to understand the role of genomic instability in systemic lupus erythematosus (SLE), an autoimmune disease with a strong genetic background. Loss of heterozygosity (LOH) can occur in human cells without evident pathological consequences. However, LOH has been reported as a model to explain the susceptibility to some classes of complex diseases. The purpose of this study was to determine the role of LOH in SLE. Blood cell lymphocytes from 23 SLE unrelated patients and 59 healthy subjects were submitted to the Genome-Wide Human Cytoscan HD Array (Affymetrix®) in order to screen for LOH regions in genomic DNA. LOH event was determined by at least 50 contiguous SNPs in a genomic region of 3 Mb. Data analysis was performed in ChAS and Plink software. An average of 4.3 (sd=5.4) LOH events per individual with a medium size of 23.2 Mb (sd=40.9 Mb) were identified in patients, while controls showed 3.2 LOH events (sd=2.6), spanning a region of 14.3 Mb (sd=16.0 Mb). Percentage of LOH in autosomal chromosomes was also higher in patients (0.7 %) than controls (0.3 %). We found 35 LOH segments that were shared by two or more subjects and 37 LOH events that occur once in SLE sample. We highlighted a potentially pathogenic LOH region of 16.5 Mb on 9p, present in two patients and absent in controls, that covers several genes involved in immune and apoptotic process. This is the first report describing LOH events encompassing these regions. Our findings show how regions of genomic instability could be potential modulators for SLE etiology. Moreover, our results strongly support that SLE patients have more homozygous loci and higher percentage of LOH than healthy individuals. Support: FAPE SP(2013/17062-9;2011/23794-7), CNPq(312547/2009-9),

1.P10

Prenatal diagnosis and molecular cytogenetic characterization of a de novo duplication of 15q24.3-q26.1

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Reported cases of duplications of distal 15q are very uncommon, in fact, there are around 50 cases described in the world, an even less de novo duplications like the present case report. Most of these mutations are the result of unbalance translocations and have an additional monosomic component. Previous authors have described a distal 15q trisomy syndrome characterized by prenatal and postnatal overgrowth, mental retardation, craniofacial and skeletal malformations, and genital abnormalities, particularly in affected males. Other authors, indicated small-size fetuses. There are only a few reported cases of pure duplication of 15q24-qter region. The type and severity of reported anomalies depend on the length and location of the duplicated region of chromosome 15q.

We present a prenatal diagnosis of a fetus with single umbilical artery, all the long bones short -2.5 SD, but with normal morphology and mineralization features. The patient is a 30-year-old female, 21+1 gestation weeks, low-risk first trimester screening and normal serology. The fetus karyotype showed a 15q duplication visible by conventional G-banding, not present in the parents' karyotypes. CGH array showed a de novo duplication 15q24.3q26.1, of 14 Mb, containing 257 genes (NCBI), 81 of them in the OMIM database.

The fetus reported here had no monosomic component, as reported in the majority of the published cases, so we can assume that the abnormal phenotype results from the duplication of the 15q24.3q21.1 segment. Our conclusion is that in case of short long bones (<p5) in a fetal ultrasound, we need to analyze their morphology and mineralization, discard a possible error in gestational dating, and make a cytogenetic study of the fetus by means of CGH array. Differential diagnoses must include constitutional short stature, intrauterine growth restriction, skeletal dysplasia and chromosomal abnormalities.

1.P11

Familial supravalvular aortic stenosis due to ELN gene disruption by a chromosome 7 paracentric inversion

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Supravalvular aortic stenosis (SVAS) is an autosomal dominant condition due to haplo-insufficiency of the ELN gene localized in 7q11.23. SVAS has incomplete penetrance and variable expressivity.

We report here a two years old boy referred for an isolated SVAS and history of heart murmur in both branch of the family.

Standard karyotype displayed an apparently balanced paracentric inversion of the long arm of chromosome 7 : 46,XY, inv(7)(q11.2q31). Fluorescent in situ hybridization (FISH) using the Williams Beuren Syndrome (WBS) critical region probe (7q11.2) and RP11-644H24 probe (chr7: 73411275-73548266, hg19) showed that the proximal breakpoint disrupted the ELN locus. Array CGH using a custom oligonucleotide array densified in the WBS region did not reveal any imbalance. Massively parallel DNA sequencing is underway in order to specify whether the breakpoint lies within the ELN gene or in its neighbourhood.

The family study showed that the chromosome 7 inversion was inherited from the father and was also present in two paternal aunts and in the paternal grand' father. The associated phenotype was highly variable, including normal phenotype, isolated heart murmur, mitral valve prolapse or typical SVAS.

Cytogenetic anomalies associated with SVAS are usually microdeletion involving 7q11.2 region that are responsible for WBS, including additional features such as facial dysmorphism and intellectual disability. Cases of SVAS due to ELN gene disruption by a balanced rearrangement have been rarely reported. The

identification of the inversion in this family is important to provide an appropriate genetic counselling and clinical follow-up.

1.P12

Evaluation of gene expression and cytogenomic study in patients with 22q11.2 deletion

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22q11.2 deletion syndrome results from a hemizygous deletion of chromosome 22 usually flanked by low copy repeat (LCR) regions, the 3 and 1.5 Mb deletions being the most frequent. The phenotype may be variable in patients with similar deletions, and similar phenotypes may also be observed in patients with different sized deletions in the 22q11.2 region, even without overlap. We conducted cytogenomic investigation by karyotyping, MLPA and genomic microarray in 29 patients. Gene expression, analysis of gene interactions and enriched biological processes were evaluated in 14 patients and 14 gender and age matched controls. Karyotypes were normal in most patients, with one showing a translocation t(6;22), resulting in 22q11.2 deletion. MLPA revealed the deletion size: 3 Mb (21 patients), 1.5 Mb (4 patients) and atypical deletions (4 patients). Deletion sizes, size refined by genomic microarray, showed breakpoint variations among patients. The study of gene expression by microarray identified reduced expression of 48 transcripts corresponding to genes of the 22q deleted region, and also of genes flanking the deleted region or genes located in other chromosomes. These data indicate a position effect and the chromatin repositioning in the nucleus, mechanisms possibly related to gene expression alteration. The coexpression evaluation by GeneMania software showed a relationship between the genes of the deleted region and those adjacent to it, between the genes of 1.5 and 3 Mb deletions regions, and between genes of chromosome 22 and of other genomic regions. These data revealed a possible biological relationship, which could explain the phenotypic similarity between patients with different sized deletions. This study contributes to the understanding of the molecular mechanisms involved in the complex network of gene regulation, which is responsible for the characteristic 22q11.2 deletion phenotype. Financial Support: FAPESP, Brazil.

1.P13

Discrepancy between cytogenetic and molecular results in two cases with isochromosomes

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An isochromosome contains two arms with identical DNA sequences and the formation of it must have happened either at meioses or as a postzygotic event. To support the theory of postzygotic formation we present these two cases:

Case 1: 30-year old gravida 2, para 1 underwent CVS at 12+1 weeks of gestation because of enlarged fetal nuchal translucency. Array-CGH was performed on DNA from uncultured CVS revealing a 5.6 Mb terminal deletion on chromosome 21q (arr[hg19] 21q22.2q22.3(42538181-48090317)x1). To estimate recurrence risk the CVS was cultured. Karyotyping and Multi-Colour FISH analyses revealed 46,XX,der(21).ish der(21)i(21); one normal chromosome 21 and one isochromosome 21 in all metaphases analyzed. Array-CGH was then applied to the cultured cells and could confirm the 27 Mb duplication and the terminal deletion of 5.6 Mb of chromosome 21q (arr[hg19] 21q11.2q22.2(15390816-42482129)X3,21q22.2q22.3(42518800-48090317)×1).

Case 2: 33-year old man referred for standard chromosomal analyses because of azoospermia. Karyotyping and FISH-analysis was performed on PFA-stimulated peripheral blood revealing 20 % mosaicism for monosomi X in combination with an iso-dicentric Y-chromosome consisting of two copies of Yp (45,X[1]/46,X,der(Y)[9].ish mos X (pter+)(DXYS129x1),i(Y)(pter++)(DXYS153++),idic(Y)(p11.1-q11.1)(DYZ3++),Xcen(DXZ1x1)[81]/X(pter+)(DXYS129x1),i(Y)(pter-)(DXYS153-),idic(Y)(p11.1 q11.1)(DYZ3-),Xcen(DXZ1x1)[19]).

Subsequently, Array-CGH was performed on DNA extracted from a new peripheral blood sample. Array confirmed a 10–15 % mosaicism for monosomi X, but could not confirm the presence of an iso-Yp chromosome expecting a duplication of Yp (arr[hg19] Yp11.31q11.221(2626115-18255512)~0-1,Yq11.221q11.23(18415668-28689881)x0).

Conclusion: These two cases illustrate that isochromosomes may be formed postzygotically when cells try to repair a terminal deletion. We hypothesise that the terminal deletions, but not the isochromosomes, were evident in our patients, and that isochromosome-formation was induced by cell culture/PFA cell-stimulation.

1.P14

Familial osteoporosis and interstitial duplication of chromosome 5

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Osteoporosis is a systemic bone disease characterized by low bone mineral density and structural deterioration of bone tissue leading to an increased risk of fractures. Genetic factors have been recognized to play an important role in osteoporosis and a number of susceptibility genes have been identified and validated. Here, we describe an 11-year-old girl affected by severe osteoporosis with vertebral fracture and intellectual disability. Her father was affected by a severe form, too. Cytogenetic investigations revealed a dicentric chromosome 5, rearranged at the centromeric level, present also in her father. The two centromeres were both active, as demonstrated by immunological staining with CENP-C antibodies. Array-CGH of the proposita showed a large proximal duplication at 5p11q11.2 band and a small distal one at 5q11.2, while her father presented a unique duplication of 5p11q11.2 bands. The duplicated region on chromosome 5 contains ITGA1 (integrin alpha 1), an osteoporosis susceptibility gene, and FST (follistatin) gene. ITGA1 encodes integrin alpha 1 chain that binds to the beta chain to form a receptor involved in cell attachment and neurite outgrowth on laminin and collagen. Proper collagen-integrin interactions are important in fracture healing, which suggests that ITGA1 plays role in the regulation of mesenchymal stem cell and cartilage proliferation. FST encodes a monomeric glycoprotein that actively participates in the regulation of bone metabolism. Experiments performed in mice overexpressing follistatin showed a decreased quality of skeleton and susceptibility to bone fractures. In conclusion, interstitial 5p11q11.2duplication involving ITGA1 and FST could play a causative role in familial osteoporosis.

1.P15

A unique case of mosaic male Turners and trisomy 13

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The incidence of trisomy 13 is 1 in 5000 births and that of Turner Syndrome (TS) is 1 in 2500 live born phenotypic females. TS is a common autosomal aneuploidy among liveborns.

We report a unique case of a male with a Turner phenotype and trisomy 13. This 25 month old Saudi phenotypically male child was referred to our outpatient clinic for a genetic workup because of dysmorphy. Parents are healthy and are non-consanguineous.

A chromosomal microarray profile was found to be consistent with a mosaic trisomy 13. Chromosomal analysis of 20 metaphases from a stimulated peripheral blood culture done in our laboratory showed the presence of two abnormal cell lines:

One had an abnormal derivative of chromosome 13 in the form of a Robertsonian translocation, (13q10), in 16 out of 20 metaphases.

The other cell line had two normal chromosomes 13 and a loss of the Y chromosome in 4 metaphases.

No other structural or numerical abnormalities were detected. This is consistent with the diagnosis of a mosaic trisomy 13 and mosaic TS.

Also FISH done in our lab to confirm the X/Y status showed 69 % of the cells with an XY signal and 31 % with only one signal for chromosome X. This result is consistent with 45,X/46,XY mosaic TS.

This is a unique case of mosaic trisomy 13 and Turner syndrome with male phenotype. Although microarrays can detect small percentage of mosaicisms up to15 %, in this case microarrays failed to detect the mosaic loss of Y chromosome.

This abstract was submitted to the 20th International Chromosome Conference 2014, University of Kent, UK.It was accepted as poster and was published as an abstract. However, the authors were unable to attend the conference and the poster was not displayed.

1.P16

Case Report: Clinical Expression of an Inherited Unbalanced Translocation in Chromosome 2

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Carriers of balanced reciprocal translocations mostly do not have recognizable phenotypic expression. However, they have an increased risk of producing gametes with unbalanced chromosomal rearrangements, leading to miscarriages or children with significant clinical expression.

We report a family comprising of healthy parents and their newborn with facial dysmorphism and feeding difficulties. Conventional GTG-banding (G-bands by trypsin using Giemsa) analysis of somatic chromosomes identified a balanced translocation, 46,XX,t(2;6)(q37.1;q25.2), in the mother and an unbalanced rearrangement, 46,XX,der(2)t(2;6)(q37.1;q25.2)mat, in the child. The child has inherited a derivative chromosome 2 with loss of the segment 2q37.1-qter and gain of 6q25.2-qter. The finding was confirmed by FISH (fluorescent in situ hybridization) and chromosomal imbalances were specified by chromosomal microarray analysis (Human CytoSNP-12 BeadChip, Illumina Inc.). The amount of deleted material on 2q was ~7.9 Mb and the gain of 6q was ~15.4 Mb.

2q37 deletion syndrome is a condition that can affect many parts of the body. This condition is characterized by weak muscle tone (hypotonia) in infancy, mild to severe intellectual disability and developmental delay, behavioral problems, characteristic facial features, and other physical abnormalities. 6q25.2-qter duplication is not yet described in the literature and it is still unclear how this aberration contributes to the clinical picture.

1.P17

Paternally inherited 7q21.3 microdeletion and maternally inherited 22q11.2 microduplication in a patient with split-hand/foot malformation, macrocephaly and developmental delay.

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We report a case of a girl born with a split-hand/foot malformation. At 3 months of age the first signs of developmental delay were noticed and an accelerated head growth had led to macrocephaly. Chromosomal microarray analysis (Human CytoSNP-12 BeadChip, Illumina Inc.) revealed two pathogenic DNA copy number variations (CNV): a 2.2 Mb deletion in 7q21.3 region associated with split-hand/foot malformation type 1 or ectrodactyly–ectodermal dysplasia–cleft (EEC) syndrome, and a 2.6 Mb duplication in 22q11.2 region associated with psychomotor developmental delay and other clinical features. The patient's father, who presents merely feet syndactyly, carries the same 7q21.3 deletion and the patient's unaffected mother is a carrier of 22q11.2 duplication.

Both CNVs, 7q21.3 deletion and 22q11.2 duplication, are well-described chromosomal aberrations with incomplete penetrance and high clinical variability. The co-occurrence of two familial CNVs complicates making genotype-phenotype correlations and providing prognosis for further development of the child.

Diagnostic results of the patient and all affected family members are presented and complexity of the phenotype will be discussed in the presentation.

1.P18

Strategies for dealing with incidental findings in diagnostic microarray-based copy number analysis

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During the past decade, chromosomal microarray-based copy number analysis has emerged as an important diagnostic tool for the evaluation of developmental delay, intellectual disability, autism spectrum disorders, and structural malformations in children. The benefit of the high-resolution detection of copy number variants offered by microarray applications comes along with an increased risk for the detection of variants of uncertain clinical significance or unsolicited findings. These include copy number variants concerning genes not related to the clinical phenotype of the respective patient that could potentially implicate a higher disease risk for offspring or other family members or present risk factors for additional diseases.

In our lab, we processed more than 470 Affymetrix Cytoscan HD arrays for diagnostic purposes over the last two years. We mainly analyzed patients with developmental delay, intellectual disability, structural malformations and dysmorphic features and detected pathogenic copy number variations explaining the disease pattern in 15 % of the samples. In 68 % of the patients, we found no evidence for a genomic imbalance causative for the clinical phenotype. Incidental findings were reported in 2,3 % of the cases (11 patients): Nine patients were found to be carriers of a recessive genetic disorder such as Cohen syndrome, Joubert syndrome or Usher syndrome due to copy number variants in respective genes. An increased risk for cardiovascular diseases was discovered in two cases due to copy number variants in genes associated with cardiac disorders.

Here we present selected cases of our incidental findings. We discuss strategies taking pre-analytical counselling, severity and prevalence of the respective disorder, characteristics of the copy number variant and the challenges of medical reporting into account, based on our experience.

1.P19

Cytogenomic investigation in patients with Oculoauriculovertebral Spectrum (OAVS) and candidate loci relevant to phenotype

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Oculoauriculovertebral Spectrum (OAVS, OMIM 164210) is a developmental disorder involving the first and second branchial arches during embyogenesis. This disorder is characterized by a wide spectrum of symptoms and physical features that may vary greatly in range and severity from case to case. The majority of cases appear to be sporadically and the etiology of the syndrome is not well understood. Some studies have detected candidate loci for OAVS by molecular cytogenetics and genetic linkage studies but no gene or locus has been specifically attributed to this spectrum. Several chromosomal abnormalities have been associated with OAVS syndrome and the chromosome region most frequently reported is the 22q11.2 region. We studied 70 patients with OAVS clinical diagnosis who met the minimal criteria (isolated microtia, preauricular tags and hemifacial microsomia) by karyotyping, genomic array and FISH. All patients showed normal karyotypes, except for one: 46,XX,inv(12)(q15q24.1). A derivative chromosome 4 from a cryptic maternal translocation t(X;4)(p22.31;p15.33) detected by FISH was found in another patient. High resolution oligonucleotide genomic arrays (Affymetrix) identified some Copy Number Variations (CNVs): 7 deletions (4p16.3p15.33, 4q13.3q21.1, 8q13.3, 10q26.2q26.3, 16p13.3, 22q11.21, Xp22.33) and 6 duplications (2q32.1, 4p16.1.1, 16p13.11, 17q11.2, and Xp22.33) in different chromosomes. The patient with a ~581 kb 22q11.21 deletion is the seventh patient ever reported with OAVS and 22q deletion, indicating that 22q11.2 region is a strong candidate locus for regulation of the body symmetry and facial development. The other CNVs are

potentially pathogenic imbalances since they include many genes described in OMIM and genomic regions which may be related to the phenotype in our patients. Therefore, CNVs and chromosomal rearrangement investigation in OAVS may potentially reveal different pathogenic mechanism in this phenotypically and genetically heterogeneous disorder. Financial Support: FAPESP, Brazil(2013/19897-0).

1.P20

Epsilon gamma delta beta thalassemia diagnosed by chromosomal microarray in critically ill neonate

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BACKGROUND: Epsilon gamma delta beta thalassemias represent a group of rare autosomal dominant haemoglobinopathies caused by deletions of the β-globin gene cluster. The most prominent clinical feature is ante- or neonatal anaemia, which might lead to severe complications. The severe anaemia resolves in a couple of months after birth. Here we describe a familial case of (εγδβ)-thalassemia caused by a novel 11p15.4 microdeletion, which is the first reported case from North-Eastern Europe.

CASE REPORT: The proband is a girl born with severe non-immune haemolytic anaemia complicated by persistent pulmonary hypertension of the newborn. The baby required blood transfusions, mechanical ventilation and extracorporeal membrane oxygenation due to critical condition. At the age of 2 months the outcome is favourable, although she remains mildly anaemic. The family history was remarkable as her older brother, father, paternal grandfather, aunt and cousin have had anaemia-related health problems of variable severity.

METHODS AND RESULTS: As a first-tier genetic test, chromosomal microarray analysis (CMA) from DNA sample of the proband was performed using Illumina HumanCytoSNP-12 array. CMA revealed a 115 kb deletion (arr[hg19] 11p15.4(5,228,708-5,343,533)×1). The deletion was found also in other five affected family members. The deleted region covers the whole β-globin gene cluster encompassing genes HBB, HBD, HGB1, HBG2, and HBE1.

CONCLUSIONS: Firstly, this case demonstrates that CMA could serve as a useful first-tier genetic test for severe life-threatening neonatal anaemia. Secondly, the detected 11p15.4 deletion is clearly pathogenic and has a size that is just slightly above the resolution limit of arrays routinely used in clinical diagnostics. Thirdly, we would like to emphasize that some rarer forms of thalassemia may also be present in Northern-European population and should be included in differential diagnosis of severe neonatal anaemia.

1.P21

A de novo interstitial 6q deletion: genotype-phenotype correlation utilizing array CGH

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Interstitial deletions of the long arm of chromosome 6 are relatively rare, with fewer than 100 cases reported. Phenotypic variation is in large part due to differences in size and location of the segmental aneuploidy. We report a patient, a girl 12 months old, with an interstitial deletion of chromosome 6q defined at the molecular level by array comparative genomic hybridization (array CGH) with CytoChip microarrays oligo 8×60 K. In our case the molecular breakpoints differed from those indicated by conventional karyotyping, demonstrating the enhanced resolution of array CGH. The patient had a deletion of 10,1 Mb involving 6q16.1–6q21. The parental karyotypes were normal. She was the first child of healthy nonconsanguineous parents. Family history was unremarkable. She presented developmental delay, craniofacial dysmorphology, and functional eye disorders, suggesting that genes affecting brain and craniofacial development are located in 6q16.2–>q21, as has been previously described in some cases in the literature. Furthermore, gene(s) for discordant phenotypic features, such as central diabetes insipidus, may reside at 6q15. Array CGH can help in further refining the deleted region and thus in the discovery of candidate genes for the phenotypic characteristics.

1.P22

Characterization of a de novo complex chromosomal rearrangement with five breakpoints associated with azoospermia

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Complex chromosomal rearrangements (CCRs) are balanced or unbalanced structural aberrations involving three or more chromosomal breakpoints with exchange of genetic material between two or more chromosomes. CCRs are rather rare event in the general population and carriers display various phenotypes (normal phenotype, infertility, malformations, mental retardation and/or congenital abnormalities). Male carriers are at risk of reproductive failure as a result of spermatogenesis disruption. Indeed, CCRs can be associated with abnormal segregation of derivative chromosomes and production of chromosomally abnormal sperm.

In this report, we describe a rare and de novo CCR associated with azoospermia in a 36 years-old man. The CCR was characterized by karyotype, FISH (fluorescence in situ hybridization) and array-CGH (microarray Comparative Genomic Hybridization) Agilent 1 M 2.1Kb resolution assays. Successive analyses by FISH allowed to estimate the location of breakpoints and thus gene mapping of these regions. Results showed that the rearrangement was more complex than initially assumed with a two-step CCR and five breakpoints were identified. The first event, insertion of a part of an inverted chromosome 12 into the short arm of a chromosome 7, involved three breakpoints located on 12p11.1q11/12q13.11, 12q21.2/12q21.33 and 7p21.2–21.3. The second event, pericentric inversion of chromosome 12, involved two other breakpoints located on 12p13.31-12p13.2 and 12q21.33/qter. The mapping associated with these breakpoints showed several genes of potential interest involved in the reproductive function such as ARL4, FOXJ2, NEDD1, SYCP3 and NFYB. Array-CGH analysis did not highlight DNA copy number variation on estimated breakpoints or elsewhere.

CCRs predispose to abnormal segregation in meiosis or gene disruption responsible for reproductive failure such as azoospermia. This case report underlines the potential of conventional and molecular techniques to specify mechanism of CCRs formation and to identify breakpoints.

1.P23

Case report: An inherited copy number loss including the ARID1B gene

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The use of Array CGH (Comparative Genomic Hybridization) as the front line laboratory approach to assess patients with intellectual disability and /or congenital anomalies has enabled the identification of new copy number variants (CNVs) and new microdeletion / microduplication syndromes.

CNVs frequently encompass a significant number of genes, and may provide evidence of association to a specific phenotype or further delineation of a critical region. However CNVs are often inherited and may highlight variable penetrance, or in the case of an unaffected parent may suggest that an alternative mechanism might be in place.

Coffin-Siris Syndrome (CSS) is characterized by intellectual disability, severe speech impairment, coarse facial features, microcephaly, developmental delay and hypoplastic nails on the fifth digits (MIM 135900). The main underlying cause has been suggested to be mutations in the ARID1B gene (MIM 614556), a gene involved in the remodelling of chromatin. However,

there are reports of ARID1B mutations in patients referred with intellectual disability with a variable set of clinical features which do not necessarily fit the clinical criteria of CSS.

Most ARID1B mutation patients have been ascertained through studies of CSS, potentially biasing the documentation of associated clinical features. Recently an attempt has been made to further characterize a subset of patients specifically showing involvement of ARID1B.

Here we present a case showing a 956 kb deletion in 6q25.3 encompassing the ARID1B gene, detected by Array CGH. This patient is affected with microcephaly, faltering growth and developmental delay. The CNV is maternally inherited. We compare this to other patients described in the literature hoping to further delineate the role of ARID1B in intellectual disability.

1.P24

Cryptic Chromosome Rearrangements Detected by BAC Genome Array-CGH.

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The use of Genome Array-CGH has recently dramatically improved the detection of cryptic chromosomal imbalances. This methodology utilizes mapped DNA sequences in a microarray platform for the detection of chromosomal deletions/duplications (Vermeesch et al. 2005; Edelmann and Hirschhorn 2009; Sagoo et al. 2009). We applied the BAC (bacterial artificial chromosome) Genome Array-CGH platform to study several patients with normal and/or abnormal chromosome karyotypes, previously investigated with conventional cytogenetic procedures, and we found further cryptic rearrangements that had escaped traditional cytogenetic analysis, because of low power resolution. Our experience shows the validity of the BAC platform as a reliable method for Genome-wide screening of chromosomal aberrations in patients with idiopathic mental retardation and/or in association with autism and epilepsy. For this study we analyzed ten patients with normal and/or abnormal karyotype by Genome Array-CGH and we found further cryptic rearrangements in five out of ten (50 %). This technique allows us to identify SLC8A3 gene (human gene for member 3 of solute carrier family 8) as a candidate gene for epilepsy (Nucaro et al., 2010). It is a sodium-calcium exchanger electively expresses in brain (Gabellini et al., 2002). The role of ionic channels is well demonstrated in epilepsy. These findings are very important to better genotype/phenotype correlation and to determine the clinical significance of these genomic imbalance. In conclusion, this study provide evidence that the BAC Genome Array-CGH platform is an important and reliable tool for detecting, mapping and characterizing cryptic chromosome rearrangements.

1.P25

SOX9 regulatory element duplication and gender phenotype modularity in a family

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We report two 46,XX SRY-negative male brothers with incomplete masculinization. Both children were born with bilaterally palpable testes and congenital hypospadias. The older brother's MRI revealed scrotal right side ovotestis, left side atrophic testis and normal size prostate. A testicular biopsy showed decreased number of Sertoli and Leydig cells and germinal cell aplasia. The histological and MRI examination of the younger brother revealed bilateral scrotal gonads with ovarian structure and behind the bladder a tubular structure resembling a closed vagina or Müller duct residue. The array-CGH analysis identified in the case of the older brother a 143 kb duplication 540 kb upstream of SOX9. The younger brother and his healthy 46,XY father harbored an identical 155 kb duplication 605 kb upstream of SOX9. SRY functions as a genetic switch of testicular differentiation while all downstream targets promote the expression of SOX9. Recent studies have shown

that high expression of SOX9 is sufficient to trigger male development in the absence of SRY. The genomic domain regulating SOX9 expression is composed of several gonadal regulatory elements and spans more than 1 Mb upstream of SOX9. The older brother's 143 kb SOX9 upstream duplication region overlaps with the minimal critical size of sex-determining region defined by Benko and Xiao. The hormone and gonadal status of this boy was more appropriate to male phenotype. The younger brother's duplicated region was away from the mentioned 584–517 kb upstream SOX9 critical region. We can hypothesize that the male differentiation has been initiated but probably the gonadal SOX9 expression wasn't sufficient to complete the masculinization. The results of our investigation suggest that gene desert regions with putative regulatory portion of the genome should be included in searches and the phenotypic impact of regulatory element alterations might differ substantially

1.P26

2p25 deletion: involvement of SNTG2 in autism or behavioral problems, and parental imprinting.

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Deletions of the terminal region of the short arm of chromosome 2 have been reported in the literature in less than 20 patients. These patients share common clinical features including early-onset obesity, intellectual disability and behavioral problems or autism. A minimal critical region of 1.97 Mb has been estimated, encompassing seven genes (SH3HYL1, ACP1, TMEM18, SNTG2, TPO, PXDN, and MYT1L). Many recent studies pointed out MYT1L gene as the main candidate for ID and obesity. We present three new patients carrying 2p25 deletions: an autistic female child with developmental delay and her depressive father, and a third unrelated patient who has psychomotor retardation and behavioral problems. Moreover, our patients do not have an overweight phenotype, highlighting indeed the role of MYT1L in obesity, as none of the deletions encompasses MYT1L. The only common disrupted gene is SNTG2, thus redefining the smallest region of overlap for autism. SNTG2 is expressed in the brain. Its product interacts with neuroligins NLGN3, NLGN4X, and NLGN4Y, which are autism-related isoforms. Finally, the parental origin of the deletions seems to be of interest, as all inherited deletions described today appear to be inherited from the paternal side. We will discuss the possible imprinting of the 2p25 region and its role in autism.

1.P27

Xq25 duplication: the crucial role of the STAG2 gene in this novel human cohesinopathy

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The Xq25 duplications syndrome has recently emerged as a distinct clinical entity. We report here on six new patients belonging to two unrelated families and harbouring an Xq25 microduplication detected by array CGH. As in previously reported cases, the phenotype of our patients is characterised by delayed milestones, speech disturbance, intellectual disability, abnormal behaviours and a characteristic facial dysmorphism. The common duplicated interval allowed further refinement of

the shortest region of overlap to 173 kb, including only one gene, STAG2, which encodes a component of the cohesin complex. We suggest that increased STAG2 gene copy number and dysregulation of its downstream target genes may be responsible for the specific clinical findings of this syndrome. Therefore, the Xq25 microduplication could be considered as a novel cohesinopathy, thus increasing the group of these disorders.

1.P28

18q Deletion Syndrome—Case Report

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Chromosome 18q deletion syndrome (De Grouchy Syndrome) is a cytogenetic disorder that results from a deletion of a part of the long arm of chromosome 18. It is classified into 2 types: the first type, a distal deletion, has a deletion up to the terminal part of the long arm, and in the second group there is an interstitial deletion near the centromere.

The distal deletion is associated with major clinical features that include postnatal growth retardation with short stature, mental retardation, microcephaly and midfacial hypoplasia. The incidence is 1:40000 live births. Cytogenetically the distal deletion is from 18q21.3 or 18q22.2 to qter.

A 2- year old male child was referred to our genetic clinic because of microcephaly, low set ears, mental retardation and bilateral undescended testes.

Peripheral blood karyotyping was done and the result was 46,XY,del(18)(q21).

Parental karyotyping was normal and no family history of similar clinical features, indicated that the deletion had occurred de novo as a random event during gametogenesis.

1.P29

Chromosomal abnormalities in Slovak couples with in vitro fertilization implantation failure

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In vitro fertilization (IVF) is a complex series of procedures used to treat fertility or genetic problems and assist with the conception of a child, but there are still many couples without achieving successful implantation. Chromosomal abnormalities, particularly translocations, are known to be implicated in various forms of reproductive failure. The aim of the study was to investigate the frequency of chromosomal abnormalities which may impact on pregnancy outcome in the survey of 79 couples with in-vitro fertilization implantation failure in Slovakia (2009–2014) using cytogenetic G-banding methods. Cytogenetic analyses revealed chromosomal abnormalities in 5/79 (6.3 %) of Slovak couples with implantation failure, of which 2/79 (2.5 %) were reciprocal translocations and 3/79 (3.8 %) were Robertsonian ano-malies. Chromosomal findings were detected in 5.0 % of women and 1.3 % of men. This study has shown that couples with otherwise unexplained repeated failure of IVF embryo implantation have a greater than expected chance of carrying a balanced chromosomal translocation. Thus it is possible that the presence of an unbalanced translocation in some gametes may predispose to preimplantation failure of embryo development, and increase the risk of repeated failure of IVF treatment. Our study has demonstrated that balanced parental translocations may be implicated in the pathogenesis of IVF implantation failure. Chromoso-mal evaluation should be considered as part of the investigation of these patients, and genetic counselling and consideration of preimplantation diagnosis should be an integral part of planning of further treatment strategies.

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1.P30

Somatic mosaicism for DNA copy number variations and copy-neutral loss of heterozygosity in the human genome

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Somatic mosaicism denotes the presence of genetically distinct populations of somatic cells in one individual who has developed from a single fertilised oocyte. Mosaicism may result from mutations of different scales that occur during postzygotic development and are propagated to only a subset of the adult cells.

The aim of our study was to investigate somatic mosaicism for DNA copy number variations (CNVs) and copy-neutral loss of heterozygosity (cn-LOH) events in fully differentiated human tissues. We studied panels of tissue samples (11–12 tissues per individual) from four subjects using high-resolution Illumina HumanOmniExpress-12 BeadChips. We observed three CNVs occurring only in a portion of the studied tissues in one out of four individuals. The length of observed somatic CNVs ranged from 123 to 796 kb and they can also be found in general population. In addition to CNVs, we detected five mosaic cn-LOH regions >5 Mb in three out of four individuals. Assuming that specific mosaic patterns of CNV and cn-LOH events are reflecting their formation during the postzygotic embryonic development of germinal layers and organ systems, we attempted to track the succession of genomic events leading to somatic genetic mosaicism.

Our results give further support to the idea that somatic mosaicism for CNVs, but also for cn-LOHs, is a common phenomenon in normal human tissues. Thus, the examination of a single tissue might produce incomplete information about CNV and cn-LOH content of a whole genome. The frequency and possible phenotypic consequences of somatic mosaicism for CNVs and cn-LOHs remain largely unknown, and obviously studies that involve larger sample cohorts can answer these questions.

1.P31

Deciphering of pathogenesis of chromosomal microdeletion and microduplications syndromes using induced pluripotent stem cells

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Chromosomal diseases have a significant impact on the congenital pathology. However, despite the long history of study and the availability of high-resolution methods for molecular diagnostics, many issues of their pathogenesis remain unclear. There are several reasons for this situation. First of all, there is a significant clinical polymorphism of chromosomal syndromes. Secondly, the presence of non-specific clinical features is common for patients with different chromosomal mutations. Additional uncertainty in the delineation of phenotype-karyotype correlations brings contiguous gene syndromes affecting a wide list of genes with different functions. Finally, an important limitation is related to the impossibility of interpreting clinical effects of chromosomal abnormalities by the analysis of peripheral blood lymphocytes. Induced pluripotent stem cells (iPSCs) with subsequent cell differentiation provide new possibilities for the study of chromosomal disease pathogenesis at the molecular and cell level. Here, we report about the production of two iPSCs lines derived from skin fibroblasts of patients with idiopathic intellectual disability having a microdeletion or microduplication at 3p26.3, affecting a single gene CNTN6 (Kashevarova et al., Mol Cytogenet. 2014). The gene product may play a role in the formation of axon connections in the developing nervous system. The proof of pluripotency was obtained by in vitro and in vivo using SCID-mice. The stability of the karyotype during reprogramming was also confirmed. Clinical features of our two patients with 3p26.3 microdeletions and one with microduplications as well as patients from the DECIPHER database were summarized. It was shown that the 3p26.3 microdeletions and microduplications are accompanied by intellectual disability, growth retardation, delayed psychomotor development, speech delay, low birth weight, microcephaly, epicanthus, wide nasal bridge, and abnormalities of the feet. The study was supported by the Russian Scientific Foundation (project 14-15-00772).

1.P32

Two patients with microdeletion and microduplication involving 1q21.1

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Molecular cytogenetics helps diagnose patients with microdeletion/microduplication syndromes. We present two cases with rearrangements involving 1q21.1.

A 9 year-old female patient was admitted for developmental delay. The patient was born to healthy non-consanguineous parents from the fifth pregnancy at the 40th week of gestation with a birth weight of 2750 g. She was able to sit without support at the fifteenth month, walk at three and half years speak at 4 years. Strabismus was diagnosed bilaterally on eye examination and she was operated at 3 years. Head circumferences was 47 cm(<-2sd), weight 17 kg(<3 percentile), length 110 cm(<3 percentile). Physical examination revealed dysmorphic facial features including bilateral epicanthic folds, deep set eyes, wide nasal bridge, bulbous nose, retrognathia. Cranial imaging showed dysmyelinated patchy areas of white matter. Routine karyotype analyses was 46,XX[72]/47,XX+mar[28], genomic analysis for copy number changes using Agilent 60 k chips revealed 1,3mb deletion at 1q21.1, further analysis showed that marker chromosome was derived from chromosome 1.

The second patient was a 5 year-old girl, born to healthy second cousin once removed parents at the 29th week of gestation with a birth weight of 940 g. Her twin had died at 24th week of gestation. She wasn't able to sit without support and walk yet. Refractive error was diagnosed at eye examination. On physical examination head circumference was 46 cm(<-2sd), weight 15 kg(3–10 percentile), length 107 cm(25–50 percentile). Developmental delay, dysmorphic facial features including bilateral epicanthic folds, deep set eyes, hypertelorism, triangular face, facial asymmetry were observed. Cranial imaging showed cerebral hypoplasia, corpus callosum agenesis, brain stem hypoplasia. Routine karyotype analyses was 46,XX. Genomic analysis for copy number changes using Agilent 60 k chips revealed 2,03mb duplication at 1q21.1.

1.P33

Case report: Small Supernumerary Marker Chromosome 15, cause of male infertility

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Introduction: Small supernumerary marker chromosomes are structurally abnormal chromosomes which cannot be unambiguously identified by conventional cytogenetics methods alone, and are generally equal in size or smaller than a chromosome 20 of the same metaphase spread. Their incidence has been estimated to be around 0.14–0.72 per 1000 in the neonatal setting, and 0.65–1.5 per 1000 in prenatal studies.

Materials and methods/ Results: We present the case of a 35-year-old male with infertility. High resolution chromosome analysis revealed a small accessory bi-satellited acrocentric chromosome found in the majority of the cells, subsequently identified as -most likely- genetically irrelevant material, part of chromosome 15, using spectral karyotyping.

Conclusion: Chromosome painting by SKY is a useful molecular cytogenetics resource, enabling to specify the origin of unknown genetic material, such as a marker chromosome: it is a single genome-wide, cost-effective method with a reasonable resolution, well suited for this situation. Ascertained through infertility in a phenotypically normal male, our case is concordant with the reports in the literature.

1.P34

SNP array analysis of 142 Czech patients with autism gives about 10 % diagnostic yield

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Autism spectrum disorders (ASD) affect over 1 % of population. They are genetically heterogeneous and have been associated with many copy number and single nucleotide variants (CNVs and SNVs). Hundreds or thousands of genes participate in ASD, most of them still remain unknown.

We examined 142 ASD patients from 133 families using Human CytoSNP-12 BeadChips (Illumina). According to the most significant aberration, the patients were classified into 5 categories (1−repeatedly reported

pathogenic aberrations, 2−likely pathogenic aberrations (some literature support), 3−unclear findings, 4 −intergenic aberrations, low-frequency polymorphisms, 5−frequent polymorphisms). The existence and inheritance of category 1 and 2 findings were validated using independent methods.

Clearly pathogenic variants found in 10 patients included: large (~10 Mb) deletions in 9p24.3-p23 and 5q23.3-q31.2, 4.2 Mb duplication in 19q13.42–43, 160 kb deletion in 9q34.3 (EHMT1), 2.9 Mb duplication of maternal 15q11.2-15q13.1 (PWS/AS), 17q12 and 12p13.33 microdeletions, 500 kb duplication of Xq28 (MECP2), and intragenic GRIN2B and NSD1 deletions.

Likely pathogenic findings in 6 patients included: 3 Mb duplication in 2q24.1, 350 kb duplication in 8q11.23 (RB1CC1, proposed new syndrome), combination of three large duplications in one patient, intragenic PTPRD deletion, maternal uniparental isodisomy (UPD) of chr1 and excess of homozygosity pointing to consanguinity. These findings are interesting for research but their potential clinical use needs more study and caution. Aberrations in category 3 or 4 are difficult to interpret using the current knowledge.

SNP arrays are an efficient tool for identifying CNVs in ASD. CNVs either have an immediate clinical effect or can suggest new causal genes. SNP arrays allow detection of homozygous regions pointing to UPD or distant consanguinity, which can direct further research towards recessive mutations.

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1.P35

Maternal MTHFR C677T Polymorphism: A Risk Factor for Meiotic I Nondisjunction of Chromosome 21

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BACKGROUND: The MTHFR C677T polymorphism may lead to DNA hypomethylation and thus represent a potential risk factor for chromosome 21 nondisjunction. The aim of the present study was to assess the association of the MTHFR C677T polymorphism with the meiotic stage of chromosome 21 nondisjunction among mothers of children with Down syndrome.

METHODS: The study included 107 mothers of children with maternally derived free trisomy 21. The mean age of the mothers was 32.1±6.5 (range 16–43) years. Short tandem repeat markers were used to determine the meiotic stage of nondisjunction. PCR-RFLP analysis was performed to detect MTHFR C677T polymorphisms. Statistical analyses were performed and reported using the Chi-squared test of independence, odds ratios, and 95 % confidence intervals. Results were considered statistically significant at $p<0.05$.

RESULTS: Meiosis (M) I nondisjunction was presented in 86 % (92/107) and MII nondisjunction in 14 % (15/107) of trisomy 21 cases. The mean maternal age was not statistically significantly different between maternal MI- and MII- derived cases of trisomy 21 (31.65±6.65 vs. 34.80±5.22, $p=0.084$). Mothers of children with MI-derived trisomy 21 had a greater frequency of the MTHFR 677CT+TT genotype compared to mothers of children with MII-derived trisomy ($p=0.040$). Mothers who are carriers of at least one MTHFR 677T allele have 3.6 fold higher risk for MI than for MII nondisjunction of chromosome 21.

CONCLUSION: These results indicate that the MTHFR 677CT+TT genotype could be a risk factor for MI nondisjunction of chromosome 21. However, further analyses on larger sample are required to provide an answer to this question.

1.P36

A Familial Interstitial 4q35 Deletion With No Discernible Clinical Effects

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Small deletions on the long arm of distal chromosome 4 do not appear to result in gross congenital malformations; the most frequently reported clinical findings include mild to moderate mental retardation, learning disabilities and minor dysmorphic features. Here we report a cytogenetically detectable familial interstitial chromosome 4 long arm deletion with no discernible phenotypic effects in a mother and her two daughters. The karyotypes of the mother and her two daughters were: 46,XX,del(4)(q35.1-q35.2). Based on the results of FISH analyses using whole chromosome specific and subtelomeric probes, the karyotype was designated as: 46,XX,del(4)(q35.1-q35.2). ish del(4)(q35-qter)(WCP4+, 36P21+, dJ963K6-). Array-CGH analysis showed an interstitial deletion encompassing 5.75 Mb in the 4q35.1-q35.2 genomic region (chr4:184.717.878-190.469.337; hg19). This is the first report of a cytogenetically detectable familial interstitial chromosome 4 long arm deletion in which there are no discernible phenotypic effects. Both our findings and a review of the literature suggest that more detailed molecular analyses are needed in cases with distal chromosome 4 long arm deletions especially those with breakpoints in the 4q35 region to establish a more precise genotype-phenotype correlation.

1.P37

Towards a molecular approach in the genetic analysis of IUFD products.

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Approximately 15 % of clinically recognized pregnancies ends in intrauterine foetal death (IUFD). Fifty percent of early IUFD is caused by aneuploidy or triploidy. Late IUFD's show more complex aberrations. Traditionally, cytogenetic testing in our laboratory was performed on cultures of chorionic villi, amniotic fluid or fetal tissue by karyotyping or interphase FISH (I-FISH) for chromosomes 13, 18, 21, X and Y. The success rate of karyotyping and I-FISH for IUFD products is low, ~50 % together, compared to molecular approaches (99 %). Moreover karyotyping and I-FISH are labour intensive. Therefore a molecular approach was introduced in the last 4 years.

From all IUFD's between 2010 and 2014, DNA was isolated and a QF-PCR was performed. In addition, an array-CGH was performed if the QF-PCR was normal. In this period, we analysed 333 IUFD products with a success rate of 96 %. An abnormal result was found in 53 samples (16 %). Thirty one out of 53 aberrations were detected with QF-PCR. Of these 19 were trisomies (13, 18, 21), 6 triploidies, 5 monosomy X and 1 XXY. Array-CGH showed one or more CNVs in 22 patients (7 %). Seven of these were presumed pathogenic or were de novo. 15 CNV's were also present in one of the healthy parents and were not considered causal of the IUFD. Thus, if a CNV is detected by array-CGH parental follow up and description of the phenotype is essential for interpretation of this CNV.

In conclusion, QF-PCR followed by array-CGH is a better approach for genetic analysis of IUFD products than traditional techniques, because the success rate is higher (96 % compared to ~50 %). Furthermore, a genetic aberration in the IUFD products is detected in a higher percentage of samples (23 vs 13 %).

1.P38

Frequency of uniparental disomy in 836 patients with pathological pehenotype

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UPD is the inheritance of a chromosome pair derived from a single parent. Based on different mechanisms, UPD can present as isodisomy (two copies of one parental homolog), heterodisomy (both homologs of one parent) or a combination thereof and can involve complete chromosomes or only chromosomal segments. The mode of inheritance can be maternal or paternal. UPD is generally thought to occur at a rate of 1:3500 live births. Besides the detection of submicroscopic deletions and duplications, SNP microarrays can be used for detection of uniparental disomy (UPD). While SNP-array analysis allows the detection of uniparental isodisomy, uniparental heterodisomy is suspected when extended homozygosity regions are found on a single chromosome, but it is confirmed through the comparison of the parent's genotype. 836 patients with autism,

ID, malformation, etc.…were included in this study to detect CNVs which are responsible of the pathological phenotype. SNP-array analysis was carried out with Beadchip (Illumina), the data were analyzed with BeadStudio, GenomeStudio and PennCNV. We detected isodisomy in 4 of 836 patients, two cases were in homogeneous form (chromosome 5 and 8, two male) and two were in mosaic (chromosome 15, one male and one female). Two cases of segmental UPD (chromosome 14 and 5q) were also detected. Furthermore UPD in other five patients (chromosome 7 three cases, one case for chromosome 14 and 15) was suspected, analysis of homozygosity regions, through the trio analysis, confirmed all five cases. In conclusion frequency of UPD in our study is 1,3 %. Our prospective target is to study frequency of segmental or homogeneous UPD with UPDio software in 132 patients and parents, who do not present extensive regions of homozygosity.

1.P39

A patient with segmental isodisomy on 5q11.2-qTER and deletion 5q11.1-q11.2

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Uniparental disomy (UPD) is a genetic condition in which the offspring receives two chromosomal homologues from a single parent. If the UPD is present on a segment of the homologues, the condition is referred to as a segmental UPD, and if the homologues are identical (copies of a single homologue from one of the parents), the condition is known as isodisomy. We report on a male patient of 2 years with ID, autism, scoliosis and normal karyotype. Analysis conducted with SNP-array (platform Illumina, Human OmnieExpressExome and software GenomeStudio) detected a submicroscopic 5q11.1-q11.2 deletion (49,457,282–53,282,649) of approximately 3.8 Mb in size. Moreover, an extensive homozygosity region was detected (53,282,979–180,693,127) in chromosome 5q11.2-qter, and UPD was suspected. It was possible to analyze only the mother, for this reason we can speculate a paternal UPD and the deletion on maternal chromosome 5q. Since autistic behaviours is the only consistent finding in our patient and two reported patients with a similar deletion in 5q the comparison can identify the critical region that is implicated in autism spectrum disorders. The isodisomy can be associated with a normal or pathological phenotype. As far as we know one other patient is described in literature with paternal segmental uniparental isodisomy and childhood onset schizophrenia, so in our patient scoliosis and intellectual disability may be caused by recessive mutations highlighted by isodisomy. Exome analysis could highlight mutations in genes on 5q responsible for the phenotype.

1.P40

Cytogenetic and array CGH findings in patients with split hand/foot malformations

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Split hand/split foot malformation (SHFM), also known as ectrodactyly, is a congenital limb malformation affecting the central rays of the autopod. It may present with syndactyly, median clefts of hands and/or feet and hypoplasia and/or aplasia of fingers, toes, tarsals and metatarsals. In severe cases, the hands and feet have a "lobster claw" appearance.

The condition is clinically and genetically heterogeneous and exhibits usually autosomal dominant inheritance with variable penetrance and expressivity. It may occur as an isolated defect or as a component of a syndrome involving ectodermic structures. The incidence has been reported to be about 1/18 000 newborn babies.

Six loci SHFM have been mapped:
SHFM 1 at chromosome 7q21.3-q22.1
SHFM 2 at chromosome Xq26 (one reported family)
SHFM 3 at chromosome 10q24 (duplication)
SHFM 4 at chromosome 3q27 (TP63 gene)
SHFM 5 at chromosome 2q31 (deletion)
SHFM 6 at chromosome 12q13 (WNT10B, autosomal recessive)
SHFM/SHFLD at chromosome 17p13.3 (BHLHA9)
Other loci have been studied.

Cytogenetic aberrations of 7q chromosome have been reported, most often deletions or translocations (balanced, unbalanced, complex), seldom inversions of 7q.

Duplications of SHFM3 are supposed to be the most frequent cause of nonsyndromic SHFM in humans.

We present two cases:

1) A patient who exhibits severe form of ectrodactyly, caused by complex aberration of chromosome 7 consisting of de novo paracentric inversion encompassed SHFM1 region and a submicroskopic deletion of 7q

2) A patient with an about 0,5 Mb large duplication of 10q24 (SHFM3) presenting only syndactyly.

Classical cytogenetics as well as array CGH revealed to be useful tools in SHFM diagnosis.

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1.P41

Marker chromosome analysis in two patients with Turner syndrome variant resembling Kabuki phenotype

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Small marker chromosomes are chromosomal fragments whose origins often cannot be determined by conventional cytogenetic methods alone and require molecular approaches for definitive characterization.

Turner syndrome presents with a characteristic mild phenotype with some degree of variability. Kabuki syndrome is a multiple congenital anomaly disorder characterized by specific facial features, mild to moderate mental retardation, postnatal growth delay, skeletal abnormalities and unusual dermatoglyphic patterns with prominent fingertip pads. The association between clinical and cytogenetic findings of Turner syndrome and Kabuki phenotype in the same individual is a rare occurrence.

We present two cases with particular oriental-like facies, one of them of Tatar ethnicity, originating from the South-Eastern Region of Romania. First patient was referred at 1 year 4 months of age with poor growth and global developmental delay. The second girl presented at 13 years of age with delayed secondary sexual characteristic development, primary amenorrhea.

GTG-banding revealed a mosaic karyotype with a cell line containing a small marker chromosome and a cell line with 45,X. Parents' karyotypes were normal.

Fluorescence in situ hybridization and molecular techniques using STR markers were performed to determine the origin of the marker chromosome and identified the very small marker as a ring X chromosome.

The presence of a marker chromosome in Turner syndrome generally implicates a sex chromosome origin. The severe phenotype of females with tiny ring X chromosomes can be explained by the inability of these chromosomes to undergo X inactivation resulting in functional disomy. Also, mosaicism resulting in the absence of the ring from some tissues, such as the brain, is important for the severity of the phenotype.

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1.P42

Maternal LINE-1 methylation and congenital heart defects in Down syndrome

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Background: Down syndrome (DS) is one of the most common chromosomal abnormalities associated with congenital heart defects (CHD); cardiac defects are present in approximately 40–60 % of the cases. The aim of this study was to determine the association between maternal global DNA methylation and occurrence of CHD in DS. The impact of MTHFR C677T polymorphism and dietary intake of folate on global DNA methylation was analyzed.

Patients and methods: The study included 94 mothers of children with DS of maternal origin. Among those mothers, 49 % (46/94) have children with DS and CHD (DS-CHD+) and 51 % (48/94) of them have children with DS without CHD (DS-CHD-). Global DNA methylation was analysed in peripheral blood lymphocytes by quantification of LINE-1 methylation using the MethyLight method. Genotyping of MTHFR C677T polymorphism was performed by PCR-RFLP.

Results: There was no significant difference in values of global DNA methylation between mothers of children with DS-CHD+ and mothers of children with DS-CHD- (P=0.951). The combination of MTHFR C677T genotype/diet significantly influenced global DNA methylation (R2=4.5 %, P=0.046). The lowest values of global DNA methylation were determined in mothers with CT+TT genotype and low folate diet. The contribution of these factors was even higher among mothers of children with DS-CHD+ (R2=9.9 %, P=0.026), particularly among those who had children with DS and septal heart defect (R2=15.4 %, P=0.018).

Conclusion: Although global DNA methylation was not significantly associated with development of CHD in DS, its influence cannot be completely excluded since the significant impact of MTHFR genotype and diet on global DNA methylation in mothers of DS-CHD+ was determined. Further analyses on a larger sample are needed to provide an answer to this question.

1.P43

Apparently balanced de novo structural rerarrangement of chromosome 10 in a boy with an abnormal phenotype

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An increasing number of apparently balanced de novo chromosomal rearrangements have been associated with phenotypic abnormalities in recent years. The use of high-resolution cytogenetic techniques enables detailed screening for cryptic imbalance of microscopically balanced de novo rearrangements in patients with abnormal phenotype.

The patient is a 1-year -old boy with retarded psychomotor development and cranium, face and neck asymmetry. A cerebral MRI scan showed a hypoplastic corpus collosum and retrocerebellar cyst. He is the second child of healthy unrelated parents. Amniocentesis was performed in the 20th gestational weeks because of a nuchal cystic hygromata was visible by fetal ultrasonographic examination. GTG-banded analysis of 20 metaphase cells from amniocytes revealed an abnormal male karyotype with a balanced de novo pericentric inversion of chromosome 10—inv(10)(p11.2q21). Conventional chromosome analysis was also performed on a peripheral blood sample from this patient shortly after his birth, which confirmed the amniocyte findings. Multicolor banding (mBAND) of chromosome 10 was performed to define breakpoints. FISH analysis revealed that the distal breakpoint on chromosome 10 was in 10q11.2. High-resolution genome-wide array analysis using Affimetrix CytoscanTM HD array platform showed copy number change in the distal breakpoint. The region 10(q11.22q11.23) contained a 5,7 Mb deletion. The deletion was considered pathogenic based on the various public web sources, including ISCA and DECIPHER. Thus the patient has apparently balanced de novo pericentric inversion of chromosome 10 accompanied by the clinically relevant CNV. The 5,7-Mb deletion is considered very likely to be causative for the abnormal phenotype.

The symbiosis of traditional cytogenetic techniques with molecular cytogenetic techniques, in association with high-resolution whole-genome CNV analysis, has permitted further investigation of cases of seemingly balanced chromosomal rearrangements with associated phenotypic abnormalities.

1.P44

An unusual combination of an atypical maternally inherited 0.3 Mb deletion in Williams-Beuren region and a de novo 22q11.21 microduplication in an infant with supravalvular aortic stenosis

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Williams-Beuren syndrome (WBS) is a rare multisystemic neurodevelopmental disorder occurring in 1/20,000 live-births. It is caused by a 1.5–1.8 Mb deletion on 7q11.23 characterized by supravalvular aortic stenosis (SVAS), intellectual disability, overfriendliness and dysmorphic facial features. The 22q11.21 microduplication syndrome has a variable phenotype which ranges from normal to abnormal and has been frequently associated with congenital heart disease.

We present a 1-month old boy referred for a possible diagnosis of WBS because of SVAS. In addition, he had bilateral pre-auricular pits. The infant passed away suddenly 10 days after referral possibly due to severe cardiac complications. Initial FISH analysis using LPU011 probe (Cytocell) revealed normal results. However, subsequent array-CGH analysis using Cytochip ISCA array (BlueGnome) revealed two different pathogenic copy number changes; an atypical deletion of 0.3 Mb within the WBS region (location: 73,187,643–73,501,025) and a duplication of 2.6 Mb within the 22q11.21 microduplication syndrome (location: 18,894,864–21,505,388). The deletion includes CLDN4, WBSCR27, WBSCR28, ELN and the first two exons of the LIMK1 gene. MLPA analysis on the patient and parents showed that the 22q11.21 duplication was de novo and the 7q11.23 deletion was maternally inherited. Family studies of the extended family are ongoing.

To our knowledge this is the first report of a combination of 7q11.23 deletion and 22q11.2 microduplication. Detailed clinical evaluation of the intellectually normal mother is currently ongoing which will provide valuable phenotype-genotype correlation contributing to the limited literature of these rare atypical deletions. The contribution of the 22q11.2 to the patient's phenotype, specifically to the severe cardiovascular problem,is difficult to evaluate, however clinical evaluation of the mother might shed some light and define the contribution of the microduplication.

1.P45

Genetic heterogeneity in patients with 22q11.2DS clinical features

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A group of 140 probands with clinical features typical of 22q11.2DS was analyzed. The most common reason for FISH analysis referral was the presence of congenital heart defects (CHD), conotruncal and non-conotruncal, facial anomalies, cleft palate, hypocalcemia, immunodeficiency and several other abnormalities. All patients previously underwent standard karyotyping to exclude other chromosomal aberrations. Those with normal karyotype underwent FISH analysis with TUPLE1/ARSA (Abbott Molecular) probe. 77 samples were concurrently analyzed by MLPA analysis with SALSA MLPA P250-B1 DiGeorge (MRC-Holland) kit. This analysis was performed in order to independently confirm earlier data, define deletion size and attempt to reveal atypical distal deletions/duplications in 22q11.2 region and deletions in other chromosomes locuses integrated in this specific MLPA kit known to cause similar phenotypes in FISH-negative subgroup. Three patients within the group were found to have chromosomal abnormalities: 48,XXXX, 46,XY,der(4)t(4;8)(q35;q22)mat, mos45,X[42]/47,XXX[8]. FISH analysis revealed 22q11.2 deletion in 43 patients, 94 patients were not deleted. Three patients with normal karyotypes were later diagnosed with Alagille syndrome (n=1) and Smith-Magenis syndrome (n=2). In the subgroup of 77 probands previously tested for 22q11.2 microdeletion by FISH MLPA analysis confirmed all 28 FISH-diagnosed cases of 22q11.2 deletion. Most patients had common recurrent 3 Mb deletion (22 cases, 79 %), the remaining patients (21 %) indicated smaller

"nested" deletions -and one patient had an elongated deletion. In our study MLPA test didn't reveal either atypical distal deletions or duplications in the remaining 49 patients, as well as other deletions in locuses known to cause comparable phenotypes. As clinical spectrum of 22q11.2DS is known to be rather broad and some features could only be recognized with child development, some patients of our group were reevaluated and found to have other genetic disorders, which explain their phenotype.

1.P46

A novel 15.5 kb deletion within the translated sequence of SHOX gene, leading to Leri-Weill Syndrome in a family with emphasizing severity in females

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Leri-Weill dyschondrosteosis (LWD) is a dominantly inherited genetic disorder characterized by short stature, mesomelia and Madelung wrist deformity. The LWD is caused by haploinsufficiency of the Short Homeobox contaning gene (SHOX), located in the 2.6 Mb pseudoautosomal (PAR1) region. At the heterozygous level most mutations found are deletions of different size, point mutations within the coding sequence or mutations on regulatory enhancers downstream of the SHOX gene. We present the clinical and molecular data of a three generation family with LWD. The index patient is a 43 year old female with disproportionate short stature, reduced arm span to height ratio (particularly mesomelic shortening), a muscular body habitus and no evidence of a classic Madelung deformity. We report a novel 15.5 kb SHOX intragenic deletion detected by microarray-CGH, followed by MLPA analysis and sequencing of the PCR product encompassing the junctions of the deletion. MLPA studies confirmed the deletion and revealed the same 15.5 kb deletion in her sister, father, daughter and son. Sequencing has determined the exact breakpoints of the intragenic deletion removing exons 3–6 containing the homeodomain region. Further evaluation of the breakpoints is ongoing to determine the possible mechanism responsible for the derived deletion. The youngest male member of the family has a very mild form of LWD, a phenomenon previously observed, that SHOX deficiency is more pronounced in females than in males and it can also exhibit inter-familial and intra-familial variable expressivity. Extended family studies, detailed clinical evaluation of the family members are ongoing which will shed more light in the phenotypic effect of this deletion.

1.P47

Relationship between heterochromatin polymorphism and FISH studies in sperm in men with idiopathic inferitility

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Heterochromatin polymorphism (HP) is a variant of a normal karyotype but which is thought to be more frequent in infertile men. It has been suggested that there is a relationship between HP and infertility, although no possible mechanism has been described. HP could be related to the existence of meiotic alterations that can be detected by Fluorescence in situ hybridization (FISH) analysis in decondensed sperm. In the present work, we studied FISH results on spermatozoa of infertile men, to evaluate the relation between heterochromatic variants and male infertility.

Blood karyotype and sperm aneuploidy (disomy and diploidy) rates for chromosomes 13, 18, 21, X and Y were evaluated in 251 infertile men without structural chromosomal rearrangements.

A total of 84 patients showed abnormalities with FISH (33.5 %) and 167 were normal. From the 251 patients, 52 patients (20,7 %) showed HP. Of these patients with HP, 22,6 % showed abnormal FISH results in contrast to 19,7 % of the patients with HP and normal sperm FISH. The most frequently observed polymorphism was 9qh+(11 %). There were no significant differences in abnormal sperm FISH analysis between

individuals with chromosomal polymorphisms and with normal karyotype (36.5 % vs 32.7 %; $p>0,05$).

Although some studies have suggested that heterochromatin polymorphism is more prevalent in the infertile population than in the normal population, our data has shown that there is no correlation between male infertility and the presence of HP.

1.P48

Interstitial deletion 2p11.2p12. Report of a patient with developmental delay and bilateral choanal atresia

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De novo interstitial deletions of the short arm of chromosome 2 are rare chromosomal abnormalities. Patients showing these kind of microdeletions have developmental delay/intellectual disability, minor facial anomalies including high forehead, frontal bossing, broad nasal bridge, abnormal ears and congenital defects such as skeletal and genital malformations. We describe the second child of a healthy and non consanguineous couple presenting at birth multiple malformations and minor facial anomalies. Because of the clinical findings, an array CGH analysis was performed using Agilent 60 K microarray oligonucleotide. The analysis detected a 9.3 Mb deletion on the short arm of chromosome 2 at band p11.2p12 spanning the bases 77,946,599–87,277,610. The five patients previously described display a minimal common deleted region which explains the clinical features shared by all of them, while their individual characteristics might be explained by the different sizes of the deletion. The common deleted region involves several genes (CTNNA2, LRRTM1, REEP1), highly expressed in the nervous system. The deletion found in this case overlaps with most of those reported in literature but our patient displays extra clinical signs such as bilateral choanal atresia and atrial septal defect. It was impossible to find any direct correlation between the genes involved in the deletion and the choanal atresia and the heart defect. The question remains open as to whether these clinical features are a consequence of the deletion or are due to a second pathogenic event. Our case emphasizes the difficulties in finding a close correlation between a large deletion and a well defined clinical picture. As only five patients with 2p11.2p12 deletions, reported in the literature are characterized by array CGH, further reports will be necessary to well define a clinical phenotype related to the 2p11.2p12 microdeletion.

1.P49

Female Sex Chromosome Abnormalities in a Cytogenetics Laboratory: what have we learnt in 30 years?

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Sex chromosome abnormalities are the most frequent cytogenetic anomalies in pre- and postnatal diagnosis. The pathologies associated with chromosomes X and Y show a large range of genetic and clinical variability and encompass many cytogenetic anomalies. However the phenotypes associated with rearrangements of the sex chromosome are generally less severe than those which are caused by the similar rearrangements in the autosomes. Infertility is frequently the only clinical manifestation in adults females with abnormalities of the X.

In 30 years, in the Cytogenetics Unit of CGMJM, 27,422 karyotypes were analyzed; of these, 8,730 female cases were selected for this study. Clinical indications for cytogenetic analysis were varied, e.g. the presence of multiple anomalies, psychomotor delay, recurrent miscarriages and clinical suspicion of Turner Syndrome. Among the selected samples, 282 showed alterations of the X chromosome (3,2 %). The abnormal results will be presented in two different perspectives: the classification of the karyotype by chromosomal abnormality and by the clinical indication. Our results will be compared with those described in the most recently published papers in medical journals.

With this work the authors present a significant number of X chromosome anomalies in Portuguese women and idendity the most frequent abnormality within each clinical group.

1.P50

HOXA cluster deletions: a clinical challenge

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HOXA cluster genes play a fundamental role in embryologic development. Deletion of the entire cluster is known to cause a clinically recognizable syndrome. The clinical manifestations may vary with different sizes of deletions of HOXA cluster and flanking regions. Smaller deletions of this region usually display mild developmental delay, characteristic facies, and hand-foot-genital syndrome, but larger deletions may cause more complex conditions, leading to difficulties of clinical diagnosis and interpretation. We report a girl born to a healthy and non-consanguineous Italian couple with a deletion involving the entire HOXA cluster at 7p15.2-p14.3. To the best of our knowledge, this is the smallest deletion involving HOXA genes reported to date. Chromosome analysis of the patient revealed an apparently normal karyotype at the 550 band level. Based on array comparative genomic hybridization, a 2.5 Mb interstitial deletion was detected at 7p15.2p14.3 (chr7: 26,333,553–28,859,312), involving the entire HOXA cluster and a small number of other genes such as SNX10, SKAP2, EVX1, HIBADH, TAX1BP1, JAZF1, and CREB5. The patient in the present study, showed a number of typical manifestations of HOXA13 deletion, such as speech delay, short stature, left vesicoureteral reflux, small feet with brachydactyly, bilateral mild hypoplasia of distal phalanges of some fingers of the hands, and some mildly dysmorphic facial features. However, our patient exhibited a number of additional features, such as bradycardia and sporadic supraventricular extra sistole, oxyhemoglobin desaturation during feeding, and laryngeal stridor. Interestingly, one of the HOXA genes, HOXA5, is known to be essential to organogenesis and function of respiratory tracts. This case provide new informations about the genotype-phenotype correlation of HOXA genes cluster deletions via the identification and characterization of the smallest deletion reported to date, furthermore opening new discussion and interpretation cues on the unusual findings detected.

1.P51

Illustration of the complementarity between CMA techniques and conventional cytogenetic techniques: a clinical case.

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Background The array comparative hybridization technology (array CGH) has been a technological revolution, which had changed our diagnostic approach todevelopmental delay pathology. This technology enables detection of sub-microscopic chromosomal aberrations and has seen its range of applications expanding in the last few years in clinical and research fields. However, the technique presents several limitations that hinder the detection of balanced rearrangements and rearrangements involving highly repeated satellite DNAs.

Results Herein we report a male patient who presented psychomotor delay and language disorder associated with facial features not obviously related to the observed copy number variation. He showed additional satellite material on the end of the long arm of the chromosome 4, clearly visible on the karyotype, although Chromosomal Microarray Analysis (CMA) revealed only a terminal 435-kb deletion of the 4q35.2. To explain the apparent discrepancy, a family investigation was carried out and indicated that, in fact, the proband carried a derivative chromosome 4 (der4) derived from a reciprocal translocation t(4;21)(q35.2;p11.2) in the mother.

In parallel, an investigation of a new pregnancy of the mother revealed a foetus with agenesis of the foetal venous system and a 47,XY,+der(21)t(4;21)(q35.2;p11.2)mat karyotype.

Conclusion With this case, we emphasise that one of the limitations of the array CGH technology is its inability to find an unbalanced rearrangements involving satellite material. This study shows that only the combined application of array-CGH with conventional and molecular cytogenetic approaches could define the exact nature of chromosomal abnormalities. Moreover, we have disclosed genes which may be involved in the phenotype of mental retardation

1.P52

Duplication 11p as a result of an unbalanced translocation 46,XY,der(6)t(6;11)(q27;p15.5)mat in a boy with Silver-Russell-Syndrome-like symptoms

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We report on a 16-month old boy who was born in the 29th week of gestation with a birth weight of 730 g. Prenatal ultrasound in the 26th week of gestation showed intra-uterine growth restriction (IUGR) and hydrocephalus. Chromosome analysis following amniocentesis revealed a normal result at the 400 band level. After birth, chromosome analysis was repeated and a FISH analysis with probes for the short and long arm of chromosome 11 was performed because of severe thrombocytopenia. Unexpectedly, no deletion 11q, but an additional signal of the short arm probe was detected—localized on the long arm of a chromosome 6. Array analysis showed a 8 Mb duplication in 11p and a 537 kb deletion in 6q. Additional molecular genetic analysis revealed a functional duplication of 11p, supposedly of maternal origin. In summary, these findings are consistent with the clinical picture of a Silver-Russell-Syndrome.

Examination of the maternal chromosomes confirmed these data—the mother is carrier of a nearly cryptic reciprocal translocation t(6;11)(q27;p15.5). Interestingly, a pericentric inversion 12 was also diagnosed; the karyotype was therefore 46,XX,t(6;11)(q27;p15.5), inv(12)(p11.2~12q13~15).

Clinical data and details of the different analyses will be presented.

1.P53

Establishment of a single-cell RNA/DNA-FISH method for detecting inactivation patterns of structural X chromosome abnormalities

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In females, X chromosome inactivation (XCI) is a gene dosage compensatory mechanism that one of the two X chromosomes is randomly inactivated in cells. XIST (X-inactive specific transcript, Xq13.2) accumulation (called an XIST cloud) on one of the two X chromosomes is the key player of XCI. The purpose of the present work is to establish a single-cell RNA/DNA-FISH method for detecting XCI and structural X chromosome abnormalities simultaneously in each individual nucleus.

The subject was a normal phenotype female with an interstitial deletion within band Xp21.2 including the GK gene (del Xp21 female). We used three kinds of BAC clones for the simultaneous 3-color RNA/DNA-FISH study onto interphase nuclei of B-lymphoblastoid cell lines; a clone consistent with the XIST gene for detecting XCI, a clone consistent with the UTX gene (Xp11.3), which is as a reference probe for both normal and abnormal Xs, and a clone consistent with the GK gene for distinguishing between normal and abnormal Xs of the del Xp21 female. We evaluated inactive X (Xi) by cloud-type XIST RNA signal, and normal and abnormal Xs by dot-type gene DNA signals on the same nucleus.

We detected cloud-type XIST RNA signal as a marker of Xi, and dot-type DNA signals of GK, UTX, XIST genes on the same nucleus by the

RNA/DNA-FISH method, which allowed us to distinguish between the normal and the abnormal Xs of the del Xp21 female. In addition, the dot-type UTX and XIST signals were observed at the cloud-type XIST RNA signal, and the other UTX, XIST and GK signals were observed at the clear area without cloud-type XIST RNA signal. We confirmed that the del Xp21 female showed skewed XCI with selective inactivation of the abnormal X.

1.P54

De novo case of a triplication of 16p

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Chromosome 16p duplication has been observed in a number of individuals, but a triplication of 16p has not been reported. Duplication 16p is usually observed as a part of complex syndromes among families with structural chromosomal rearrangements. Described duplications of the proximal 16p arm varies from small, interstitial duplications to whole arm duplications. The phenotype of individuals with duplication varies from normal to severe, depending on the size of the duplication and potential additional chromosomal rearrangement.

Here we present the clinical and cytogenetic findings in a 6-year-old female referred for genetic evaluation because of psychomotor delay and dysmorphic features, including low set ears, long philtrum, gothic palate, wide spaced nipples, clinodactyly and epileptic seizures. Cytogenetic examination of GTG banded metaphases showed a female karyotype with additional material on the short arm of chromosome 16. Extended molecular cytogenetic analysis (arrayCGH) showed female molecular karyotype with four copies of the 16p13.11p11.2 region. Size of the amplification is 11.8±0.1 Mb. The proband's karyotype can be reported as: 46,XX,add(16)dn.arr[hg19]16p13.11p11.2(16,525,289–28,318,164)x4. Proband's parents have normal karyotypes.

The size and boundaries of structural chromosome anomalies arising de novo are usually difficult to define, based only on classical cytogenetic analysis, due to its limited resolution. We have compared the clinical features of our proband to other patients carrying a duplication of proximal part of 16p as described in the literature. Four copies of genes on chromosome 16p11.2-p13.11 seem to result in greater developmental disturbance than are reported in patients with three doses of the same genes. This is supported by an animal model as well, where research on mice showed that tetrasomy of chromosome 16 had much more deleterious effects on embryonic development than did trisomy of the same chromosome.

1.P55

Distichiasis in a patient with a deletion downstream FOXC2 gene

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Heterozygous mutation in the coding sequence of FOXC2 gene causes a clinical syndrome combining lymphedema and distichiasis which is a double row of eyelashes requiring ablation with a risk of keratitis. Less than hundred patients were hitherto described and some with renal abnormalities and diabetes mellitus. Several patients suffered from lymphedema with distichiasis without FOXC2 gene mutation. One of them carried a deletion distal to FOXC2 gene with distichiasis, bilateral vesicoureteral reflux, glomuvenous malformations but also microcephaly and intellectual impairment, suggesting a regulatory region of FOXC2 gene in this locus (Butler et al., 2012). More over some studies showed a potential regulatory region in 16q24, close to FOXC2 gene (Sholto-Douglas-Vernon, 2005—Brice, 2002).

Here, we report a 20 year-old girl patient with distichiasis, myopia, left bifid kidney with vesical urethral reflux, dysmenorrhea with septate uterus, diabetes mellitus, and mild intellectual delay. She was the only case in her family, without parents' consanguinity. Array CGH study identified a 693 kb deletion located at 550 kb to FOXC2 gene (Agilent—180 K)

encompassing five genes, three pseudogenes and one unknown open frame region.

We describe here the second patient with a 16q24 cryptic deletion which could confirm Butler et al. hypothesis concerning the regulation of FOXC2 gene by this region. This case allowed us to better map the potential regulatory region of FOXC2 gene.

1.P56

A case report: 13q21-qter deletion with digital anomalies, duodenal atresia and anal atresia

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The 13q-syndrome is caused by a deletion of the long arm of chromosome 13.

We report a newborn female infant who was delivered at 33 weeks of gestation by Cesarean Section,with a birth-weight of 830 g and severe symmetric intrauterine growth retardation. The mother was a 19 year old woman, G2P1A0C1, who was followed-up during her pregnancy at a 1st level state hospital, initially with normal findings then with a suspicion of a cystic lesion in the abdomen of the fetus. The father had previously had a child from his first marriage who had died 40 days after birth because of a major cardiac anomaly. The mother of our patient was transferred to the university hospital due to preterm rupture of membranes and a risk of congenital abnormality. The baby required PPV at birth and her initial examination at NICU revealed hypertelorism, flat nose bridge, low-set ears, typical bilateral anterior ear tragus, pointed chin, kliteromegali, four digits in each extremity and bilateral absence of thumbs, and anal atresia. Imaging studies including plain abdominal radiographics, abdominal USG, Doppler echocardiography, and transfontanel USG demonstrated duodenal atresia, interatrial septal aneurysm, bilateral mild renal pelvic dilatation and agenesis of corpus callosum. She was operated for duodenal atresia, malrotation and anal atresia but postoperatively developed multiorgan failure and died on day 5. Karyotyping using her peripheral blood revealed the chromosomal abnormality, 46,XX,del(13)(q21.4). Quantitative gene screening using Multiplex Ligation dependent Probe Amplification was performed for aneuploidy and we found heterozygous deletion 13q21.4-q34. Both parents were subsequently evaluated by a clinical geneticist and were karyoptyed; both had normal karyotypes.

1.P57

5q distal deletion detected by arrayCGH in a girl with learning disability and congenital anomalies

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Interstitial deletions of the long arm of chromosome 5 are rare. Clinical features of patients bearing these deletions allowed genotypic classification into proximal deletions, encompassing the region from q15 to q22, and distal deletions, encompassing the region from q22 to q31. The clinical presentation of distal deletions has been fairly well established but genotype-phenotype correlations have proven hard to define. This lack of correlation may be due to difficulties in cytogenetically assigning breakpoints in this region due to its banding pattern.

Here we describe an eight year old girl with a de novo 12.15 Mb interstitial deletion of 5q22.3q23.2 region detected by arrayCGH (NimbleGen CGX 135 K, PerkinElmer). She presented at birth with cleft palate and feeding difficulties. Posterior clinical examinations revealed a patent ductus arteriosus, inguinal hernia and hearing problems, she had speech delay, along with specific learning difficulties and deficits in attention, motor control and perception (DAMP). Craniofacial dysmorphic features, including dolichocephaly, prominent ears and downslanting palpebral fissures, were also present.

Cases reported in the literature with 5q distal deletions are usually larger or have a more distal location than the present case. This 12 Mb deletion with breakpoints well defined by aCGH greatly contributes to a more accurate genomic mapping of clinical features.

1.P58

French collaborative survey of 749 patients with 22q11 deletion

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The 22q11.2 deletion syndrome (22q11DS) is the most recurrent human microdeletion syndrome. The deletion results more commonly from non allelic homologous recombination (NAHR) occurring during meiosis and mediated by LCR. About 90 % of patients have a 3 Mb deletion spanning LCR22-A to LCR22-D called the « typically deleted region » (TDR). 22q11DS is associated with a wide spectrum of abnormalities, but little is known about its incidence.

Methods and population: Clinical and cytogenetic data on the cases diagnosed from 1995 to 2012 were collected in 38 French cytogenetic laboratories. 749 cases postnatally diagnosed were collected.

Results: Most cases were diagnosed by FISH (98,0 %) and few case (2.0 %) by array-CGH with a deletion size ranging from 745 to 2904 kb for the latter. Only 8/15 patients diagnosed by array-CGH had a deletion involving LCR22-A and LCR22-D corresponding to the TDR.

The most frequent reasons for referral in postnatal cases were heart defect (48.6 %), facial dysmorphism (49.7 %) and developmental delay (40.7 %). The signs motivating the genetic analysis are different according to age at diagnosis and will be described. Some patients with 22q11DS and atypical phenotype are fortuitously found with array-CGH. The deletion was inherited in 15.4 % of cases mainly of maternal origin.

Conclusion: This is the largest cohort of patients with 22q11DS reported to date in the same population. We have contributed to the evaluation of the frequency of 22q11DS diagnosis by cytogenetics laboratories. There are at least 108 affected patients (including fetuses) per year in our French population of about 66 Million people. The clinical signs at diagnosis are highly variable and change with age at diagnosis.

1.P59

Genotype-phenotype correlation in female gonadal dysgenesis—experience of Genetics Department of "St. Spiridon" Hospital, Iasi, Romania

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Gonadal dysgenesis could be caused by numerical or structural abnormalities of the gonosomes; the main findings are infertility (sterility) and underdeveloped sexual characteristics.

Material and methods: Our consists of 7 patients (adolescent or adult) with features suggesting Turner syndrome (short stature, primary amenorrhea, failure of sexualisation) clinically diagnosed in the Genetics Department of "St. Spiridon" Hospital, Iasi, Romania. These patients were referred from November 2013 to January 2014 and confirmed by cytogenetic analyses—Barr test and chromosomal analysis (using cultures of lymphocytes and GTG bands). In one case we used also FISH.

Results: In 6 cases, the final diagnosis was Turner syndrome (one 45X, one Xp-, two Xq-, one i(Xq), and one r(X)). One case of central hypogonadism with ovarian failure had a 46,XX/47,XX,+mar karyotype. The Barr test was not always concordant with the karyotype.

The most common clinical finding in our patients with Turner was short stature varying from −2,7 to −4,8 standard deviations except in one case with Xq deletion where the stature was normal. The stature most affected was in patient with X ring chromosome which presents also a development delay and a very rare congenital anomaly in Turner syndrome—callosal agenesis (described just in five cases associated with Turner syndrome).

In the case of central hypogonadism with ovarian failure the cytogenetic finding was a mosaicism with normal line and a line with supernumerary marker chromosome which was confirmed by FISH to be an X derivative chromosome.

Conclusions: The karyotype with or without FISH is an appropriate method to confirm the diagnosis in all cases with gonadal dysgenesis.

1.P60

Upstream deletion of PITX2 detected by SNP array in a child with Rieger syndrome

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Rieger syndrome (RS) is a rare autosomal dominant disorder characterized by specific ocular, dental and umbilical anomalies. Mutations in PITX2 and FOXC1 genes explain about 50 % of RS. Deletions of coding exons and more rarely chromosomal translocations have been described. Very recently a deletion involving the upstream non coding region of PITX2 in two RS patients (Volkmann et al. 2011, Reis et al. 2012) has been reported. The conserved non coding elements upstream of the gene were showed to be involved in regulation of pitx2 expression in a zebrafish model.

We report a girl aged 13 months who presents unilateral eye anomalies including posterior embryotoxon, irido-corneal adhesions and pupillary anomaly associated with periumbilical skin and anal anteposition. To confirm the suspicion of RS, Sanger sequencing of PITX2 and FOXC1 were performed but no mutation was detected. The Affymetrix SNP array analysis revealed a de novo 4q25 deletion of 950 kb which contains no gene. The deletion began 194 kb upstream of the PITX2 gene removing cis-regulatory elements of PITX2.

The identification of this additional case highlights the interest of using whole-genome chromosomal microarray analysis to detect copy number variation in gene desert region and the importance of screening the upstream regulatory region of PITX2 involved in RS.

1.P61

Are there better clinical criteria for requesting molecular analysis for Prader-Willi syndrome diagnosis?

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Prader-Willi syndrome (PWS) arises from lack of gene expression in paternal chromosomal segment 15q11-q13 which can result from deletion (65–75 %), maternal uniparental disomy in chromosome 15 (20–30 %) or from a defect in genomic imprinting (~5 %). The diagnosis of Prader-Willi syndrome (PWS) is a challenge because several of the signs and symptoms overlap with those of other genetic syndromes, particularly during early childhood. The aim of this work was to examine the association between laboratory tests and the clinical criteria established by Holm et al. (1993) and Gunay-Aygun et al. (2001) for diagnosing PWS, and to assess the clinical criteria that are important when selecting patients for molecular screening of PWS. Forty-five patients suspected of having PWS were evaluated. Methylation-based PCR (MB-PCR) technique was used as well as GTG-banding, FISH and microsatellite analysis. Forty-five patients suspected of having PWS were evaluated, 13 were MB-PCR positive: 10/13 patients met both clinical criteria of Holm et al. (1993) and Gunay-Aygun et al. (2001), 2/13 met one or the other set of criteria, and one patient met none of the criteria. Short stature ($\chi 2=7.89$, $P=0.004$) and feeding problems ($\chi 2=4.70$, $P=0.03$) were the only clinical characteristics that differed significantly between patients with negative and positive MB-PCR results. Despite the small sample size of this study, our results indicate that the use of short stature and feeding problems (poor suck), rather than obesity and mental retardation, as screening criteria for subsequent MB-PCR testing, can increase the chances of detecting PWS.

1.P62

Genetic diagnosis of autism spectrum disorder (ASD) by array CGH.

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Autism comprises a clinically heterogeneous group of disorders—collectively referred to as features of impaired social relationship, impaired language and comunication, and repetitive behaviors or a narrow range of interests. Currently, an etiology can be identified for between 15 and 20 % of individuals with autism; in the others the cause remains unknown.

Actually, array comparative genomic hibridization (aCGH) has replaced high resolution chrmosome analysis as the test of choice for the evaluation of any child with (ASD). For that, we are implement the array CGH thecnique as first tier test for genetics studies of children with ASD.

In this study we present the results of array CGH obtained in 75 patients clinically diagnosed as ASD. The Nimblegen CGX Cytogenetic Microarrays platform, suplied by PerkinElmer, was performed.

From the total 75 patients, 59 (78,7 %) were males and 16 (21,3 %) females with a rate nearly 5 to 1 between males and females, similar to the rates described previously. Pathologycal results were obtained in 8 patients (10,7 %). When the study was performed by sex, we obtained pathologycal results in 5 males (8,5 %) and in 3 females (18,7 %), the higher proportion of pathologycal results in females when compared with males in our results, can be due to the small number of cases and can not be considered stadisticaly sibnificant.

Ours results with array CGH for genetic study of ASD are in concordance with the results previously obtained in other laboratorios and they are improved significantly the genetics diagnosis for ASD patients in our laboratory.

1.P63

Application of array comparative genomic hybridization in Hungarian patients with intellectual disabilities and multiple congenital anomalies

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Whole genom array comparative genomic hybridization (CGH) is recommended as first-tier test for the postnatal evaluation of individuals with intellectual disability, developmental delays, autism spectrum disorders and multiple congenital anomalies. Array CGH has increased the diagnostic yield of 15–20 % in clinical cytogenetic laboratories. In this study our aim was the application of array CGH in clinical diagnostics of patients with intellectual disability, multiple congenital anomalies and accompanying dysmorphic features. Cytoscan 750 K oligonucleotid array (Affymetrix) was succesfully implemented in a cohort of 18 patients. The identified copy number variants were confirmed by fluorescence in situ hybridization, multiplex ligation dependent probe amplification and G-banding. In 14 patients with normal karyotypes the array CGH did not revealed genomic imbalances. Clinically significant copy number variants (CNVs)were found in 4/18 patients with the size ranging from 0.9 to 15 Mb. Two patients had CNVs associated with known syndromes: 3q29 microduplication and 2q32q33 microdeletion syndrome. Two cases presented rare genomic aberrations: unbalanced subtelomeric translocation t(8;18)(p23.3;q22.3) causing partial deletion of 18q22.3 and duplication of 8p23.3; de novo unbalanced ins(8;6)(q22;q21q25) with multiple microdeletion on 6q and concomitant microdeletion of Xp22.1. All patients presented multiple congenital abnormalities. Our results further demonstrate the usefulness of chromosomal microarray as first-tier genetic test in the clinical diagnostics of patients with unexplained intellectual disabilities, developmental delays and dysmorphic features.

1.P64

CTNNA3 Deletions—Susceptibility to Autism Spectrum Disorders?

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Introduction: Autism spectrum disorders (ASD) are neurodevelopmental conditions showing extreme genetic heterogeneity. The CTNNA3 gene (alpha T-catenin) maps to 10q21.3 and codes for a protein with multiple cellular roles including cell adhesion. The CTNNA3 gene is one of the largest genes in the human genome and is located in a common fragile site (FRA10D). Fragiles sites are considered hot spots for genomic instability, associated with neuropsychiatric diseases including autism.

Material and Methods: 522 patients were analysed using the Agilent 4x180K microarrays and cytogenomics 2.9.2.4 software. The main clinical indications were intellectual disability, ASD, epilepsy, and multiple congenital abnormalities.

Results: Partial deletions of CTNNA3 were found in 7 of the 522 patients studied and were classified as VOUS. The CNVs sizes ranged between 23 Kb and 76 Kb with genomic intervals between 68,087,319 and 69,235,883 (GRCh37), involving exons 9 to 10, and intron 6, 11 and 13 of CTNNA3 gene. Additionally, 2 out of 7 cases showed other relevant CNVs that were reported as pathogenic or presumably pathogenic.

Conclusions: Recent studies from Bacchelli et al. (2014) showed that heterozygous exonic deletions in the CTNNA3 gene are not pathological however, homozygous or heterozygous compound exonic deletions/mutations affecting α-catenin function may contribute to ASD pathogenesis. Only 3 of 7 patients with CTNNA3 deletions presented ASD, all involving deletion of exons 9 and 10. Screening for additional mutations in CTNNA3 is warranted to elucidate the phenotype in these cases.

1.P65

Combined Wolf-Hirschhorn and Beckwith-Wiedemann syndrome in a girl with a 4p deletion and a 11p duplication: a recurrent double syndrome

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The co-occurrence of specific chromosome abnormalities, which are responsible for recognizable syndromes create phenotypic effects that more or less reflect and combine the characteristics of each individual one. Such mixed phenotypes may be particularly difficult to clarify in newborns and infants when the particular symptoms are not yet that distinctive. We present the case of a 6-week old girl with various such ambiguous abnormalities in whom a CytoScanHD SNP-array (Affymetrix®) analysis revealed a 3,98 Mb heterozygous deletion of telomeric 4p and a 3,4 Mb duplication of telomeric 11p regions. Such abnormalities are typically encountered in Wolf-Hirschhorn (WHS) and, depending on the parental origin of the duplicated segment, in specific forms of Beckwith-Wiedemann (BWS) or Russel-Silver syndrome (RSS), respectively. To distinguish between the later two options we performed a multiplex methylation-specific PCR analysis of the promoter regions of the H19 and KCNQ1OT1 genes that enabled us to quantify the copy numbers of the methylated and unmethylated alleles. This analysis revealed that the paternal allele was duplicated, which is a known but extremely rare cause of BWS. This result also implies that the girls unbalanced deletion/duplication abnormality may have been inherited from her father who presumably carries a balanced t(4p;11p). The respective analyses to verify this notion are currently underway. Our notion is further supported by the fact that the one or the other parent of 4 from 5 cases with an analogous unbalanced translocation and a mixed WHS/BWS or WHS/RSS phenotype reported so far was a balanced translocation carrier. The sixth case with such an unbalanced t(4p;11p) reported herein suggests that such recurrent abnormalities result from nonrandom breakpoint events the underlying predisposing genomic structure of which still needs to be determined.

1.P66

Complex chromosome rearrangement involving Xp21 band in a girl with a syndromic Duchenne muscular dystrophy.

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Complex chromosome rearrangements (CCR) are rare structural chromosome aberrations involving more than two breakpoints located on more than two chromosomes. Most of them are reported to be de novo. In such cases, CCR involve more breakpoints and more chromosomes than in familial cases. CCR can be found in phenotypically normal patients presenting, however, recurrent miscarriages or infertility. CCR can also be found in patients with phenotypic abnormalities due to a chromosome imbalance or to genes disruption at the breakpoints. Conventional karyotype generally allows their identification. However, molecular cytogenetic methods can reveal subtle rearrangements. We report, here, the identification of a de novo CCR involving chromosome X, 13 and 15 associated with a translocation between chromosome 6 and 11, in a girl presenting a delayed development and a symptomatic muscular dystrophy. FISH was performed to characterize this rearrangement, with use of probes that hybridize in DMD gene region. We found that DMD is disrupted by the breakpoint in Xp21.We also performed array-CGH that revealed no cryptic imbalance.

Duchenne muscular dystrophy usually affects males. However, females are also affected in rare instances. The majority of them have been associated with a skewed X-inactivation pattern. Of particular importance have been those in whom the manifestation of the disorder has been associated with de novo X;autosome translocations. Although these females carry two X chromosomes and therefore have two dystrophin gene loci, they manifest DMD because of a skewed X-inactivation pattern. Whereas one gene is disrupted by the translocation and lies on the active X, the other lies on the intact, inactive X chromosome. Here, our patient presented also a developmental delay probably due to the disruption of one or more other genes located in the multiple breakpoints.

1.P67

Characterization of a rare analphoid supernumerary marker chromosome in mosaic

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Analphoid supernumerary marker chromosomes (SMCs) are a rare subclass of SMCs C-band-negative and devoid of alpha-satellite DNA. These marker chromosomes cannot be identified unambiguously by conventional banding techniques alone being necessary to apply molecular cytogenetic methods in favour of a detailed characterization.

In this work we report an analphoid SMC involving the terminal long arm of chromosome 7, in 9 years-old boy with several dysmorphic features and severe development delay.

Cytogenetic analysis revealed a mosaic karyotype with the presence of an extra SMC, *de novo*, in 20 % of lymphocytes and 73 % of fibroblast cells.

FISH analysis with alpha-satellite probes for all chromosomes, whole chromosome painting probe for chromosome 7, and D7S427 and TelVysion 7q probes, allowed establishing the origin of the SMC as an analphoid marker resulting of an invdup rearrangement of 7q36-qter region.

Affimetrix CytoScan HD microarray analysis, redefined the SMC to arr[hg19] 7q35(143696249-159119707)×2~3, which correspond to a gain of 15.42 Mb and encloses 67 OMIM genes, 16 of which are associated to disease.

This result, combined with detailed clinical description, will provide an important means for better genotype-phenotype correlation and a more suitable genetic counselling to the patient and his parents, despite the additional difficulty resulting from being a mosaic (expression varies in different tissues). Analphoid SMCs derived from chromosome 7 are very rare, with only three cases reported so far. With this case we hope contribute to a better understanding of this type of chromosome rearrangements which are difficult for genetic counselling.

1.P68

Pallister-Killian syndrome caused by mosaicism of abnormal chromosome 12 but not isochromosome 12 p

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CASE: 22 month old baby was referred to a pediatrician for lack of independent ambulation. At 27 months he could free run.

MATERIAL AND METHODS: Cytogenetic analysis on patient's peripheral blood was performed, finding an additional material of unknown origin in the telomeric region of the short arm of chromosome 12. Whole chromosome painting was made.

Array CGH technique 60 Kb was performed to identify the origin of this material and genes involved.

Karyotypes from parents and healthy sister of the patient were analyzed.

RESULTS:

Karyotype: 46,XY,add(12)(p12)[7]/46,XY[7]dn. WCP: additional material of chromosome 12.

Parents and healthy sister had normal karyotypes.

CGH array : arr[hg19] 12913.33(230.421-697.269)×1,12p13.33p11.1(835.365-34.756.209)×3 Terminal deletion of 467 Kb in short arm of chromosome 12, 12p13,33 region, and interstitial region amplification of 34 Mb in 12p13,33-12p11,1 region. Both anomalies modify the structure and / or change doses of reference genes RefSeq, involved in diseases with OMIM number.

DISCUSSION: Interstitial amplification of 34 Mb identified on the short arm of chromosome 12 is associated with Pallister-Killian syndrome (OMIM: 601803).

Clinical descriptions of patients with a triplication of 12p show the following: newborns have a high weight and a high risk of hypoglycemia. Facial anomalies consist of a short neck, flattened face, anteverted nostrils and everted lower lip. The patients have severe mental retardation involving language and learning and have sleep and behavior disorders.

It should be noted that our patient has a duplication of 12 in a mosaic form.

DECIPHER describes two patients with overlapping duplication with our case who have feeding difficulties, coloboma, prominent nasal septum, micrognathia and tricuspid regurgitation.

ISCA describes two patients with mental retardation, psychomotor retardation and short stature.

Bibliography: De novo trisomy 12p in twin girls with different levels of mosaicism, Am J Med Gen A. 2013 Jul; 161A(7): 1702–5.

1.P69

Cytogenetic Approach to Male Infertility Diagnosis: the importance of a karyotype.

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Introduction: Infertility is a relatively common problem that affects about 15 % of all couples, with a male-factor identified in approximately half of the cases. Although numerous causes may contribute to this occurrence, genetic basis including chromosomal abnormalities and/or genetic syndromes may give rise to gene defects and therefore affect the chances of normal pregnancies. The examination of male infertility is complex, comprising detailed family history, physical examination, semen analysis, hormonal screening and genetic analysis of somatic cells.

Material and Methods: The study was carried out between 01.01.2013 and 31.12.2014, in 146 infertile males, referred to our Cytogenetics Unit for azoospermia, recurrent miscarriages, hypogonadism, infertility, oligoasthenospermia (OAT). Chromosomal analysis of peripheral blood lymphocytes was performed according to standard protocols.

Results: Among the 146 infertile men studied, 15 revealed constitutional chromosomal abnormalities: 11 cases of Klinefelter Syndrome (47,XXY); three with apparently balanced translocations [t(4;6); t(1;11); t(20;22)] and one mosaic (45,X/46,XY).

Conclusions: The present review showed the results and chromosomal abnormalities found in infertile men, using standard cytogenetic methods, during a period of 2 years. The anomalies were found in 10,2 % infertile males and the most common abnormality was Klinefelter Syndrome, which is in agreement with previously published data. The authors emphasise the importance of cytogenetic studies as a fundamental approach to evaluate male infertility and improve fertility prospects, particularly with the use of intracytoplasmatic sperm injection (ICSI) techniques and the support of appropriate genetic counselling.

1.P70

Discrepancies in results of standard cytogenetics and cytogenetic/molecular evaluation of patients with sex chromosome abnormalities

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The aim of our study was reevaluation of karyotypes in patients with sex chromosome abnormalities and/or Disorders of Sexual Development (DSD). We examined 453 individuals: 110 Turner syndrome (TS) and 93 Klinefelter syndrome, 19 46,XX/46,XY karyotype, 21 XX and 55 XY gonadal dysgenesis, 14 ovotesticular DSD, 56 46,XX-males, and 85 males with Y chromosome abnormalities and/or mosaicism.

Conventional karyotyping was done by standard technique. FISH with DXZ1, DYZ3, WCPX, WCPY and LSI SRY probes was performed on metaphase chromosomes and interphase nuclei of peripheral lymphocytes and buccal smears. Aneuploidy of 13, 18, 21, X and Y chromosomes and a chimerism were evaluated by QF-PCR for 20 autosomal and 5 gonosomal loci. Y-microdeletions were detected by mPCR for SRY, ZFX/ZFY and 20 Yq markers. X chromosome heterozygosity was evaluated by analysis of 13 X-chromosome loci.

The karyotypes were refined or redefined in 13.2 % patients. Mostly, it was due to hidden mosaicism and false positive detection of Y chromosome in the karyotype. The highest percentage of discrepancies was detected in XX/XY- and X/XY-mosaics (10.5–22.4 %), XX-gonadal dysgenesis (9.5 %) and TS (8.2 %). Markers were X- or Y-derivates (50 %:50 %) in TS

patients, and Y-derivate in all male carriers. SRY-negative amplification was found in 9 % XY-gonadal dysgenesis patients, 19 % 46,XX-males were SRY-positive. Cryptic X;Y translocations were found in (SRY+)XX-males and 46,X,del(X)(q22) female with DSD. Yq11.2 microdeletions or cytogenetically detected structural Y abnormalities were found in 40 % individuals with Y chromosome mosaicism, including polysomic cases, all of them were mosaics. AZF deletions were detected in all idicYp chromosomes, TS females with cryptic Y-mosaicism, and two-thirds of Y-derivative markers. There is overlapping of certain gonosomal abnormalities between some DSD forms mainly by sex chromosome rearrangements and/or mosaicism.

1.P71

An assessment of three cases with distal 15q duplication

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Patients with duplications of distal 15q have rarely been reported in the literature. A 15q25-qter trisomy syndrome, characterized by unusual features of prenatal overgrowth, tall stature, macrocephaly and craniosynostosis was proposed by previous authors (Zollino et al. 1999, Am J Med Genet 87:391–394). We report three new patients with a duplication of 15q23qter (46,XY,der(9)(9qter→9p22.1::15q23→15qter)). The first case was a fetus with abnormal ultrasonographic findings of ventriculomegaly and bilateral hydronephrosis. Although conventional cytogenetic and molecular cytogenetic analyses from amniocytes revealed the abnormality, the parents decided to continue the pregnancy. Postnatal examination of the child revealed dysmorphic findings as asymmetric face, hypertelorism, prominent philtrum, cleft palate. The second case, a 4-year old girl, was referred because of her dysmorphic features including down slanted palpebral fissures, flat nasal bridge, microretrognathia and cleft palate. The third case, a 6 year old girl, was referred because of suspicion of an overgrowth syndrome. The physical examination revealed a flat nasal bridge, long and pointed chin, long and big hands and feet. However, the clinical features of the case were not consistent with an overgrowth syndrome. The unbalanced karyotypes of the first and second cases resulted from a balanced t(9;15) translocation in the mothers who were from the same village. The unrelated father of the third case from a different city was a carrier of the same translocation. The breakpoints of the rearrangements were determined by array-CGH and FISH analyses. None of these patients had craniosynostosis or overgrowth as previously reported.

1.P72

Molecular and clinical delineation of the 2p15-16.1 microdeletion syndrome and proposal of a new candidate gene for microcephaly

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Interstitial 2p15-p16.1 microdeletion is a rare syndrome previously reported in 14 patients to our knowledge. It is characterized by moderate to severe intellectual disability, autism spectrum disorder, short stature, dysmorphic features, structural brain anomalies, congenital organs defects and microcephaly in some cases. It is considered a contiguous gene syndrome involving deletion of several genes. Deletions overlapping this region previously reported are variable in size (from 203 kb to 6.9 Mb) with non recurrent breakpoints. We report here three additional patients with a 2p15p16.1 microdeletion identified by SNP-array analysis (Cyto12-SNP, Illumina) or CGH-array (Agilent 8x60K): one prenatal case and two postnatal cases. The prenatal and one of the postnatal cases shared an overlapping 2p15p16.1 deletion of 105 kb (chr2:61671686-61777241, hg19) including only XPO1 and the distal end of USP34. Both patients presented features overlapping the clinical

spectrum of the previously described 2p15p16 microdeletion syndrome including mild intellectual disability in the postnatal case, dysmorphic facial features and brain structural abnormalities. We confirm that one or both genes are probably involved in facial dysmorphic features, cognitive impairment and brain structural abnormalities observed in the syndrome.

The third patient we report on is a 4 years-old male with an heterozygous de novo 427 kb deletion at 2p16.1 (chr2:60624940-61051867, hg19) containing BCL11A and PAPOLG and a phenotype characterized by speech delay, autistic traits, stereotyped behavior but without microcephaly.

Considering previous reported deletions in the 2p15p16.1 region and our three new cases, we precise the genotype-phenotype correlation of the microdeletion syndrome. Moreover, as the REL gene is the only gene shared by all patients having a microcephaly, we suggest that this gene could be considered as a candidate gene for microcephaly associated in the syndrome.

1.P73

Genomic imbalances identified by array CGH in cases with chromosomal mosaicisms

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At present, array CGH-based techniques are widely used in clinical genetics because they are extremely powerful for the delineation of chromosomal breakpoints, identification of new microdeletion/microduplication syndromes, characterization of extra SMC (supernumerary marker chromosome), derivative chromosomes and uncovering genomic variations in healthy and disease associated population. Furthermore, the sensitivity of array CGH has been documented to detect placental and somatic genomic mosaicism. Since the application of this method is performed on genomic DNA extracted directly from uncultured tissues or unstimulated peripheral blood cells, the detection of low-level mosaicism, missed by conventional methods, is more likely and the percentage rate of the cell lines included are undistorted.

From our experience, we present three cases of mosaicism, two prenatal and one postnatal, that array CGH has facilitated to 1) explicate two abnormal cell lines placental mosaicism, 2) identify the origin of a SMC in mosaicism and 3) reveal the existence of a second normal cell in a clinical case with moderate signs of Williams Syndrome.

BAC array-CGH (Illumina Cytochip Focus Constitutional) was applied and the data were analysed by BlueFuse Multi software.

Intercellular variation due to chromosomal mosaicism is frequently hidden and is likely to be involved in the genetic diversity and human pathogenic conditions. Until recently, due to technical difficulties, the significance of chromosomal mosaicism remained under investigation. Today, molecular cytogenetics has the potential to detect low-level mosaicism and thus, future studies can provide new insight for the uncovering of the role of chromosomal mosaicism in human diseases that will assist in more informed genetic counseling and patient management.

1.P74

Microdeletion of 2q24.2 region: clinical and molecular analysis based on a new case

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Microdeletion of 2q24.2 is a rare genomic disorder. To date, 12 comparable submicroscopic deletions involving this region have been described. Although this chromosomal aberration is associated with a heterogeneous phenotype, some clinical traits (intellectual disability, developmental delay, hypotonia, joint hyperlaxity, intrauterine growth restriction, and facial dysmorphy) seem to be recurrent.

Here, we report on the case of a 4-year-old girl referred for genetic counseling because of psychomotor retardation, speech delay, heart defect (atrial septal defect), and dysmorphic features (plagiocephaly, high forehead, short and upslanting palpebral fissures, hypertelorism, dysplastic low-set ears, short nose with flat nasal bridge, long philtrum, high palate, micro-retrognathia). The pregnancy was complicated by maternal infection of the upper airways in the first and third trimester and intrauterine fetal hypotrophy. In the neonatal period, the patient presented with feeding problems and poor weight gain. At the clinical examination, hypotonia, joint laxity, long fingers and toes as well as abnormal position of the anus were observed. Ophthalmologic issues included post-inflammatory changes on the fundus of the right eye and strabismus (surgically corrected). The girl showed poor social interactions and lack of eye contact.

Whole-genome oligonucleotide microarray analysis revealed a 4.93 Mb deletion of 2q24.1q24.3 region (chr2:159,684,613–164,611,097; hg19) encompassing 19 OMIM genes; some of them (RBMS1, PSMD14, TBR1, SLC4A10, DPP4 and KCNH7) could be strong candidate genes for the genotype-phenotype relationship.

In conclusion, this study contributes additional information for the 2q24.2 microdeletion. Our results also confirm that haploinsufficiency of genes within the deleted region might play a role in the etiology of this genomic disorder. Moreover, the clinical heterogeneity in the patients harbouring this chromosomal syndrome could be explained by incomplete penetrance or variable expressivity.

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1.P75

Diagnostic utility of arrayCGH in epilepsy and autism—Slovenian experience

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Technical advances in genetic diagnostics have finally enabled us to analyse the entire genome at once and identify disease-associated copy number variants (CNV). Epilepsy and autism spectrum disorders (ASD) are the two most prevalent subgroups of neurodevelopmental disorders. By twin and family studies it was shown that an important part of occurence of both disorders can be explained by genetic factors and up to date, numerous genes and some microdeletions/ microduplications have been linked to each of the 2 disorders. Structural variants play a significant role in 5–10 % of ASD or epilepsy cases. If there are additional clinical findings, these percentages are even higher. Using array comparative genomic hybridization (CGH) we analysed 170 children with epilepsy (n=80) and ASD (n=90). In our cohort, we found a patogenic CNV in 5/90 (5.6 %) ASD cases and 12/80 (15 %) epilepsy cases. Additional 11/90 (12.2 %) ASD cases and 4/80 (5 %) epilepsy cases had CNV classifed in the group of variants of unknown significance. The diagnostic yield is greater in the group of patients where epilepsy co-occur with additional clinical features, such as developmental delay, congenital anomalies and unexplained epileptic encephalopathy and it has been reported to be as high as 20–30 % (1,2). Indeed, in 10/12 of our patients with reported pathogenic CNVs, epilepsy was accompanied by developmental delay.

Interestingly, some copy number variations are risk factors for both epilepsy and ASD. For example microdeletions in 15q11.2, 15q13.3, and 16p13.11, described in epilepsy patients, also confer a risk for autism (and for other neurodevelopmental disorders, as well). We found all three CNVs in our epilepsy patients and none of them in ASD group. Our results add to growing evidence of expected discovery rate and important non-recurrent CNVs in both groups of patients.

1.P76

A new chromosomal rearrangement resulting in Axenfeld-Reiger syndrome

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Axenfeld -Rieger syndrome (ARS) is a developmental disorder that associates eye abnormalities and multiple congenital malformations. An abnormal migration of neural crest cells results in dysgenesis of the anterior segment of the eye. Other manifestations are mild dysmorphic features, dental, cardiac and umbilical anomalies. ARS is inherited in an autosomal dominant manner with high penetranceof FOXC1 and PITX2 mutations or deletions. However, over 50 % of cases remain of unknown cause.

Herein, we report an ARS associated with epilepsy in a two year old boy. Clinical examination found some dysmorphic features and growth delay. Ophthalmologic examination revealed a bilateral corectopia, irishypoplasia and a bilateral embryotoxon, consistent with ARS eye disorders. Whole genome microarray analysis using Nimblegen 135 K array showed a 4.55 Mb de novo deletion on 4q25q26 bands (nt 112,009,675 to nt116,558,399 bp – hg18). The deleted region encompasses 9 OMIM genes and its distal boundary is located 230 Kb upstream of PITX2. In Silico Search we found out the presence of regulatory sequences of PITX2 in this region. PCR analysis confirmed the deletion of these sequences. This is the first case of ARS resulting from a deletion of PITX2 gene regulatory sequences. Further studies are carried out to assess the disruption of PITX2 expression

1.P77

48,XXYY syndrome: case report

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Introduction: 48, XXYY syndrome, which affects 1 in 18,000 to 1 in 50,000 neonates, is a numerical chromosomal abnormality associated with meiotic nondisjunction during maternal or paternal gametogenesis and advanced age. This syndrome is characterized by facial dysmorphism, congenital malformations, mental deficiency and severe behavioral and psychiatric disturbances. The increased stature of affected individuals and the presence of hypogonadotropic hypogonadism led to this condition being considered a variant of Klinefelter syndrome, although both are now recognized as distinct conditions. In this report, we describe a case of 48,XXYY and the paternal origin of the extra chromosomes.

Case report: First child of a young, nonconsanguineous couple. Maternal depression during pregnancy. Post-term cesarean section with perinatal hypoxia. The child showed neurophyschomotor delay, learning deficiency, convulsions and autism spectrum disorders. Large stature, macrocrania, no eunuchoid habitus, dysphemia. Testicles 10 mL (p50), Tanner stage III pubertal development. Karyotype 48,XXYY, with XYY of paternal origin. Normal hormone levels. No fragile X chromosome.

Discussion: Aneuploidies of paternal origin are less common and are usually associated with advanced age. Mental deficiency and behavioral disturbances, the most important characteristics seen here, are attributable to the 20 % of genes on the X chromosome that escape inactivation and/or gene imprinting in maternal/paternal chromosomes. Unlike Klinefelter syndrome, the increased stature was not caused by primary hypogonadism. This case highlights the importance of investigating the paternal origin of extra chromosomes and the mechanism involved in aneuploidy in order to establish a prognosis and provide appropriate genetic counseling.

1.P78

Cytogenomics Array Database (CAGdb)—a useful tool for cytogeneticists

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The Cytogenomics Array Group (CAG) began as an informal network of colleagues formed to exchange

information between laboratories regarding constitutional microarrays and rare case findings. In order to facilitate this exchange, we created a web-accessible database (CAGdb) to house each laboratory's abnormal cases, which could then be freely shared in a de-identified fashion with all participating laboratories. CNV details, interpretation comments, parental and other follow-up data, and clinical features (all fully stripped of patient identifiers) are collected and shared for each case in this site. This database provides a useful laboratory tool to track experience with in-house microarray cases, network with colleagues, and to share experience with rare findings. Additionally, this resource is meant to facilitate submission to other larger-scale data collections in order to aid in syndrome discovery efforts and make information on rare cases more broadly available.

1.P79

Cytogenetic and Molecular Studies of Sudanese Patients with Disorders of Sexual Development

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Disorders of sexual differentiation occur in 1–2 of every 10,000 births; these present in several forms e.g. male and female pseudohermaphroditism, gonadal dysgenesis, and true hermaphroditism. The present study aimed to explore and characterize cytogenetics, molecular alterations, hormonal profile, and clinical aspects of different types of intersex in Sudan. This study comprised 70 patients: 18 raised as males, 52 as females. Medical history, physical examination, culturing of lymphocytes, chromosomal analysis, PCR, hormonal profile, ultrasonography, laparoscopic examination when required, histopathology, and psychological assessment were done. Most patients were above the age of 17 years (40 %) followed by patients younger than 2 years (33 %). The most affected Sudanese tribe was Jaaliyin tribe (19 %). Half of the patients were referred for genital ambiguity. Other complaints like primary amenorrhea, failure to thrive and short stature were reported. Thirteen patients (19 %) were satisfied with their sex of rearing, 5 patients (6 %) were not satisfied, 16 patients (23 %) could not decide; 36 patients (51 %) were children. Final diagnosis was reached in 55 patients: 23 patients diagnosed as male pseudohermaphrodites, 2 as testicular feminization syndrome, 14 as complete gonadal dysgenesis, 8 as female pseudohermaphrodites, 6 as true hermaphrodites, 1 as Smith-Lemli-Opitz syndrome and 1 Down syndrome, 15 patients were still undiagnosed by the end of the study.

As conclusion, multidisciplinary approach remains the best for management of such cases. Midwives should be well trained. Circumcisions of intersex patients should be prohibited. Most of the patients were of low social class thus a non-profit body should shoulder this responsibility.

2. Tumor Cytogenetics

2.P1

Improved chromosomal abnormality detection rates in patients with multiple myeloma by karyotyping, fluorescence in situ hybridization and chromosome microarray analysis using plasma cell enrichment

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Aim: To ascertain the efficacy of utilising enriched plasma cells (PCs) for analysis of bone marrow (BM) samples of patients with morphologically-confirmed Multiple Myeloma (MM)

Materials and methods: Sixty-three diagnostic BM samples were analysed by FISH, 59 by karyotyping and 20 by CMA. The FISH assay employed a 9-probe panel comprising FGFR3/IGH, CCND1/IGH, MAF/IGH, RB1, TP53, 1q21, and centromeric probes for chromosomes 9, 11 and 15. The CMA assay utilised a 4x180K chip (Agilent SurePrint G3 Cancer CGH+SNP). PCs were enriched by antibody complexes linked to targeted PCs to the magnetic particles (EasySep™/RoboSep™, STEMCELL™) and used for FISH (enriched-FISH) and CMA (enriched-CMA).

Results: By karyotyping, only 32 cases (54.2 %) were abnormal. With FISH performed on whole BM (standard-FISH), 53 cases (84.1 %) were abnormal. With enriched-FISH, 61 cases (96.8 %) were abnormal. Fourteen cases were analysed by standard-CMA with 9 being abnormal (64.3 %). However, of the 12 enriched-CMA that were performed, 100 % were abnormal.

Discussion: Karyotyping yielded a modest abnormality detection rate owing to the indolent nature of PCs in culture. The FISH assay is independent of cell division and consequently a much higher abnormality rate was obtained. However, enumeration of signals from whole BM invariably includes the scoring of non-PCs. When PCs were enriched for FISH, the abnormality rate was further improved. The abnormality rate obtained by standard-CMA on whole BM was only 10 % better than karyotyping. This was due to the lower limit of assay detection involving mosaicism <20 %. Indeed, when CMA was performed on enriched PCs, the abnormality detection rate reached 100 %.

Conclusion: This study reaffirms the importance of utilising enriched PCs for FISH and CMA assays in patients with MM.

2.P2

Extraskeletal Ewing sarcoma of the parapharyngeal space with a unique translocation, t(19;22)(q13.4;q12.2)

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Ewing sarcoma (ES) is a group of small round blue cell neoplasms comprising the second most frequent primary bone malignancy in children and young adults. Around 15 % of ES is estimated to arise in extraosseous sites. Approximately 90 % of ES demonstrate the translocation t(11;22)(q24.1;q12.2) creating a fusion gene between EWSR1 and FLI1. Other translocations are rare and mainly described as fusions between EWSR1 or EWSR1-related genes.

We present a case of a rapidly enlarging mass of the left parapharyngeal space, located in the left carotid space, displacing the carotid bifurcation anteriorly. The patient was taken to the operating room for excision of the mass and neck dissection.

Histopathological examination revealed a small blue round cell neoplasm with multiple foci of perineural invasion involving the sympathetic chain, and metastasis to two cervical lymph nodes at levels 2 and 3. The tumor was strongly positive for S100 and focally positive for synaptophysin and O13 (CD99). The chromosome analysis of the resected tumor showed a t(19;22)(q13.4;q12.2) confirmed by fluorescence in situ hybridization analysis using ZNF443 (19p13.13), CRX (19q13.3) and the EWSR1 (22q12.2) locus specific unique sequence DNA probes. The patient was treated with chemotherapy followed by autologous stem cell transplant. To our knowledge, this is the first extraskeletal ES exhibiting this translocation.

Rare translocations have the potential to aid in the diagnosis and are valuable for correct tumor classification and therapeutic purposes. Although t(11;22)(q24.1;q12.2) anomaly is the hallmark of ES, the fusion product of EWS-FLI1 by itself does not transform human cells and other mutations may appear to be necessary to understand the cell origin of ES. Further studies as well are needed to determine the importance of this chromosome band involvement in ES and other malignancies.

2.P3

Four genetic lymphoma-specific events (MYC, BCL2, BCL6 and CCND1) identified in a B high grade lymphoma case

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The term « double/triple hit lymphoma » depicts rare and aggressive lymphomas displaying either BCL2 and/or MYC and/or BCL6 gene rearrangements. To our

knowledge, these rare lymphomas have been described only twice with four specific genetic events (MYC, BCL2, BCL6, CCND1). Here we describe the third observation. A 79 years old patient suffering from paraesthesias was admitted for polyneuritis in a context of poor general condition. Clinical examination showed the presence of many scattered nodes and skin infiltration. A skin arm nodule biopsy revealed large proliferating cells (CD10-, CD20+, CD23-, BCL2+, BCL6+, Ki67 80 %) consistent with a DLBCL, NOS. Blood cell film examination showed the presence of pathological lymphocytes suggestive of a high grade lymphoma. The flow cytometry (CD5 dim, CD10-, CD19+, CD20+, FMC7+, CD22+, CD23-, CD148+) may be in favour of pleomorphic mantle cell lymphoma (MCL) variant diagnosis. Blood cytogenetic study found a complex hyperdiploid karyotype (47 chromosomes) with a t(3;22)(q27;11) translocation (BCL6/IGL), structural abnormalities of a chromosome 8 resulting in juxtaposition of 5'MYC and BCL2, a derivative chromosome 18 from a t(14;18)(q32;q21) translocation (IGH/BCL2), and a t(11;14)(q13;q32) complex translocation (IGH/CCND1). Overexpression of cyclin D1 was detected in the white blood cells and in the skin lesion. The patient was treated with a chemotherapy combining rituximab, ifosfamide, cytosine arabinoside and intrathecal methotrexate and died 4 months after the diagnosis. This third case of quadruple hit lymphoma underlines the complexity of the classification of such aggressive malignancies, particularly DLBCL with expression of cyclin D1, CCND1 chromosomal rearrangement and CD5 positivity. Some suggest the existence of a "gray zone" in which morphologic, clinical and genetic features are insufficient to segregate these lymphomas between blastoid MCL and cyclin D1-positive DLBCL.

2.P4

Molecular cytogenetic analysis of important prognostic genetic markers among pediatric patients with neuroblastoma in Slovakia.

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One of the most common solid tumors in young children is neuroblastoma. It is a typical example how important role play genetic aberrations in determining the status, disease progression, in discovery of new treatment options and generally overall patient survival. Non-random genetic changes associated with neuroblastoma are important prognostic factors and also they are an integral part of disease diagnosis.

Since 2008 untill 2014, we have analyzed 80 samples of pediatric patients with age between 1 month to 18 years (median=21 months) with diagnosis of ganglioneuroblastoma and neuroblastoma. Samples were collected from three children's cancer centers within Slovakia. The primary samples were native or formalin-fixed paraffin-embedded (FFPE) tumor tissues and also the cultures of bone marrow. We proceed according to International Neruroblastoma Risk Group (INRG) Convention and we have focused our analysis on the main independent prognostic factors—status of MYCN (2p24) oncogene, tumor cell ploidy and segmental chromosomal aberrations (SCA) as loss of 1p, 11q and gain of 17q. All samples were analyzed with I-FISH and they were compare with MLPA results. Our primary goal was to monitor the occurrence of above mentioned selected genetic aberrations in correlation with age and available clinical data.

This is the first major study of genetic markers of neuroblastoma in Slovakia.

2.P5

Bone marrow failure conditions due to chromosome anomalies

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Two children are reported with bone marrow (BM) failure but without a precise diagnosis. Patient n. 1 is a 3-year-old female with a severe anaemia associated with several dysmorphic/malformative symptoms. The standard karyotype was normal, but array-CGH showed a 4 Mb duplication of chromosome 1 short arms of and a 2 Mb deletion of chromosome 8 long arms. The anomaly was constitutional. The lacking region of chromosome 8 included the locus RUNX1T1 which is relevant in transcription repression of several genes with a role in myelopoiesis. The expression of RUNX1T1 in the BM was down-regulated. We postulate that the chromosome imbalances are the cause of BM failure and severe anaemia, besides of all extra-haematological signs.

Patient n. 2 is a female of less than one year of age with severe pancytopenia, in absence of other symptoms. The karyotype showed a constitutional change that had been already diagnosed prenatally, and that was interpreted as a balanced translocation t(2;8). No imbalances were disclosed by array-CGH. FISH with libraries and a number of BAC probes showed that the rearrangement was in fact an insertion of two adjacent portions of chromosome 2 short arms into the long arms of chromosome 8. The RUNX1T1 gene was intact, but included in the portion between the two insertions of material from chromosome 2. The expression of RUNX1T1 in BM was highly up-regulated, probably by position effects, and this was interpreted as the cause of the pancytopenia.

The two cases reported here, together with other similar ones already reported by our group, demonstrate that a possible and not infrequent cause of BM failure is a chromosome anomaly, both balanced or unbalanced, either constitutional or acquired.

2.P6

Chronic lymphocytic leukemia with 8q24 aberrations: a distinct disease subgroup?

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Cytogenetic aberrations have important prognostic implications in CLL. Abnormalities of chromosome 8q24 are rare and their prognostic significance remains unclear. In a series of 668 patients with CLL successfully karyotypes in our Department over the last 12 years, 8 (1.2 %) carried 8q24 aberrations. These were 7 males and 1 female with a median age of 68 years (range 38–80). Binet stage at diagnosis: A-4/B-2/C-1 (unknown, 1). Seven cases had a typical CLL immunophenotype, while the remaining case was considered as atypical. Six and 2 cases carried IGHV-unmutated and mutated genes, respectively. In all cases, conventional cytogenetic analysis was performed prior to treatment; in 6/8 cases, this analysis was conducted at diagnosis, while one case was studied at both diagnosis and disease progression. Six cases carried complex karyotypes including 8q aberrations; one case carried t(8;22)(q24.1;q11); the remaining case showed normal karyotype at diagnosis but developed an abnormal clone at progression showing a t(8;13)(q24;q14). Four cases had involvement of chromosome 17p aberrations and two del(11q) as detected by classical cytogenetic study and confirmed with FISH. Deletion 13q14 was detected by FISH in 6/8 cases; 3/6 carried monoallelic deletion, 2/6 carried biallelic deletions and one carried monoallelic and biallelic deletions. Amplification of the c-myc oncogene was found in 3/4 cases using a MYC break apart rearrangement probe; the remaining case showed one split signal because of translocation. After a median follow up 48 months (range 17–154) six of seven patients with available data progressed and two of them died. In summary, abnormalities 8q24 are rare and associated with complex karyotype and poor prognosis.

2.P7

Prognostic significance of unsuccessful cytogenetics at the time of diagnosis in acute myeloid leukaemia

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Diagnostic karyotype is one of the most powerful independent prognostic factors in acute myeloid leukemia (AML). Conventional cytogenetic analysis is unsuccessful in ~10 % of AML patients. The prognostic significance of unsuccessful cytogenetics (UC) or unperformed cytogenetics (UPC) on the outcome of AML patients remains unclear. Recently, two studies showed that UC predicts for poor outcome. Between 1998 and 2013, 393 patients below 74 years of age with AML received intensive treatment. The frequencies of UC and UPC were 4,6 and 3 %, respectively. No significant differences were noted in the baseline characteristics between the patients with UC and complete cytogenetic analysis. The early death rates differed between the cytogenetic subgroups ($p<0.001$) with the highest rates in patients with UC (11 %) followed by high-risk (HR) AML, intermediate risk (IR), favorable risk (FR) and UPC. However, the complete remission rate was 55,55 % in UC and 66 % in UPC and was the same compared with the patients in the successful karyotyping group (66,6 %, p=ns) and especially with IR AML (68,5 %). The 5-year overall survival(OS) rates were 27,8 % for UC and 33,3 % for UPC, whereas the corresponding rates for FR, IR and HR AML patients were 66,3, 25,9 and 6,2 %, respectively ($p<0.001$). The probability 5-year OS was 85 % in UC patients who received allogeneic hematopoietic cell transplantation(HCT) vs 0 % in UC patients without HCT. Higher relapse rates and shorter disease free survival (DFS) was observed at the subgroups of UC and HR cytogenetics ($p<0.001$). In summary, the patients in UC subgroup have higher rates of early death and shorter DFS, thus UC should be considered a high-risk feature. Intensive treatment such as allo-SCT could change the poor outcome.

2.P8

Monosomy 7 and ring/marker chromosome derived from chromosome 7 in a patient with MDS-RAEB-2: A case report

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The most frequently observed cytogenetic abnormality is monosomy 7 or deletion of chromosome 7 in MDS. But very few MDS cases with ring chromosome 7 have been reported. In this case report; a 30-year old woman with the history of blood transfusion was referred to hospital of Akdeniz University with complaining about weakness. Peripheral blood count showed anemia and thrombocytopenia. A bone marrow (BM) biopsy was performed to patient because of unclarified anemia and thrombocytopenia. The BM biopsy showed decrease in granulocyte series and abnormal localization, was reported as normocellular BM with dysmegakaryopoiesis. BM myeloblast percentage was determined 14,3 % by flow cytometry immunophenotyping. Chromosomal analysis revealed 46,XX,ring(7)(p22q22)[7]/46,XX,-7,+mar(7)(p13p22?)[7]/46,XX[23]. FISH analysis results indicated that marker chromosome was derived from the chromosome 7 with deletion of 7q22 and 7q31 locus. The patient was diagnosed as MDS-RAEB-2 (IPSS:3,highrisk). AML induction type-chemotherapy was initiated (7+3, 100 mg/m2 ARA-C, 12 mg/m2 Idarubicin). Minimal residual disease (MRD) was determined as 5,3 % at BM sample on the 28th days after treatment. After therapy, cytogenetic analysis revealed 46,XX,del(7)(q22)[5]/46,XX,-7,+mar(7)(p13p22?)[2]/46,XX,ring(7)(p22q22)[1]/46,XX[4] and confirmed by FISH. HIDAC treatment regimen was initiated for re-induction therapy and MRD was determined as 3,5 % in patient. The patient was than treated with 1 cycle of azacitidine for consolidation therapy. The decision of allogeneic hematopoietic stem cell transplantation was received to patient from a HLA full compatible sibling donor and initiated a myeloablative therapy regimen. Cytogenetic analysis revealed 45,XX,-7[6]/46,XX[9] and monosomy 7 was also detected in 31 % of interphase nuclei by FISH. The treatment process of patient is still continued at stem cell transplantation unit. To our knowledge,our patient is the fourth MDS patient having ring7 in the literature.

2.P9

Identification of two novel fusion partners of the MECOM gene in acute myeloid leukemia

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In acute myeloid leukemia (AML) patients, the MECOM (MDS and EVI1 complex locus) gene, located at chromosome band 3q26, can be rearranged with a variety of partner chromosomes and partner genes. Here we report two rare cases of chromosomal translocations involving MECOM – t(3;10)(q26;q21) and t(3;6)(q26;q25) – found in two patients with de novo AML.

Our aim was to identify the fusion partners on chromosomes 10q21 and 6q25 and to characterize the precise nucleotide sequence of the chromosomal breakpoints, enabling us to design a clone-specific real-time PCR assay for sensitive minimal residual disease (MRD) monitoring in these patients.

Karyotypes were investigated by molecular-cytogenetic techniques (mFISH/mBAND), and involvement of the MECOM gene was confirmed by FISH with the use of a commercially available probe set. The derivative chromosomes were isolated using fine-needle microdissection followed by whole genome amplification (WGA). Amplified chromosomal fragments were sequenced on the GS-Junior next-generation sequencing platform. The reads obtained were aligned to reference sequences of the respective chromosomes using in-house developed software. The last mapped reads from both chromosomes were used as docking sites for primers for long-range PCR to amplify the putative breakpoint. The long-range PCR products were directly sequenced using Sanger sequencing to reveal the precise nucleotide sequence of the breakpoint.

Using a combination of cytogenetic and molecular approaches, we mapped both translocations to the single nucleotide level, revealing a fusion of the MECOM gene (3q26.2) and C10orf107 (10q21.2) in t(3;10) and LOC101928923 (6q25) in t(3;6).

The procedure described here enabled us to identify two previously unreported fusion partners of the MECOM gene as well as to perform real-time PCR-based monitoring of MRD.

2.P10

Targeted arrayCGH for determining the deleted region on chromosome arm 6q in chronic lymphocytic leukemia

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Background: Cytogenetic abnormalities have improved the current chronic lymphocytic leukemia (CLL) risk stratification. 6q deletion is known to occur at a relatively low frequency in CLL (3–6 %) and its prognostic significance remains controversial, as is the extent of deletion and the candidate genes in the deleted region.

Aim: The objectives were to analyze a group of CLL patients with chromosome 6q deletion using arrayCGH, determine the minimally deleted region (MDR) and the candidate genes located therein, attempt to determine their relative gene expression, and evaluate the clinical characteristics.

Methods: Peripheral blood/bone marrow samples from a group of 1147 CLL patients in three haematology centers in the Czech Republic (Olomouc, Plzen and Brno) were analyzed using conventional cytogenetics and FISH. ArrayCGH with chromosome 6 specific and oligonucleotide microarrays (Agilent, Santa Clara, USA) and gene expression of FOXO3, NF-kB, TBX21, IL-2 and BCL10 were assessed by quantititative RT-PCR.

Results: 6q deletion was found in 87 (7.58 %) patients, arrayCGH was applied in 69 patients. MDR of 1.4 Mb was determined in the 6q21 region spanning eight genes (FOXO3, SOBP, SCML4, SEC63,

OSTM1, NR2E1, LACE1, and ARMC2). Expression profiling of FOXO3 revealed lower number of FOXO3 transcripts in CLL patients with 6q deletion than in those without 6q deletion ($p=0.03$) and healthy subjects ($p<0.0001$). Clinical evaluation of the deletion showed that 6q deletion was more prevalent in males, the patients had unmutated IGVH and more advanced clinical stages (Binet B and C).

Conclusion: Our study confirmed the low frequency of 6q deletion, determined the MDR with low expression of the FOXO3 gene and higher occurrence of 6q deletion in patients with unfavorable clinical course.

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2.P11

Comparison between cytogenetics, FISH and a-CGH in CLL evaluation

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Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous B-cell lymphoid neoplasm, where the main clinical relevant genetic lesions consists in chromosome gains and losses.

The SurePrint G3 Cancer CGH+SNPs 4x180K platform (Agilent Technologies) was used to study 25 CLL patients, in addition to conventional cytogenetics and FISH with the FDA-approved panel including probes specific for ATM and TP53 genes, D13S319(13q14)/LSI13q34 loci and chromosome 12 centromere (Abbott).

Genetic alterations were detected by cytogenetics, FISH and a-CGH in 72, 72 and 92 % of the cases, respectively. Conventional cytogenetics detected 2 balanced translocations and 1 inversion, not revealed by a-CGH. An overall concordance of 92 % was observed between FISH and a-CGH results. In 2 cases the D13S319 (13q14) deletion detected by FISH was not revealed by a-CGH probably due to the low percentage of deleted cells (<10 %). Two cases were normal with the three methods. FISH detected 13q14 deletion, encompassing DLEU gene, in 28 % of patients and a-CGH showed in 42.8 % that the deletion comprised also the RB1 gene, known to be associated to a worse prognosis.

The most recurrent anomalies identified only by a-CGH with a proved or possible prognostic significance included losses of 3p26.3-p25.3, 6q21, 9p21.3, 10q22.2-q26.3, 14q24.2-q32.33 and 15q14-q15.1. LOH was observed at 5q23.3 and 20q12-q13.2 both in 8 % of the cases.

Based on our preliminary results, we would suggest to perform a-CGH and conventional cytogenetics that can assure the identification of balanced rearrangements with a diagnostic significance. Moreover, when a-CGH turn to be normal, FISH should be performed, at least for TP53 deletion associated to the worst prognosis, possibly present in a very low percentage of tumor cells.

2.P12

The ASXL1 gene alterations in patients with 20q deletion

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Deletion 20q is a recurrent abnormality observed in myeloid disorders. In our previous study we showed a fusion of the ASXL1 and TSHZ2 genes resulting in an isochromosome of a deleted 20q in a patient with myelodysplastic syndrome. The aim of this study was to determine the frequency of ASXL1 alterations in del(20q) cases.

Fluorescence in situ hybridizations (FISH) with locus specific probes for 20q11 and 20q12 regions (Abbott

Molecular, Kreatech Diagnostics) confirmed the cytogenetically observed deletions of 20q in a cohort of 25 patients with myeloid malignancies. Metaphase FISH mapping with set of 7 bacterial artificial chromosome (BAC) probes (BlueGnome) distributed in 20q11.21 and 20q13.2 was used for determination of the breakpoints. Array comparative genomic hybridization (CytoChip Cancer 180 K, BlueGnome) was performed on DNA samples of bone marrow cells of 14 patients with suspected ASXL1 gene deletion to find out the gene copy number variation.

A weak signal of RP11-358N2 BAC probe (20q11.21) suggesting the proximal breakpoint of the deletion in the ASXL1 gene was observed in 8 patients (32 %). In 7 patients (28 %) the signal of this probe was not present on the derivative chromosome confirming the ASXL1 gene deletion. In the remaining 10 patients the proximal breakpoint of the deletion was determined bellow the ASXL1 gene. The distal breakpoint in the TSHZ2 gene was found in one patient only.

In our study, ASXL1 gene was altered in 60 % of patients with 20q deletion. Mutations of the ASXL1 gene are associated with a poor clinical outcome and determination of the ASXL1 gene alteration in del(20q) cases may have a prognostic impact. The relations with clinical data should be studied in a larger cohort of patients.

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2.P13

5′RUNX1-3′USP42 chimeric gene in acute myeloid leukemia can occur through an insertion mechanism rather than translocation and may be mediated by genomic segmental duplications

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The runt-related transcription factor 1 (RUNX1) gene is a transcription factor that acts as a master regulator of hematopoiesis and represents one of the most frequent targets of chromosomal rearrangements in human leukemia.

The t(7;21)(p22;q22) rearrangement generating a 5′ RUNX1-3′USP42 fusion transcript has been reported in two cases of pediatric acute myeloid leukemia AML) and further in eight adult cases of myeloid neoplasms. We describe the first case of adult AML with a 5′ RUNX1-3′USP42 fusion gene generated by an insertion event instead of chromosomal translocation

Conventional and molecular cytogenetic analyses allowed the precise characterization of the chromosomal rearrangement and breakpoints identification. Gene expression analysis was performed by quantitative real-time PCR experiments, whereas bioinformatic studies were carried out for revealing structural genomic characteristics of breakpoint regions.

We identified an adult AML case bearing a ins(21;7)(q22;p15p22) generating a 5′RUNX1-3′ USP42 fusion gene on der(21) chromosome and causing USP42 gene over-expression. Bioinformatic analysis of the genomic regions involved in

ins(21;7)/t(7;21) showed the presence of interchromosomal segmental duplications (SDs) next to the USP42 and RUNX1 genes, that may underlie a non-allelic homologous recombination between chromosome 7 and 21 in AML.

We report the first case of a 5′RUNX1-3′USP42 chimeric gene generated by a chromosomal cryptic insertion in an adult AML patient. Our data revealed that there may be a pivotal role for SDs in this very rare but recurrent chromosomal rearrangement.

2.P14

ADAMTS2 gene dysregulation in T/myeloid mixed phenotype acute leukemia

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Mixed phenotype acute leukemias (MPAL) include acute leukemias with blasts that express antigens of more than one lineage, with no clear evidence of myeloid or lymphoid lineage differentiation. T/myeloid (T/My) MPAL not otherwise specified (NOS) is a rare leukemia that expresses both T and myeloid antigens,

accounting for less than 1 % of all leukemias but 89 % of T/My MPAL. From a molecular point of view, very limited data are available on T/My MPAL NOS.

In this report we describe a T/My MPAL NOS case with a complex rearrangement involving chromosomes 5 and 14, resulting in overexpression of the ADAM metallopeptidase with thrombospondin type 1 motif, 2 (ADAMTS2) gene due to its juxtaposition to the T cell receptor delta (TRD) gene segment.

Detailed molecular cytogenetic characterization of the complex rearrangement in the reported T/My MPAL case allowed us to observe ADAMTS2 gene overexpression, identifying a molecular marker that may be useful for monitoring minimal residual disease.

To our knowledge, this is the first evidence of gene dysregulation due to a chromosomal rearrangement in T/My MPAL NOS.

2.P15

Dicentric chromosomes in bone marrow cells of patients with acute myeloid leukemia

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Dicentric chromosomes have been described in many hematological diseases including acute myeloid leukemia (AML). Two mechanisms of formation have been observed: telomere fusion and dicentric translocation. High instability of dicentrics during cell division is usually prevented by secondary chromosomal changes leading to an inactivation of one of the two centromeres. Missegregation of dicentrics can produce monosomies which are predictors of poor prognosis in AML. Although dicentric chromosomes are known in cancer cells, highly relevant to global genome instability, their frequency is usually underestimated due to the limitations of metaphase cytogenetic methods.

The aim of this study was to revise karyotypes of 19 AML patients with dicentric chromosomes found by conventional G-banding or mFISH using FISH with (multi)centromeric probes (Abbott Molecular, Kreatech, Metasystems). FISH analyses confirmed 21 out of 27 expected dicentrics and revealed ten others which were not cytogenetically detected before. Most frequently involved chromosomes were: 7, 17 (n=5), 13, 18, 19, 20 and 22 (n=4). In one case, FISH proved only one centromere in suppossed dicentric chromosome, whilst the second centromere was excised and observed as a small marker chromosome.

In conclusion, we demonstrate the necessity of molecular cytogenetic studies to identify the presence and role of dicentric chromosomes and consequent monosomies in cancer. Neither classical cytogenetics nor mFISH is always sufficient to reveal the presence of dicentrics, especially in cases when intercentromeric distance is too small. The evaluation of dicentric chromosomes and monosomies at diagnosis and during the course of the disease contributes to the understanding of unclear mechanisms involved in development and progression of leukemic transformation and will point out to the prognostic significance of dicentric chromosomes in AML.

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2.P16

Looking for what is not there: An interesting case of pediatric B-ALL solved by SNP array

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Acute lymphoblastic leukemia (ALL) represents nearly 25 % of all pediatric cancers, making it the most common malignancy. Several cytogenetic abnormalities are well characterized in in pediatric precursor B-cell ALL (pre-B ALL) and have a known prognostic value, such as high hyperdiploidy (51–65 chromosomes) with double trisomy 4 and 10 which is very frequent and of

favourable prognosis. On the contrary, the presence of near-haploidy or hypodiploidy in pre-B ALL is rare and is associated with a very poor prognosis. Unfortunately, the presence of a hyperdiploid or near-triploid clone resulting from endoreduplication of the near-haploid or hypodiploid clone may mask the latter, causing the pre-B ALL to be misclassified as being high hyperdiploid. We present here the case of a 7 year-old male diagnosed with pre-B ALL in whom standard chromosome analysis showed the following karyotype: 52,XY,+X,+Y,+14,+?14,+21,+21,inc[4]/52,XY,+X,+Y,+14,+21,+21,+?mar,inc[4]/46,XY[3]. Interphase FISH analysis did not detect any of the most common structural rearrangements in pediatric pre-B ALL, nor did it detect the presence of a near-haploid clone. SNP array analysis on an EDTA blood sample confirmed the observed gains of chromosomes X, Y, 14 and 21 but also demonstrated a marked skewing of the alleles on the other chromosomes. These results were consistent with duplication of a single homologue of all chromosomes except X, Y, 14 and 21 in the abnormal leukemic cells due to endoreduplication of a masked near-haploid clone. We have now changed our procedure so that DNA is extracted from an EDTA blood sample from all new pediatric leukemic patients in order to allow further analysis by SNP array if required.

2.P17

Analysis of cytogenetic characteristics in Slovak patients with myelodysplastic syndrome

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Myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal disorders characterised by dysplasia and innefective haematopoiesis in one or more of the major myeloid cell lines. MDS is a neoplastic disease of bone marrow characterized by non-random chromosomal abnormalities. The aim of the study was to analyze the results of cytogenetic examination of 205 patients with MDS in the Prešov region (1990–2014). Cytogenetic analysis were performed from cultured cells of bone marrow without stimulation using GTG banding method and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN). Chromosome analyses in patients with MDS declosed structural and numerical abnormalities in 58 (28.3 %) of samples. 62.1 % of patients with MDS have only one chromosomal anomaly, in 15.5 % two chromosomal anomalies was identified, and 22.4 % have the complex karyotype. Trisomy of chromosome 8 was detected in 25.9 %, deletion of chromosome 5 in 52.2 %, chromosome 7 deletion in 25.9 % and chromosome 20 deletion in 8.6 %. Cytogenetic analyses revealed in addition to frequently reported chromosomal anomalies novel and rare chromosomal abnormalities. The results of our study proves that cytogenetics is still a valuable tool in MDS genetic testing, providing important clues for diagnosis, treatment and clinical managment of patients. Detailed detection of cytogenetic changes may brings insights into molecular mechanisms underlying myelodysplastic syndromes.

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2.P18

Primary and recurrent diffuse astrocytomas: compared genomic profiles revealed acquisition of aberrations with relevant biological potential.

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Diffuse astrocytomas represent heterogeneous group of glial tumours with highly variable biological behaviour.

Despite radical surgical resection and multidrug chemotherapy, recurrent lesions appear after asymptomatic period in majority of patients and additional intervention is necessary. The possibility that tumour may develop novel aberration with relevant biological properties is often neglected. In this study we represent two cases of diffuse astrocytomas where additional cytogenetic and epigenetic markers with potential influence on cell proliferation or differentiation were detected at relapse.

The biopsies taken from primary and recurrent tumours of two patients with astrocytoma were analysed using I-FISH with VYSIS locus specific and centromeric probes (Abbott), SNP array (HumanCyto SNP-12 Bead Chip, Illumina), and MLPA (P370-A1 kit, MRC-Holland) to assess copy number variations and the IDH1 mutations status. Methylation of MGMT and MLH3 promoters was investigated by methylation-specific MLPA (ME011 kit).

Both cases were characterised by features specific for diffuse astrocytomas: R132H mutation of IDH1 gene in both, UPD of 17p and/or deletion of CDKN2A gene in one of them. These markers were present in primary and recurrent lesions. The additional aberrations, mainly deletions or amplifications of chromosomal segments (such as deletion of RB1 gene or trisomy 7), acquired UPD and hypermethylation of MGMT and/or MLH3 promoters were detected in the recurrent lesions.

The IDH1 mutation was the primary event as previously reported. According to our observation the methylation of promoters is the latter event which may along with additional aberrations further disrupt cellular proliferation and/or differentiation. Larger cohort of patients with molecularly analysed recurrent lesions is necessary for global view of tumour biological behaviour and may help to define new therapy targets. Supported by IGA MZ CR NT/13212-4, RVO-VFN64165, PRVOUK-P27/LF1/1.

2.P19

Highlighting of NR3C1 deletion by array-CGH in relapsed childhood ETV6-RUNX1 positive acute lymphoblastic leukemia

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Glucocorticoid therapy plays a fundamental role in the management of lymphoid malignancies, especially in the acute lymphoblastic leukemia. Indeed, glucocorticoids induce apoptosis in lymphoid progenitor cells. Several genes play an important role in glucocorticoid-induced response and in particular the NR3C1 gene which codes for human glucocorticoid receptor. NR3C1 gene deletion is associated to primary glucocorticoid resistance and therefore could represent a poor prognosis marker in childhood acute lymphoblastic leukemia.

Here we report an 8-years-old boy with relapsed ETV6/RUNX1-positive ALL 3 years after his diagnosis. At relapse, we also identified by FISH the (12;21)(p13;q22) translocation, and the R banded karyotype highlighted additional clonal abnormalities such as an interstitial heterozygous deletion of the long arm of the chromosome 9 and a trisomy 21. The high resolution array-CGH analysis detected additional copy number variations that were cryptic on karyotype :

- the NR3C1 heterozygous deletion on the long arm of chromosome 5 : del(5)(q31.3q31.3)
- the partial trisomy of long arm of chromosome X : dup(X)(q26q28)

At relapse, as at the diagnosis, assessment of corticosensibility to J7 of prephase of chemotherapy showed corticoresistance.

The presence of NR3C1 deletion in leukemic cells clone could explain the persistent corticoresistance and suggests that the relapse could be due to persistence of an ancestral clone with NR3C1 deletion. Additional studies are presently being performed in order to identify the presence of ancestral clone on diagnosis.

In conclusion, the high resolution array-CGH analysis may have an impact on the management of ALL. Indeed, array-CGH allows to highlight the presence of copy number abnormalities of genes with a role in resistance to treatment (eg NR3C1 in 5q31.3, NR3C2 in 4q31.22, BMF in 15q15.1…) and might help to guide future personalized therapies.

2.P20

Molecular genetic changes in gliomas and their impact on progression in patients with low grade gliomas

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Diffuse gliomas are the most common primary central nervous system malignancies. Classification of these tumors is based on WHO used traditional methods, including cellular morphology, presence of mitoses, vascular proliferation and necrosis. Using these, tumors are classified in astrocytic, oligodendroglial or oligoastrocytic categories and specified by a tumor grade. Despite the progress in diagnosis and treatment, gliomas are almost incurable since radical resection is not possible due to their diffuse nature. It is expected that the therapy would be based—among others—on the knowledge of genetic changes in the individual types of tumor (chromosomal abnormalities, activating mutations of oncogenes and mutations associated with loss of tumorsuppressor gene function).

In the period of 2007–2012, we examined 127 native astrocytic tumor samples and 20 oligodendroglial or oligoastrocytoma tumor samples. In astrocytic tumors, FISH analyses of EGFR and MDM2 oncogene statuses, CDKN2A, PTEN, TP53, RB1 tumorsuppressor gene statuses were performed. In oligodendroglial or oligoastrocytic tumors, status of 1p/19q codeletion and status of tumorsuppressor genes CDKN2A and PTEN were determined by FISH.

In 30 low grade astrocytoma samples, 13 samples were found with chromosomal aberrations associated with high grade gliomas (deletion of tumorsuppressor genes CDKN2A, PTEN, monosomy of chromosome 10, amplification of EGFR gene). In 20 tumors within oligodendrogliomas and oligoastrocytomas, 6 tumors with histopathological gr. II showed changes associated with gr. III (deletion of tumorsuppressor genes CDKN2A, PTEN). It seems that progression or relapse of disease in these patients occur earlier than in patients without aberrations associated with high grade gliomas. This findings are on the border of statistical significance (p=0,0519), possibly due to the small number of patients with low grade gliomas in the study group.

2.P21

Acquired uniparental disomy of 17p is associated with mutations of TP53 gene in patients with MDS and complex karyotypes.

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Cytogenetic aberrations are the most important prognostic factors in patients with myelodysplastic syndromes (MDS). The complex karyotypes are associated with the poorest prognosis. However, the pathogenic mechanisms leading to origin of complex aberrations at diagnosis of MDS are poorly understood. Segmental acquired uniparental disomy (aUPD), occurring as a result of mitotic recombination, might confer increased genomic instability and play a fundamental role in tumorigenesis. The aim was to evaluate the frequency of aUPD of 17p in bone marrow (BM) cells of newly diagnosed MDS patients with complex karyotypes and to assess its correlation with mutations of TP53 gene (17p13.1).

Genomic DNA isolated from diagnostic BM samples of 68 MDS patients with complex karyotypes (≥3 aberrations) previously proved by G-banding and/or mFISH was analyzed by aCGH/SNP (BlueGnome). Next-generation sequencing on the GS Junior system (Roche) was used to detect TP53 mutations.

The overall incidence of aUPD17p was 13/68 and its average size was 8–20 Mb always encompassing TP53 gene. Homozygous mutations of TP53 were detected in all aUPD17p positive patients (nine missense mutations in DNA-binding core domain, two frameshift mutations, one nonsense mutation, and one substitution in intron 4). The most common chromosomal imbalances were loss of 5q (11/13), 3p (8/13), 7q (6/13), monosomy 7 (2/13), and 12p deletion involving ETV6 gene (8/13).

We confirmed that aUPD17p is a frequent nonrandom finding in MDS with complex karyotypes and is strongly associated with homozygous mutations of TP53 gene. A heterozygous TP53 mutation and following recombination event give rise to homozygosity of mutated gene. This phenomenon may be one of trigger mechanisms of clonal evolution and cause rapid disease progression in high risk MDS.

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2.P22

Identification of a novel cryptic t(4;21)(p16;q22) associated with 5q abnormalities in a pediatric case of acute myeloid leukemia

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The runt-related transcription factor 1 (RUNX1) gene, located in chromosome 21q22, is a transcription factor that acts as a regulator of hematopoiesis and represents one of the most frequent genes involved in chromosomal translocations in human leukemias with nearly 20 identified partner genes. Here we report a 14-year-old boy with newly diagnosed AML-M0. He presented with asthenia probably due to a very low hemoglobin level (4.2 g/dL). Blood smears revealed 80 % immature cells and the bone marrow examination showed 95 % blasts positive for CD13, CD34, CD117, CD7 and CD56. Conventional cytogenetics identified an abnormal clone with 5q deletion. EGR1 (5q31) and CSF1R (5q33-q34) deletions in chromosome 5 were confirmed using FISH probes. Systematic interphase FISH using LSI RUNX1/RUNX1T1 showed three signals for RUNX1. FISH studies on metaphases showed the extra RUNX1 signal on the short arm of chromosome 4. Further characterization using the subtelomeric 4p probe showed a cryptic t(4;21)(p16;q22) translocation. Our case appears to share similarities with acute myeloid leukemia cases with cryptic t(7;21)(p22;q22) recurrent translocation including aberrant CD56 and CD7 lymphoid antigen expression, loss of 5q material as an additional abnormality, absence of hyperleukocytosis and severe macrocytic anemia. The macrocytic anemia may be related to 5q deletion and might be associated with the haploinsufficiency of the ribosomal genes on the long arm of chromosome 5, such as RPS14, as described in 5q- myelodysplastic syndromes. To the best of our knowledge, this was the first report of a novel cryptic t(4;21)(p16;q22) translocation involving RUNX1 gene associated with 5q abnormality in a childhood AML. Additional studies are being performed in order to identify the RUNX1 partner gene implicated in this translocation.

2.P23

IGH abnormalities detected by I-FISH in selective CD138+ plasma cells in multiple myeloma

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Multiple Myeloma (MM) is a malignant accumulation of monoclonal plasma cells (PC) with low proliferation activity in the bone marrow (BM). Conventional cytogenetic analysis of MM has been hampered not only by a low level of bone marrow

infiltration with PC and by a low mitotic index of PC in vitro, but also by the fact that many translocations affecting the IGH locus in 14q32 region are cytogenetically cryptic in this disease and not visible in light microscopy.

MM exhibits a wide variety of numerical and different structural IGH changes involving 14q32 region translocations with numerous partner chromosomes, which might be used for clinical classification.

The main method to detect the chromosomal abnormalities in MM is Interphase Fluorescent In situ Hybridization (I-FISH) on PC. Enrichment of PC by CD138 positive selection prior to I-FISH is one way to enhance diagnostic sensitivity.

The aim of this study was to characterize the rearrangements of chromosome 14 in group of 109 patients with MM. The specific probe for chromosome 14q32.33 (IGHC/IGHV) was used to detect chromosomal aberrations. Forty six of the patients (43 %) had at least one type of abnormality and the most frequent was IGH rearrangement (24 %).

In this group of patients with 14q32 rearrangement additional probes were further applied to detect t(4;14)(p16.3;q32.3) (LSI FGFR3/IGH), t(11;14)(q13;q32.3) (LSI CCND1/IGH), t(14;16)(q32.3;q23) (LSI IGH/MAF) or t(14;20)(q32.3;q12)(LSI IGH/MAFB).

Interestingly, 21 of the patients (19.2 %) showed only one signal of IGH, and the effect of this loss on the clinical phenotype is currently being studied.

Our data illustrate that I-FISH together with CD138+ PC enrichment improve the cytogenetic diagnosis in MM and provide additional tool for MM analysis.

2.P24

Molecular cytogenetic analysis of selected chromosome 8 regions in patients with multiple myeloma and its comparison at the time of diagnosis and following relapse/progression

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Multiple myeloma (MM) is a hematologic malignancy characterized by accumulation of terminally differentiated plasma cells in the bone marrow. The genome of MM clonal plasma cells is characterized by genetic changes of prognostic importance. Among the high-risk abnormalities include hypodiploidy with immunoglobulin heavy-chain (IgH) translocations, including the genes CCND3, MAF, MAFB and FGFR3/MMSET. Disease progression is accompanied by a number of secondary chromosome aberrations, the most common being MYC rearrangements, del(13q), del(17p), and the deletion of 1p and/or amplification of 1q. The aim of our study was to evaluate and summarize the frequency and types of aberrations on chromosome 8, the relation to the disease course, and finally, to compare the findings with the clinical characteristics. The analysis was performed on 62 patients with MM at the time of diagnosis and at relapse/progression. We used FICTION and FISH methods with specific probes for detecting c-MYC, TRAIL-R1, -R2 genes aberrations. Chromosome 8 aberrations were found in 53 % of the patients. At relapse/progression, we detected c-MYC gene rearrangements in 14 patients, numerical changes in c-MYC gene in 23 patients and deletion of region 8p21.3 in 12 patients. Up to ~73 % of these aberrations were detected also at the time of diagnosis. Clinical evaluation of our cohort revealed that the patients with chromosome 8 aberrations were in advanced clinical stage, with predominance of monoclonal IgG. The aberrations of chromosome 8 were detected not only at relapse/progression but also at the time of diagnosis, with worsening clinical course of the disease. Their frequency and prognostic significance demonstrate the importance of their inclusion in routine FISH examinations.

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2.P25

May HER2 amplification be a molecular marker for a subgoup of familial papillary thyroid cancer?

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Introduction and aim. The majority of papillary thyroid carcinoma and follicular thyroid carcinoma are sporadic (sPTC and sFTC), although familial tumors may account for approximately 9 % of thyroid carcinoma

cases. Whether familial papillary thyroid carcinoma (fPTC) has a more severe phenotype than sPTC is still debated, and no specific gene(s) involved in fPTC has been identified so far. Recently, various types of novel molecular alterations have been reported in sPTC, such as ALK and ETV6 genes rearrangements, and HER2 amplification (HER2amp) in association with telomere shortening (Pérot et al., 2014; Ricarte-Filho et al., 2013; Sugishita et al., 2013). BRAF inversion was already reported in sPTC (Ciampi et al., 2005). On the other hand, our group reported short telomeres and chromosome instability in fPTC (Cantara et al., 2010). These observations prompted us to investigate the frequency of the novel molecular alterations in sPTC and fPTC.

Results. Interphase FISH retrospective study was carried out on nuclei isolated from formalin fixed paraffin embedded (FFPE) sections. 14 sPTC and 17 fPTC (some of which were previously studied by our group for telomeres length) were examined using home-brew probe (HER2) and commercially available probes (BRAF, ETV6 and ALK). We found that HER2amp was higher in fPTC compared to sPTC (p=0.01). No rearrangement of the other tested genes was observed. Histologic sections of cases positive for HER2amp were then evaluated: HER2amp was mainly present in scattered cells.

Conclusions. Our preliminary findings indicate a higher HER2amp frequency in fPTC and pinpoint the need of studying additional cases to verify the potential of HER2amp in molecularly characterize a subgroup of fPTC. If confirmed, our finding open considering trastuzumab therapy effectiveness in refractory thyroid carcinoma positive for Her2amp. Fund: PRIN N.2012Z3F7HE.

2.P26

Jumping translocations in a 13-year-old child with RUNX1/RUNX1T1-positive acute myeloid leukemia

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Jumping translocations (JT) are a rare cytogenetic phenomenon, the word jumping referring to the translocation of the same segment of the donor chromosome on to various recipient chromosomes, creating multiple related clones in an individual. It has been described as a constitutional abnormality, in solid neoplasms and rarely in various hematological malignancies including acute myeloid leukemia (AML), acute lymphoblastic leukemia, myelofibrosis, chronic myeloid leukemia, lymphomas, and multiple myelomas [McGrattan, 2010; Manola, 2008]. In these patients JT occurred as a secondary event leading to progression of disease either as a secondary AML evolving from a previously existing myeloproliferative neoplasm and myelodysplastic syndrome or a relapse of a disease previously in remission. JT have commonly been described in elderly adults.

We report a rare case of JT occurring in a 13-year-old girl with RUNX1/RUNX1T1-positive and very high expression WT1+ AML implicating its probable role in disease progression. We revealed JTs in bone marrow cells examined at the time of first chemoresistant relapse of AML. The findings of conventional cytogenetics were confirmed by fluorescence in situ hybridization (FISH) with locus specific probes. For precise description of chromosomal parts involved in JTs, multicolor FISH were performed (MetaSystems). Cytogenetic analysis revealed JTs with chromosomal segments 17q21-17qter translocated to the pericentromeric regions of chromosomes 13,14,15 and to regions 1p36 and 2q37. The patient has been performed allogeneic SCT from haploidentical donor, but child died after progression of leukemia.

JTs form a heterogeneous group of rare events involving probably random chromosomal segments, they reflect the general chromosomal instability of malignant cells. However, precise analysis of chromosomal breakpoints involved in JTs on a larger series of patients could contribute to explain their mechanism and connection with pathogenesis together with the determination of clinical significance of these aberrations.

2.P27

Cytogenetic analysis in myeloid sarcoma

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Myeloid sarcoma (granulocytic sarcoma, chloroma) is an extramedullary mass composed of cells of the myeloid lineage. Myeloid sarcoma is reported in 3–8 % of patients with acute myeloid leukemia and occurs concomitantly, following, or, rarely, antedating the onset of systemic bone marrow leukemia. Potentially it can affect every organ, more often it is found in the „sanctuary sites", which are difficult to target with chemotherapy. Myeloid sarcoma has been described in association with a variety of chromosomal aberrations, complexity of the cytogenetic findings indicates poor prognosis. Most common translocation in myeloid sarcoma is t(8;21) and core binding factor involvement is also frequent. During the last 20 years 240 patients with acute myeloid leukemia were treated in the First Department of Medicine, University Pecs, Hungary. Nineteen of them (7,9 %) had myeloid sarcoma. We report on cytogenetic data of 15 patients, where cytogenetic analysis were available. When bone marrow involvement was present, cytogenetic examination was performed on bone marrow aspirates. Where adequate samples were available, conventional karyotyping, as well as fluorescent in situ hibridization was performed on cells of extramedullary involvement. Most common aberrations were +8; and complex abnormality (3-3 cases). We also detected translocation t(9;11), t(8;21) and t(8;22).

2.P28

Acute promyelocytic leukemia with isochromosome 17q and cryptic PML-RARA successfully treated with all-trans retinoic acid and arsenic trioxide

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Acute promyelocytic leukemia (APL) is a curable form of leukemia characterized by typical morphology, high prevalence of severe bleeding events and distinct chromosomal aberrations, most commonly t(15;17). However, approximately 9 % of APL patients harbor other translocations involving chromosome 17, such as t(11;17)(q23;q21), t(5;17)(q35;q12-21), t(11;17)(q13;q21) and der(17). All-trans retinoic acid (ATRA) and arsenic trioxide (ATO) have a specific targeted activity against the PML-RARA fusion protein. ATRA and ATO have recently been shown to be non-inferior to chemotherapy and ATRA as induction therapy for non-high risk APL. The clinical significance of non t(15:17) APL-related aberrations is controversial, with conflicting reports regarding sensitivity to modern, targeted therapy. Isochromosome 17q (iso(17q)) is a rare cause of APL, usually associated with t(15:17). There is no published data regarding the efficacy ATO based therapy for APL patients with iso(17q). We report a patient with APL with iso(17q) as the sole aberration, without overt t(15;17) translocation but with a cryptic PML-RARA transcript, who was treated with ATRA and ATO after failure of chemotherapy and achieved complete remission. To the best of our knowledge this is the first published report of isolated iso(17q) with cryptic PML-RARA transcript as a cause of APL and the first reported iso(17q)-related APL successfully treated with an ATO containing regiment.

2.P29

Potential of aminolevulinic acid/photodynamic therapy to induce DNA damage on MCF-7 and HepG2 cell lines

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Background: Photodynamic therapy (PDT) is a clinically approved and promising therapeutic procedure involving

the administration and irradiation of a photosensitizer such as 5-aminolevulinicacid (ALA) to produce singlet oxygen. The aim of the present work is to evaluate cytotoxic and genotoxic effects induced by ALA/PDT on adenocarcinoma breast cancer (MCF-7) and hepatocellular carcinoma (HepG2) cell lines.

Methods: ALA (0.5 and 1 mM) was applied to MCF-7 or HepG2 cell lines for 4 h before exposure to laser irradiation (633 nm; 0.25 W) for 4 min and then incubated for 24 h. Cytotoxicity was evaluated with trypan blue. The potential to induce DNA damage was recorded by alkaline comet assay and micronucleus test.

Results: ALA/PDT reduced cell viability in a concentration dependent manner. ALA/PDT IC50 reached to 1,74 mM in MCF-7 cells and reached to 5.09 mM in HepG2 cells. Also, ALA/ PDT induced DNA damage and micronuclei in MCF-7 and HepG2 cell lines and percentages of DNA damage with comet tail and the proportion of tail length were increased highly significantly in comparison to non-treated cells.

Conclusion: ALA/PDT induced cytotoxic and genotoxic effects on MCF-7 and HepG2 cells. ALA/PDT is effective in cancer treatments as it targets DNA directly leading to induction of DNA damage as evaluated with Comet assay and micronucleus test. Further experimental studies are needed to investigate mode of cell death by ALA/PDT which enables direct DNA damage.

2.P30

Trisomy 5 as a sole cytogenetic abnormality in acute lymphoblastic leukemia

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Numerical chromosomal aberrations are commonly seen in acute lymphoblastic leukemia with specific gains of chromosomes (hyperdiploidy), which are associated with an excellent prognosis. Trisomy 5 as sole or primary chromosomal abnormality is very rarely detected. A review of the literature shows only 15 patients and 11 of them were children. Some studies suggest that this rare cytogenetic abnormality may be associated with a poor outcome. We present a case of acute lymphoblastic leukemia (ALL) with trisomy 5 as primary chromosomal abnormality. A 32-year-old male was presented in September 2013 with leukocytosis, anemia and thrombocytopenia. Smear of peripheral blood revealed 81 % blasts and bone marrow aspirate was hypercellular with 91 % blasts. The case was classified as common ALL according immunophenotype markers Tdt+,CD19+,CD10+,CD34+. The cytogenetic study showed:47,XY,+5[15] / 48,sl,+13[2]/47,XY,+del(5)(q13q31)[2]/46,XY[1] karyotype. The patient attained complete hematological remission after one course of induction therapy with CVAD (Cyclophosphamide, Vincristine, Adriamycin and Dexamethazone). Minimal residual disease analysis of the bone marrow by flow cytometry showed 10 % lymphoblasts. He received consolidation treatment (4 cycle) and MRD was after the first cycle 1,5 % and after the second cycle 0,1 %. In March 2014, he received allogeneic hematopoietic cell transplantation from HLA-mishmatced unrelating donor in CR with positive MRD. Nowadays the patient is alive in complete remission and maintains complete donor chimerism. Despite hematological remission at the end of induction chemotherapy, our patient had evidence of minimal residual disease and this may have adverse prognostic significance. A review of this rare abnormality confirm our findings that trisomy 5 could be associated with a poor prognosis. These data is probably recommend to support the indication of intensive therapies such as allo-SCT in first remission.

2.P31

Evaluation of TEL/AML1 fusion and additional abnormalities involving TEL and/or AML1 genes using FISH technique in patients with childhood acute lymphoblastic leukemia

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Childhood Acute Lymphoblastic Leukemia (ALL) is a malignant disorder of lymphoid progenitor cells with a peak incidence among 2–5 years of age and is the most common type of childhood leukemia. The t(12;21)(p13;q22) translocation occurs in 25 % of childhood B-cell precursor ALL. In this study, bone marrow samples were obtained from 165 patients with childhood ALL. We analyzed t(12;21) translocation and other related abnormalities by fluorescent in situ hybridization technique using ETV6(TEL)/ RUNX1(AML1) ES dual color translocation probe and conventional cytogenetics analyses were performed. TEL and AML1 related chromosomal abnormalities were determined in 42 (25.5 %) of 165 patients with childhood ALL. Structural changes were detected in 33 (78.6 %) and numerical abnormalities in 9 (21.4 %) of a total of 42 abnormal karyotypes. Frequencies of FISH abnormalities in pediatric ALL cases were as follows; 8,5 % for t(12;21)(p13;q22) TEL/AML1 fusion, 6.0 % for AML1 amplification, 3.0 % for tetrasomy/trisomy 21, 1.8 % for TEL deletion, 1.21 % for TEL deletion with AML1 amplification, 1.21 % for TEL amplification with AML1 amplification, 0.6 % for poliploidy, 0.6 % for AML1 deletion, 0.6 % for diminished TEL signal. The most frequent structural abnormality was t(12;21) translocation followed by AML1 amplification and TEL deletion while most frequent numerical abnormality was trisomy 21.

2.P32

Evaluation of MDM2 gene copy number alterations in chronic lymphocytic leukaemia patients with fluorescent in situ hybridization

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The aim of this study was to evaluate MDM2 gene copy number alterations in chronic lymphocytic leukaemia(CLL) patients with fluorescent in situ hybridization(FISH) technique by using murine double minute 2(MDM2) gene-specific probe sets, and also to evaluate the possible relationship between this amplification and the disease course, indication for treatment and drug resistance.

Copy number alterations of the MDM2 gene located on chromosome 12 were investigated by FISH using metaphase chromosomes obtained from bone marrow aspiration samples for routine chromosome analysis in 40 CLL and 20 chronic myeloid leukemia(CML) patients followed-up at Akdeniz University Department of Haematology. Additionally, routine FISH and conventional cytogenetics results of the patients were evaluated.

MDM2 gene amplification was not observed in the patient cohort (CLL) or in control patients (CML). Trisomy 12 FISH signal pattern was observed in 12 out of 40 CLL patients (30 %). Furthermore, chromosomal alterations detected by routine FISH analysis in 40 CLL patients were as follows; 17p13 deletion in 16 cases (40 %), 13q14 deletion in 13 patients (32.5 %), 11q22.3 deletion in 6 patients (15 %), and 6q23 deletion in 1 case (2.5 %).

Evaluation of MDM2 mRNA expression levels in CLL patients might be conducted due to absence of MDM2 gene copy number alterations. Additionally, evaluation of SNP309 G/G single nucleotide polymorphism in advanced stage CLL patients without MDM2 gene amplification might provide important clues for progression disease.

27 out of the 40 CLL patients (67.5 %) were classified as having early stage disease. We suggest that in the case of progression to advanced stage CLL during the follow-up period,re-evaluation of MDM2 gene copy number alterations will be more useful to explain the relationship between copy number alterations of MDM2 and CLL.

2.P33

The process of malignant transformation in a child with acute myeloid leukemia.

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On the basis of genetic changes found in cytogenetic and molecular studies and depending on the response to treatment, the stratification of patients with acute myeloid leukemia to therapeutic groups of risk is performed.

The study reports data of a 5 years old boy with acute myeloid leukemia (AML) type M5. Cytogenetic examinations of bone marrow with the use of classical method G-banding revealed translocation among three chromosomes: 4, 9 and 11.

The FISH (fluorescent in situ hybridization) study was carried out on metaphase slides and interphase nuclei. The MLL gene rearrangement was detected with use of the LSI MLL Dual Color, Break Apart Probe (Vysis). The existence of the fusion gene (RUNXT1/RUNX1) was excluded.

Further investigations used whole chromosome painting FISH on 4, 9 and LSI MLL Dual Color, Break Apart Probe. To improve genetic diagnosis we applied high resolution comparative genomic hybridizations (HR-CGH).

Hybridization method detected deletion of part of p arm of chromosome 9-ish cgh dim(9)(p13p21). The study was confirmed by FISH study results with using of LSI p16 probe. No changes were detected in a test for the presence of an internal tandem duplication of the FLT3 gene .

The above studies can determine the direction in which malignant transformation proceeded. The original rearrangement had been the translocation (4;11) than appeared the secondary translocation (4;9) and the translocation (9;11) with 9p21 deletion. The following karyotype was received: 46,XY,der(4)t(4;11)(q21;q23),der(9)t(4;9)(q21;p13)der(11)t(9;11)(p22;q23).

Due to complex irregularities of karyotype and renewal of blasts, it was decided about hematopoietic cell transplantation after the first remission. Due to lack of related donor, it was decided to take cells from unrelated one.

The treatment was carried out without complications. Currently the patient is 4 months after transplantation in the.

2.P34

Application of fluorescence in situ hybridization to detect MYCN amplification on paraffin-embedded tissue sections of neuroblastoma

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Neuroblastoma is a malignant tumor in children aged 0–15 years.

It accounts for 7–10 % of all childhood tumors and 30–50 % of all infants tumor. Research of the molecular biology and cytogenetic are a necessary tool to evaluate the advanced stage of the disease and besides the patient's age, stage of disease, tumor histology, location of primary tumor and specific biochemical parameters, are the very important prognostic indicators, classifying patients to a specific therapeutic group.

The most common cytogenetics changes including deletions of chromosome 1p, gain of chromosome arm 17q, amplification of the oncogene MYCN, ploidy changes in tumor cells.

The aim of this study was create a method to evaluation MYCN amplification on paraffin- embedded tissue sections of neuroblastoma patients. Paraffin embedded tissue sections were previously digested respectively and prepared to apply a molecular probe, using fluorescent in situ hybridization (FISH) technique.

The direct fluorochrome- labeled, dual color DNA Probe for the MYCN gene locus (2p24) and α-satellite DNA identifying the centromeric region of chromosome 2 (Vysis LSI N-MYC (2p24) SpectrumGreen/CEP 2 SpectrumOrange Probe) was used to estimate MYCN amplification.

Amplification MYCN is detected in 20–30 % neuroblastoma patients. It is the strongest marker of poor prognosis. It is strongly correlated with advanced stage of disease, rapid tumor progression, resistant to chemotherapy and poor outcome independent of patient age and stage of advanced disease.

The test material were the paraffin embedded tissue sections with fresh remove tumors, as well as archival sections 32 patients with an established clinical diagnosis of neuroblastoma.

Positive results of MYCN amplification were obtained in 9 patients paraffin-embedded tissue sections, which represents an 28.125 % of all tested patients in the analysis.

2.P35

Molecular cytogenetic analysis of recurrent chromosomal aberrations in diffuse large B-cell lymphoma (DLBCL).

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DLBCL is the most common non-Hodgkin lymphoma in adults and clinically and genetically it is a heterogeneous disease. DLBCLs are associated with number of genetic aberrations. However, the prognostic significance of some of the recurrent changes, such as BCL2, BCL6 or MYC gene rearrangement and deletion of 6p or 17p13, has been demonstrated.

In our study, we analysed the frequency of BCL6 and MYC genes rearrangement and BLIMP1 gene deletion by fluorescent in situ hybridization (FISH). Multicolor FISH (mFISH) and array comparative genome hybridization (arrayCGH) were used to detect recurrent balanced and unbalanced changes in the karyotypes. This analysis was performed on 59 patients.

Translocation of BCL6 gene was observed in 9/49 (18 %) patients with the translocations' partner from chromosomes 2 (IgK gene), 10, 12 and 14 (IgH gene). A total of 14/54 (26 %) patients had copy number changes at MYC gene, while none of the patients had MYC rearrangement. Deletion of BLIMP1 was part of the complex karyotype in 8/46 (17 %) patients. We performed detailed analysis of 24 patients with complex karyotype and confirmed that chromosomes 1, 3, 6, 9, 10, 14 and 18 were frequently affected by translocations. By using arrayCGH, we examined 38 patients and found recurrent gains at 1q, 6p, 7, 13q and losses at 6p, 6q and 17p. A comparison of the histological prognostic groups with genetic changes shows that GCB-DLBCL is associated with rearrangement of chromosome 14, 18, and BCL2/IgH positivity. In the group of nonGCB-DLBCL, the frequently rearranged chromosomes were 3, 6, 7, 9, 10 and X. We confirmed that DLBCL is, genetically, a heterogeneous disease with number of recurrent cytogenetic aberrations that were, in our cohort, detected in 43 (72.8 %) patients.

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2.P36

FISH and arrayCGH analysis of chromosome 13q deletions in patients with chronic lymphocytic leukemia (CLL)

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Cytogenetic abnormalities have confirmed prognostic importance in CLL. The most frequent aberration is deletion of 13q14 detected in ~50 % of patients by fluorescence in situ hybridization (FISH). As a single aberration, 13q14 deletion is associated with favourable prognosis. However, the prognostic value of other changes such as 13q14 biallelic deletion, monosomy 13 or chromosome 13 translocation remains controversial.

The aim of our study was to perform arrayCGH analysis in a group of patients with 13q14 deletion to determine the extent of the deletion and the common deleted region (CDR). Additional objectives included

comparison of the findings in patients with single aberration with those with 13q14 deletion and other changes including complex aberrations, and also compare them with certain clinical parameters.

We used FISH with a panel of CLL specific probes (Abbott, Illinois, USA; Metasystems, Althusheim, Germany) and arrayCGH with oligonucleotide microarray (Agilent, Santa Clara, USA) to perform the analysis in total of 55 CLL patients with 13q14 deletion (20 pts with single aberration, 10 patients with an additional aberration and 25 patients with complex changes).

ArrayCGH determined the CDR of 1.67 Mb at 13q14.2-13q14.3, containing MIR15a, MIR16-1, DLEU1, DLEU2, and DLEU7. We observed that the large 13q deletion (Type I—including RB1) and the higher percentage of altered nuclei are frequently associated with unmutated IgVH status and complex changes.

Our analysis of 55 CLL patients confirmed significant heterogeneity of the extent of the deleted region on 13q and the determined CDR. Small number of patients in the study has not allowed the determination of clinical significance of deletions of 13q yet.

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2.P37

Clonal evolution in chronic lymphocytic leukemia: Combination of FISH and conventional cytogenetics after stimulation with CpG oligonucleotides and interleukin-2

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Chromosomal abnormalities are important prognostic factors in chronic lymphocytic leukemia (CLL). Clonal evolution (CE), defined as acquisition of new cytogenetic aberration, may occur in 17–26 % of CLL patients and is associated with shorter overall survival. The aim of our study was to prospectively assess CE frequency by FISH and conventional chromosome banding (CBA) after stimulation with CpG oligonucleotides and interleukin-2. The role of other prognostic factors and therapy in the development of poor-prognosis CE (new deletion 17p, new deletion 11q or new complex karyotype) was also evaluated.

140 patients (86 men, 54 women, median age 64 years) with previously untreated CLL were evaluated by CBA and FISH (11q, 13q, 17p deletions, trisomy 12, 14q32 rearrangement). Peripheral blood samples of each patient were provided for baseline and follow-up testing. Mutation status of IGVH gene and expression of CD38 and ZAP70 were also analysed.

CE was detected in 15,7 % (22/140) of patients using FISH, in 28,6 % (40/140) using CBA and in 34,3 % (48/140) of patients by combining both methods. The median time to CE occurrence was 16 (range 6–52) month. Poor-prognosis CE was detected in 15 % (21/140) of patients and was significantly associated with previous CLL treatment (p=0,013). In 10/21 cases CE was shifted into a poor-prognosis group according to the hierarchical FISH model, while in the remaining 11 patients, poor risk CE was presented by complex karyotype without evidence of 17p deletion.

CBA after stimulation with CpG oligonucleotides and interleukin-2 provides more complex information about cytogenetic abnormalities in CLL than FISH, suggesting that many patients acquire new abnormalities during the course of their disease, especially after treatment.

2.P38

Cytogenetics in multiple myeloma: Abnormalities of Chromosome 1

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Background: Multiple myeloma (MM) is a B-cell malignancy of the plasma cells characterized by complex cytogenetic aberrations. Chromosome 1 abnormalities are found in almost half of MM cases and are associated with aggressive disease. The most common structural changes that involve chromosome 1 are 1p deletion and 1q gain.

Patients and Methods: Between 2009 and 2014, 27 MM patients (6 women, 21 men, median age 68) showed chromosomal abnormalities by conventional chromosome banding (CCB). Bone marrow aspirates were processed using short time cultivation (24–48 h) without stimulation. A minimum of IGH rearrangement and 13q deletion was performed in all cases by cytoplasmic immunoglobin light chain staining (cIg FISH). Chromosome 1 abnormalities were confirmed by CCB, cIg-FISH or FISH and multicolor FISH.

Results: Chromosome 1 aberrations were detected in 15/27 patients. Deletions were identified in 11/15 patients: 1p deletions as a result of unbalanced translocation (8/11), 1p intersticial or terminal deletions (2/11) and 1q deletion (1/11). Gains were caused by unbalanced translocation in 10/15 patients: 1q12-qter (5/10), 1q21-qter (3/10), 1q22-qter (1/10) and 1p21-qter (1/10). Trisomy 1 was presented in 1/15 patient. Deletion of 1p as well as gain of 1q were were presented in 7/15 karyotypes.

Summary: The 1p was preferentialy involved in deletions that affect regions 1p11 and 1p13. The 1q gains were mostly in regions 1q12-qter and 1q21-qter. The 1q12–q23 region cover a large number of possible candidate genes. Our results support the importance of chromosome 1 abnormalities in the patogenesis in MM.

2.P39

Cytogenetic and FISH studies of B-cell lymphomas

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Double-hit B-cell lymphomas (DHL) are rare subtype of lymphomas usually associated with highly aggressive clinical behavior and poor prognosis. This heterogenous category is characterized by spectrum of morphological, phenotypic, genetic and pathological features overlapping with Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), B-lymphoblastic lymphoma/leukemia (B-LBL) and acute lymphoblastic leukemia/lymphoma (ALL). DHL are genetically defined by obligatory MYC (8q24) rearrangement in combination with another genes, involved in development of lymphomas, such as BCL2 (18q21), BCL6 (3q27), CCND1 (11q13) or BCL3 (19q13). The mechanism of proto-oncogenes activation includes translocation or amplification, usually a part of complex karyotypes. Here we report interesting de novo cases of patients with DHL in term of classical and molecular cytogenetics. Details of cytogenetic and FISH analysis will be presented.

2.P40

Evaluation of Danusertib effects on cell viability and cytomorphological parameters in cancer stem cell lines from glioblastoma multiforme

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Glioblastoma multiforme (GBM) is a grade IV astrocytoma and the least successfully treated solid tumor: current therapies provide a median survival of 12–15 months after diagnosis, due to the high recurrence rate. Glioma stem cells (GSCs) are believed to be the real

driving force of tumor initiation, progression and relapse. Better therapeutic strategies GSC-targeted are needed.

A number of potential molecular targets for the identification of novel anticancer drugs interferes with the cell cycle. Danusertib is a small molecule with strong activity against Aurora kinases, a protein kinases family overexpressed in a variety of human cancers and correlated, also in GBM, with chromosomal instability, tumor aggressiveness and poor prognosis.

In this study, we analyzed the effect of Danusertib exposure on cell viability, proliferation and cytomorphological parameters, by means of different assays (MTT, trypan blue and clonogenic assays, mitotic index and ploidy determination, morphological analysis), in four GSC lines from GBM. GBM2 cell line showed a loss and G166 a gain of Aurora kinases genes, while GliNS2 and G179 cell lines showed no variation.

Results showed that response to Danusertib exposure was heterogeneous among GSC lines. Cell viability and proliferation were significantly reduced in GBM2 and G179 cell lines, while G166 and GliNS2 were resistant. The analysis of cell and nuclear morphology in GBM2 and G179 cell lines highlighted the presence of large multinucleated cells and an increase in the number of polymorphic nuclei and micronuclei. At last conventional cytogenetics evidenced a significant increase in ploidy in GBM2 and G179.

Expression and mutational analysis of Aurora kinases and the study of chromosome segregation errors are in progress in order to deeply understand the heterogeneous response to Danusertib treatment on our GSC.

2.P41

Chromosomal abnormalities in chronic myeloid leukemia patients at diagnosis and during follow up.

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Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder characterized by the presence of the Philadelphia (Ph) chromosome.

AIM: To investigate the chromosomal abnormalities present in CML patients at diagnosis and during follow up and to evaluate their long—term response.

MATERIALS AND METHODS: 47 newly diagnosed CML patients (median age 56 years (range 26 to 89 years) with a male:female ratio 1.14 : 1) treated in our Hospital between 2001 and 2014 were monitored. Conventional cytogenetics and/or Fluorescent in situ Hybridization (FISH) were performed both at diagnosis and during follow up. Molecular analyses were used for the monitoring of patients and Molecular Response (MR) was defined according to the International Scale.

RESULTS:. At diagnosis, cytogenetic studies showed 40/47 (85.11 %) patients with the standard Ph translocation, 4/47 (8.51 %) patients with a variant Ph translocation and 2/47 (4.25 %) patients with Additional Chromosomal Abnormalities (ACAs) (one with del(7)(p13p15), ider(22)(q10)t(9;22)(q34;q11) and one with +8). In one patient (2.13 %) with normal karyotype, FISH revealed the presence of a masked Ph. During follow up, other chromosomal aberrations were detected in 4/47 patients: –Y (n=1) and +8, i(17q) (n=1) in the Ph+clone and –Y (n=1) and +8,+21 (n=1) in Ph- patients. Complete and Major MR were obtained in 28 and 12 patients respectively, while no response was observed in 4 patients. However, 3 patients have not yet been tested for response. These results were obtained irrespectively of CML phase or treatment used.

CONCLUSION: Our findings are consistent with other studies, confirming that variant Ph translocation, ACAs and masked Ph are rare events in CML. Conventional cytogenetics, FISH and molecular studies are of great importance at diagnosis and monitoring of CML patients as they provide useful information with prognostic and therapeutic implications.

2.P42

An evaluation of array comparative genomic hybridisation (aCGH) as a method of improving the detection rate of genetic aberrations in myelodysplastic syndrome patients.

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Myelodysplastic syndromes (MDS) consist of a heterogeneous group of haematopoietic stem cell disorders characterised by dysplastic and ineffective blood cell production. Although karyotyping is considered the 'gold standard' in the cytogenetic characterisation of MDS, approximately 40–50 % of the patients exhibit a normal karyotype. Recent studies have reported the ability of aCGH to detect cryptic copy number alterations and regions of copy-neutral loss of heterozygosity (cnLOH) in MDS.

DNA was extracted from stored fixed pellets from 32 diagnostic MDS patients with both normal and abnormal karyotypes. Samples were anonymised and processed using the Agilent 4x180K Cancer CGH & SNP array. 31/32 samples provided an acceptable diagnostic array result. Array CGH detected additional cryptic copy number alterations and large cnLOH regions, not detected by karyotyping.

Array CGH identified a crytic copy number loss (confirmed by FISH) on chromosome 21q, encompassing part of the RUNX1 gene. In contast to ALL, the literature suggests that partial deletions of RUNX1 are a common finding in MDS. Large terminal regions of cnLOH on chromosome 4q were idenified in 2 MDS patients with an apparently normal karyotype. Recurring cnLOH on chromosome 4q have previously been reported in MDS patients and these regions are known to include the tumour suppressor gene, TET2. Array CGH also detected a large cnLOH region on chromosome 17p that included TP53. Subsequent sanger sequencing revealed a TP53 Lys132Gln mutation. A balanced translocation present in one patient was not identified by aCGH.

Array CGH identified all except one of the karyotypic abnormalities in our patient cohort, helped to define cytogenetic breakpoints and identified cryptic copy number alterations and cnLOH regions not previously detected.

2.P43

Rare recurrent t(15;21) translocations disrupting RUNX1 in myeloid leukemia.

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RUNX1 (21q22), encoding a transcription factor crucial for hematopoiesis, is described as a target of multiple translocations in leukemia. More than 55 cytogenetic bands are described as partners, although only 21 of them were identified.

We here describe four cases [three AML (cases 1, 2 and 3) and one CML (4)] showing a t(15;21) translocation disrupting RUNX1. FISH analyses revealed that the breakpoints were respectively located between exons 6-7 (case 4) and 7–8 (cases 1 to 3) of the gene. The mapped breakpoints on chromosome 15 showed recurrence in cases 2, 3 and 4 within the basic helix-loop-helix TCF12 transcription factor gene at 15q21.3. The juxtaposition of TCF12 to RUNX1, with an opposite transcriptional orientation, however, excluded the origin of a fusion transcript. Another breakpoint was mapped in case 1 within SIN3A (15q24.2) encoding for a transcriptional regulator. The translocation juxtaposed the 5′end of SIN3A to the 3′end of RUNX1, with the same transcriptional orientation. RNA-Seq and RT-PCR experiments confirmed the occurrence of a SIN3A/RUNX1 fusion transcript. In the same case, we identified another out of frame chimera fusing the 5′ end of RUNX1 to the long non-coding RNA UBL7-AS1 gene, interrupted at intron 1. Since the two genes showed an opposite transcriptional orientation, a submicroscopic inversion event should be envisaged to explain the genesis of such fusion gene.

Thus, we report two novel t(15;21) translocations involving RUNX1, one of them as recurrent. Interestingly, in case 1, we identified two chimeric genes involving the 5′ and the 3′ of RUNX1 respectively fused with UBL7-AS1 and SIN3A. Further experiments are presently in progress to elucidate the pathogenetic role of the newly described and, in one case, recurrent rearrangements in myeloid leukemia.

2.P44

Translocation t(6;9)(p22;q34) in a patient with acute myeloblastic leukemia

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INTRODUCTION: The t(6;9)(p23;q34) occurs with an incidence of 1–5 % in adult patients with acute AML with poor prognosis. The t(6;9) is reported mostly in young adults. The symptoms are due to the cytopenias often seen in leukemia.t(6;9) results in a chimeric fusion gene between DEK(6p23) and CAN/NUP214(9q34). Prognosis is usually poor, and complete remission is achieved with conventional chemotherapy in only 50 % of the cases.

MATERIAL AND METHODS: We performed FISH analysis including PML/RARα,AML1/ETO,inv16,del5q,MLL, del7q,del20q,p53 probes. Karyotype analysis of bone marrow was applied for the patient. Immunophenotypic analysis with flow cytometry was was made in the diagnose stage.

RESULTS A 35 years old male was diagnosed with AML-M1.He came to our attention for a marked leukocytosis(28.7×103/μl),anemia(Hb 7.0 g/dl),thrombocytopenia (102×103/μl) and hepatosplenomegaly. According to FISH analysis all the regions were normal. Conventional cytogenetic analyses detected a 46,XY,t(6;9)(p23;q34) karyotype. The patient was treated with Idarubicin HCL(12 mg/m2×3 days) and Cytarabine(100 mg/m2×7 days). Then based on the absence of complete response (CR),the same protocol was repeated. Allogeneic hematopoietic stem cell transplantation (HSCT) was planned for this patient.

CONCLUSION: Although t(6;9) is mostly associated with subtypes of AML(M1,M2,M4),considered by some researchers to be a separate disease entity because of its distinct clinical and morphologic features and poor prognostic implication. The presence of this karyotype is accepted to be an indicator for allogeneic hematopoietic stem cell transplantation (HSCT). Molecular follow-up of minimal residual disease is helpful in managing the disease. WHO classification of hematopoietic tumors emphasizes the importance of prognostic impacts of cytogenetic abnormalities. AML with t(6;9)(p23;q34) is a rare status but associated with distinctive morphologic and clinical features.

2.P45

Translocation t(12;22)(p13;q11) in a patient with AML M1

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Introduction Translocations involving the band 12p13 are one of the most commonly observed chromosomal abnormalities in human leukemia and myelodysplastic syndrome.

ETV6 gene located in 12p13 is often involved in this chromosomal rearrangements.

At present 28 translocations have been characterized. t(12;22)(p13;q11), one of these translocations, has been reported in very few cases so far. Patients reported as t(12;22)(p13;q11) were diadnosed with AML M0, M2, M4, AML on MDS,CML (Ph-).

We present here the first case of acute myeloblastic leukemia without maturation (FAB type M1) with t(12;22)(p13;q11).

Material and Methods Cytogenetic analysis, AML-MDS FISH panel (del 5q, PML/RARα, TP53 deletion, AML/ETO, MLL breakapart, del 7q,CBFβ/MYH11translocation, del20q), TEL(ETV6)/AML1 and BCR/ABL FISH analysis were performed on bone marrow and peripheral blood samples.

Results The patient diagnosed with AML M1 was 62 years old. There was no abnormality in the AML-MDS FISH panel. The result of cytogenetic analysis performed on bone marrow sample was 46, XY, t(12;22)(p13;q11). BCR/ABL and TEL(ETV6)/AML1 FISH analysis were performed to define breaking points on derivative chromosomes 22 and 12. BCR signals were normal on bone marrow cells but the rearrangement of ETV6 (12p13) region was observed in 15 % of peripheral blood cells examined. Molecular analyzes were planned.

Conclusion We identified t(12;22)(p13;q11) involving ETV6 gene in a AML M1 patient with cytogenetic analysis and FISH method. In the literatue, t(12;22)(p13;q11) in hematologic malignancies is associated with MN1-ETV6 fusion gene. MN1 is a transcription co-activator of the retinoic acid receptor (RAR). RAR is a key regulator of proliferation and differentiation of blood cell lineages. The MN1-ETV6 fusion protein is a dominant-negative suppressor of MN1. Thus, MN1-ETV6 fusion gene contributes to the development of leukemia.

To our knowledge, AML M1 with t(12;22)(p13;q11) is the first case in the literature.

2.P46

Cytogenetic studies on a chronic myeloid leukemia case developing into acute myeloid leukemia after treatment with imatinib.

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A 34-year-old male was diagnosed with CML in April 2006. All metaphases in the bone marrow showed t(9;22)(q34;q11.2). After one year of daily treatment with 400 mg imatinib no Philadelphia-chromosome positive metaphases were found in the bone marrow. FISH showed 2.5 % of the 200 interphase nuclei positive for BCR/ABL1. Surprisingly, a monosomy 7 was detected in 15 out of 20 metaphases, whereas at this time no clinical signs of MDS or AML were present. Three months later, the bone marrow showed 3 clones: 46,XY,t(9;22)(q34;q11.2)[2]/45,XY,-7[13]46,XY[5]. There were still no morphological signs of MDS or AML. After three years, monosomy 7 was detected in all metaphases and by FISH in 90 % of 200 interphase nuclei. Again FISH for BCR/ABL1 was negative. Bone marrow cytology showed AML. The patient received induction therapy with cytarabine and daunorubicine and one cycle of amsacrine and cytarabine. Then cytogenetic studies of the bone marrow revealed 3 clones: 45,XY,-7[3]/47,XY,+Y[5],46,XY[22]. FISH was positive again for BCR/ABL in 2,5 % of the 200 interphase nuclei. In June 2012 the patient underwent a double cord transplantation and remained in remission for 17 months. In November 2013 the patient had a relapse of the AML with the following karyotype: 45,XY,del(6)(q13),-7[9]/46,XY[17]. FISH analysis revealed 33 % cells with monosomy 7, but no presence of BCR/ABL1. The patient received two cycles of chemotherapy and in February 2014 an allogeneic stem cell transplant from a related donor.In Feb.2015 the patient is still in complete remission.

Transformation of a Ph-negative clone with monosomy 7 and no clinical signs of MDS into overt AML is a rare but recurrent phenomenon. So, patients showing monosomy 7 during imatinib treatment should be closely monitored both cytogenetically and clinically for signs of MDS or AML.

2.P47

Loss of the Y chromosome in myelodysplastic syndromes

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Introduction: The clinical association between loss of the Y chromosome and MDS has been debated, because both phenomena are related to aging. The objective of this study was to asses if the the mean age is related to the loss of the Y chromosome.

Material and Methods. From 1986 to July of 2014, 875 patients were diagnosed of Myelodysplastic Syndrome (MDS) in the Hospital Clinic of Barcelona and 275 (55 %) were male. ANOVA was used for age comparison between groups.

Results. Twenty-six (6 %) patients showed a loss of the Y chromosome, in most of them as a sole abnormality. According to the WHO classification about half of these 28 patients were affected of refractory cytopenia with multilineage dysplasia (RCMD) (n=16). The mean age in patients with loss of the Y chromosome was 77 years old (95 %CI: 74 to 80), significantly higher than that observed in patients with either a normal karyotype (71 y.o.; 95 %CI: 70 to 73) or with an abnormal karyotype without loss of the Y chromosome (61 y.o.; 95 %CI: 65 to 70).

Conclusion. Based on our results, the loss of the Y chromosome is observed more frequently in a group of elder MDS patients.

2.P48

FISH as a useful tool for diagnosing a hematologic disorder

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In myelodysplastics syndromes (MSD) Fluorescence In Situ Hybridization (FISH) is a powerful tool for patient prognosis. We describe a case in which this technique was used to something more.

85-year-old patient referred to our service for Monoclonal gammopathy with undetermined significance (MGUS) to reject progression.

Marrow cytology showed a moderately reactive with mild dysplastic changes.

Flow cytometry detected slight alterations in erythroid size accompanied of 0.6 % of proerythroblasts, relative to the total cellularity. Myeloid cell population moderately hypogranular without antigenic alterations. 0,7 % clonal plasmatic cells. Final immunodiagnosis: Monoclonal gammopathy MGUS type in the context of discrete cellular dysplasia possibly associated with MDS.

The pathologist reported a hypercellular bone marrow (60 %) with mild reticulin fibrosis (grade 1/3) and osteoesclerosis. Hyperplasia was found in all three series. Granulocytic series with proper maturation; red series of dispersed location and megakaryocytic series constituted by large forms that are not added. Blast percentage did not exceed 3 %. The pathologist's definitive diagnosis: clinical and laboratory correlation is necessary for diagnosis of myelodysplastic syndrome. Plasmacytosis interstitial (15 %) CD138 +, CD56 -, Lambda+. Compatible diagnosis with MGUS.

Conventional cytogenetics showed a normal karyotype. FISH analyses were performed with probes from Vysis: LSI EGR1/D5S23, D5S721 Dual Color Probe, D7S486/CEP7 FISH Probe Kit, CEP 8 (D8Z2) SpectrumGreen Probe, TP53 /CEP 17 FISH Probe Kit and LSI D20S108, 20q12, Spectrum Orange. 20q deletion was found in 13 % of cells.

With all the data collected, the final diagnosis was Refractory anemia with 20q deletion (IPSS-R: very low, 1)+Monoclonal gammopathy with undetermined significance IgA Lambda.

In this case the molecular cytogenetics has not only served like prognostic indicator, it was the real key tool for diagnosis.

2.P49

Genetic alterations distinguishing molecular subtypes of papillary thyroid tumors may influence the behaviour of corresponding stem-like cancer cells

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Introduction and aim. RET/PTC1 activation and BRAFV600E mutation distinguish two molecular subtypes of papillary thyroid tumors and confer distinct biological behaviour to the associated carcinoma. These genetic alterations are maintained in the TPC-1 and B-CPAP PTC-derived cell lines, respectively. From these cell lines (CLs), we have recently isolated thyrospheres (ThySp), with stem-like tumor cell properties, still bearing the genetic alterations of the parental adherent cells (AdhC). To explore the possible influence of the specific alterations on ThySp behaviour, we compared some functional and molecular aspects of ThySp isolated from the two CLs. Specifically, we compared self-renewal (SR) and sphere-forming efficiency (SFE), expression of stemness (OCT4/Nanog/ABCG2/ALDH1-A1) and epithelial/ thyroid differentiation markers (CK19/TTF1/PAX8) in both AdhC and ThySp.

Results FISH and real time-PCR confirmed RET/PTC1 and BRAFV600E in TPC-1 and B-CPAP ThySp, respectively. B-CPAP ThySp maintained SR and SFE for a significantly higher number of generations compared to TPC-1 ThySp. The main difference in the molecular behaviour between the CLs was the lack

of expression of early and late differentiation markers in TPC-1 AdhC and ThySp. Indeed, CK19 marker was present in B-CPAP AdhC but not in ThySp, which expressed TTF1 at lower level than AdhC. ALDH1-A1 was negative in both TPC-1 ThySp and AdhC, higher expression was seen in B-CPAP ThySp than AdhC.

Conclusions. Our molecular and functional findings indicate that B-CPAP express a wider panel of stemness markers and that B-CPAP ThySp rather than TPC-1 ThySp contain cells behaving as stem-like tumor cells. On the strength of our results, we cannot ruled out the hypothesis that BRAFV600E and RET/PTC1 may influence the functional and molecular features of B-CPAP and TPC-1 ThySp. Fund: PRIN N° 2012Z3F7HE and RAS.

2.P50

Chromosomal aberrations, mutations and polymorphisms as prognostic factors in endometrial cancer development

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Objectives: The primary end-point of the research project, undertaken in a group of patients with endometrial hyperplasia, was an identification of the earliest and specific genetic changes which could be associated with an increased risk of transformation into malignancy. The secondary end-point of the reported research was a characteristics of genetic processes, associated with advanced malignant formations.

Material and methods: The studies involved forty-four (44) female patients, including five (5) with no histopathologically confirmed hyperplastic features, twenty-six (26) with histopathologically confirmed endometrial hyperplasia and thirteen (13) patients with diagnosed carcinoma of the endometrium. The studies were supported by the use of a custom-made 4x180K microarray of BlueGnome.

Results: The patients with no endometrial hyperplasia presented with copy number variations (CNV), which are normally found with varying frequency in the genome of the healthy female population. A significant genome imbalance was identified in the twenty-six (26) (100 %) patients with diagnosed hyperplasia and in eleven (11) (84.6 %) with diagnosed endometrial cancer. Other, not yet reported changes were also observed in characteristic regions of the genome: 14q32.33 and 15q11.2.

Conclusions: A study of chromosomal instability, which determines the type and extent of chromosomal changes, is sensitive enough to uncover critical regions and, consequently, isolate genes or groups of genes supporting the formation and development of endometrial malignancy. The degree of genome imbalance can thus be considered as a risk factor for endometrial cancer.

2.P51

Distrubution of chromosomal abnormalities in adult acute myeloid leukemia patients: A single center study

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Acute myeloid leukemia (AML) is a heterogenous group. Cytogenetic abnormalities are found in more than 50 % of adult AML patients. Cytogenetics has significant role in classification of AML according the World Health Organization (WHO) classification.

In this study, we present our cytogenetic data of 303 adult AML patients retrospectively. The study included 16 years and older patients (range 16–89, median 45) who were reffered to our laboratory between 1996 and 2014. Cytogenetic analysis was carried out succesfully in 247 patients (81.5 %). We detected clonal chromosomal abnormalities in 55 % of analyzed patients.

Reccurent abnormalities for AML; t(8;21) and t(15;17) were found in 4 and 3.2 % of the patients respectively. −5/del(5q) were found in 5.26 % of the patients while -7/del(7q) were 7.28 %. 11q23 rearrangements were present in 2.4 % and +8 were detected in 4.45 % of the patients. Abnormalities of 12p, −13/del(13q) and del(9q) were observed in 2 % of the patients each. t(4;19)(q21;p or q13), which was not reported previously in AML with these breakpoints, was observed in two patients in our study.

Most of the abnormalities reported above were part of a complex karyotype. In 12.5 % of the patients found complex karyotype. Recent studies identified monosomal karyotype (MK) which is defined by presence of a clone with at least two separate autosomal monosomies or one monosomy beside one or more structural abnormalities. MK were shown in 36 (14.5 %) of our patients. The majority of these patients also had a complex karyotype. MK was reported ~10 % in all AML and up to 20 % in older patients by previous studies.

In accordance to literature, we concluded that frequency of balanced abnormalities were more common in younger group (16–34 years) and frequencies of unbalanced abnormalities, complex karyotypes and monosomal

2.P52

13q deletion and survival of patients with light chain multiple myeloma

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The recent advances in investigative techniques that have helped refine the diagnostic work up in multiple myeloma (MM) includes use of serum free light chains, and use of cytogenetic and FISH to determine prognosis. 14q rearrangements, del13q, amplification of 1q21 and del17p are especially important prognostic markers which are used for risk stratification for MM patients. Although most MM cases are characterized by the detection of a monoclonal immunoglobulin in the serum, in about 15 % of the patients, no complete monoclonal immunoglobulin can be detected in the serum; these patients produce large amounts of light chains. These patients are commonly referred to as having light-chain MM. In most of these patients, large amounts of monoclonal light chains are detected in the urine and to a lesser extent in the serum. These light-chain MM (LC-MM) patients have in two thirds of the cases a κ-LC type, whereas the remaining one third of the patients produces λ-LC. Although improved predictive risk stratification models which have included international staging systems as well as FISH-detected chromosomal changes used for guiding treatment algorithms in MM, the prevalence of genetic prognostic factors for these LC-MM cases is unknown. Because of the prognostic significance of 13q deletions in multiple myeloma, we sought to define the frequency and significance of this abnormality in LC-MM patients tested in the routine clinical laboratory. We reviewed laboratory characteristics of patients with LC-MM and with monoclonal gammopathy who had FISH analysis for 13q deletion performed as part of their routine clinical testing using the standard screening multiple myeloma panel employed at our laboratory. Of the bone marrow specimens from monoclonal gammopathy patients, 10 % had 13q deletions. The corresponding frequencies were significantly different between MM and LC-MM groups: 6 and 20 %, respectively.

2.P53

Follow-up of del(5)q MDS patients with or without other chromosomal abnormalities

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Myelodysplastic syndrome is the most common hematologic malignancy characterized by ineffective hematopoiesis leading to persistent peripheral blood cytopenia and predisposition to acute myeloid leukemia whereas interstitial deletion of chromosome 5q is the most common cytogenetic abnormality in this

syndrome. Lenalidomide, is a second generation, immunomodulatory drug that was approved for treatment of transfusion dependent anemia in patients with lower risk MDS with interstitial deletion of the long arm of chromosome 5. The experiences with lenalidomide in patients with lower risk MDS with deletion 5q showed cytogenetic responses, independent of the presence or absence of additional cytogenetic abnormalities. Therefore 5q deletion is drawing increasing attention.

We analyzed clinical and laboratory characteristics and follow up information of 50 MDS patients with 5q deletion. All these patients were scored according to the revised International Prognostic Scoring System (R-IPSS). In our study, 9 patients (18 %) had isolated del5q and the remaining 41 patients (82 %) had additional chromosomal abnormalities. In our study the most frequent chromosomal abnormality concomitant with del(5)(q) was 7q deletion. During the follow-up transformation of myelodysplastic syndrome into acute myeloid leukemia rates in the patients with del(5)(q) alone and del(5)(q) with other chromosomal abnormalities were 66.7 and 65.9 % respectively. Twenty nine of the patients were under the treatment and three of them received lenalidomide therapy. In the group of del(5)(q) alone 22 % of the patients were in remission with a 12 months follow up. At the same period, the remission rate was 7 % the in patients with del5q and other chromosomal abnormalities. The median survival was 20 months in patient group with del(5)(q) alone, and 12 months in the group with additional abnormalities accompanying del(5)(q).

Here, we present genetic abnormalities and clinical implications of 50 MDS patients with del(5)(q).

2.P54

The influence of CpG-oligodeoxynucleotide/IL2 stimulation on quality of routine cytogenetic analysis in CLL

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Chromosome banding analysis in CLL is hampered by a low in vitro proliferation rate of B-CLL lymphocytes. Combination of CpG-oligodeoxynucleotides and IL-2 (CpG-IL2) has been proposed for stimulation of B-lymphocytes division in growth media. We assessed how the introduction of CpG-IL2 influences the routine cytogenetic analysis in CLL.

24 samples (peripheral blood - 7, bone marrow aspirate - 17) were cultivated in parallel with or without addition of CpG-IL2. Mitotic indexes were determined in each sample to evaluate proliferation rates. To evaluate the influence of CpG-IL2 stimulation on cytogenetics results we analyzed two groups of different samples (55 each) which were cultivated with or without addition of CpG-IL2. FISH analysis for recurrent aberrations (del(11q), +12, del(13q), del(17p), IGH rearrangement) was done in all samples. In 36 samples of each group G-banding analysis was additionally performed.

When we evaluate proliferation rates of same samples cultivated with or without addition of CpG-IL2, we found that mitotic indexes were significantly increased in blood samples only ($p<0,05$). The importance of CpG-IL2 stimulation was revealed when the results of banding analysis were evaluated. Unsuccessful banding analysis has been reduced from 22 % to less than 3 %. Since the effect of CpG-IL2 on malignant B-lymphocytes is selective the proportion of false negative results was considerably reduced. Proportion of normal karyotypes in patients with recurrent abnormalities detected by FISH has decreased from 20 to 3 %. Additionally, proportion of CLL patients with chromosomal aberrations that were revealed only by banding analysis has increased from 14 to 33 %.

Our study confirmed that cultivation of CLL samples with CpG-IL2 stimulation is mandatory when banding analysis is performed. It enables fulfillment of the latest ECA quality standards for bone marrow aspirates as well as for peripheral blood.

2.P55

Study of fluorescence in situ hybridization in malignant melanoma

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Morbidity and mortality rates of malignant melanomas are reported to be increasing every year. Since there are a subset of cases where histopathologic discrimination between malignant melanoma and melanocytic nevi may be exceedingly difficult, genetic and genomic analysis have yielded new opportunities for improvements in diagnostic accuracy for the distinction of nevus from melanoma and better selection of patients affected by melanoma for targeted treatment. In this study, we aimed to determine the ability of FISH analysis targeting CCND1, MYB, RREB1, CEP6 regions in the samples from 18 malignant melanoma and 24 melanocytic nevi. The results showed copy number alterations in 15 and 4 cases with malignant melanoma and melanocytic nevi, respectively. In contrast to the literature, the CCND1 gene amplification was significant ($p<0,001$). The other aim of the study was to determine the relation between the copy number alterations and prognosis of the diseases. The FISH analysis specific to EGFR, P16, P53 and MDM2 genes were performed. The EGFR gene amplification showed significant difference between malignant melanoma and nevi cases ($p<0,001$). No relation between genomic copy alterations and prognostic values (ulcer, lenf infiltration etc.) was seen. We concluded that CCND1, MYB, RREB1 and EGFR alterations have a potential to discriminate between malignant melanoma and melanocytic nevi in challenging cases.

2.P56

Isochromosome 5p—a rare chromosome abnormality in myeloid disorders

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Although loss of chromosome 5q is a common abnormality in patients with AML and MDS, isochromosome of chromosome 5p is a rare abnormality. Two types of i(5p) have been reported: type 1 is formed by loss of 5q and gain of 5p and type 2 includes extra i(5p) and two normal homolog chromosomes 5. To date, i(5)(p10) has been reported in 24 cases. Of them, 11 were type 1 and 13 were type 2. Here we reported 2 cases with i(5p). The first case who had been treated for bladder cancer before developed MDS, diagnosed as RAEB-II. Cytogenetic and FISH analyses of the bone marrow revealed an aberrant karyotype, showing type 1 i(5p) and a deletion in 12p. Following two cycles of successful treatment with 5-azacytidine, the case died at home and therefore the death reason could not be determined. In the second case, the diagnosis of AML was established. Cytogenetic and FISH analyses showed both type 2 i(5p) and trisomy 8. The case died due to severe pneumonia. The presence of type 2 i(5p) accompanied by trisomy 8 could have been reported in only 9 cases. It is controversial whether i(5p) is the seconder abnormality. In the second case, the clones with either pure i(5p) or pure trisomy 8 were seen. Although poor prognosis of the patients with i(5p) clones have been reported, reporting and collecting data of rare chromosomal abnormalities will add information to disease progression and prognosis.

2.P57

Frequency of genetic changes in pediatric leukemias diagnosed with the help of FISH at Paraná state hospital

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FISH is a valuable tool for the identification of genetic changes, because leukemic blasts often give poor quality metaphases or none at all. In this sense, FISH is recommended by WHO as a method that can be used on interphase nuclei, and replace techniques that have false negative results, technical problems and to confirm other techniques. This research aimed to evaluate the incidence of genetic alterations presented in pediatric patients diagnosed with acute leukemia, and associate these changes with age, gender and blood test results, classical cytogenetics and immunophenotyping. Thus, a quantitative cross sectional study was performed with

retrospective data collected from medical records from beginning FISH analysis, from January 2013 to December 2014. Seventy four samples were analyzed, 58 (79 %) of which from the Hospital Infantil Pequeno Príncipe and 16 (21 %) from the Hospital de Clínicas da Universidade Federal do Paraná; 36 (48 %) samples were obtained from male patients and 38 (52 %) from female patients. The analysis by FISH showed 20 samples with alterations, being 2 BCR/ABL1 positive, 6 MLL positive and 5 ETV6/RUNX1 positive samples. The incidence of MLL gene rearrangement, BCR/ABL1 and ETV6/RUNX1 gene fusions was 30, 10 and 25 %, respectively. Data analysis indicated that the fusion gene BCR/ABL1 had similar frequencies in all age groups up to age 15, the fusion genes ETV6/RUNX1 and MLL had the highest until the fifth year of life and decreasing with age; but ETV6/RUNX1 was the most frequente finding in this study. Two BCR/ABL1 positive cases with a confirmation by cytogenetics. Six MLL positive but 2 cases confirmed by cytogenetics. 5 ETV6/RUNX1 positive cases had no translocation by cytogenetics. The results of PML/RARα were negative by FISH.

2.P58

Atypical proximal deletions of the long arm of chromosome 20 retaining D20S108 locus in myeloid malignancies.

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Deletion of the long arm of chromosome 20 (del20q) is a common finding in myeloid disorders. The 20q deletion breakpoints are heterogeneous and the commonly deleted region (CDR) is different in various myeloid pathologies but overlaps within 20q12 and D20S108 locus. Our objective was to focus on and characterize the deletion size of atypical proximal 20q deletion in 2 myeloid patients using fluorescent in situ hybridization (FISH).

In the past 10 years, we identified two patients with myeloid malignancies and atypical 20q deletion (a 74-year old male with myelodysplastic syndrome and a 84 year-old male with acute myeloid leukemia with multilineage dysplasia).

In order to assess the del20q CDR, we performed FISH using the 20q12 probe (Vysis, Abbott, Rungis, France) and a library of Bacterial Artificial Chromosome (BAC) clones located in bands 20q11 and 20q12.

The CDR encompassed 5.6 Megabases (Mb), between bands q11.1 and q11.23, and 8.6 Mb, between bands q11.21 and q12, for patients 1 and 2, respectively. No recurrent breakpoint was found. D20S108 locus was retained and 20q deletions were large, interstitial and proximal in 2 cases.

In our patients, the 20q deleted regions included several genes, notably *HCK*, *ASXL1* and *DNMT3B*. Haploinsufficiency of these genes could play a role in myeloid disorder.

20q deletion is a recurrent cytogenetic abnormality with a favorable outcome according to the revised International Prognostic Scoring System. Although very rare, atypical proximal del20q is a recurrent abnormality. It could be underestimated without use of FISH probe focusing on D20S108. Whether the leukemia process and prognosis of this anomaly is identical to the classic 20q deletion in myeloid pathologies remain to be established.

2.P59

Associations between HER2/neu,TOP2A, chromosome 17 copy numbers, and CDH1 and GSTP1 gene promotor hypermethylations of patients with breast cancer

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Introduction: Breast cancer is an important public health problem worldwide. The HER2 /neu protooncogene is amplified and overexpressed in approximately 25–30 % of invasive breast carcinomas. DNA topoisomerase 2-alpha enzyme controls and alters the topologic states of DNA during transcription. GSTP1 plays an important

role in protecting cells from cytotoxic and carcinogenic agents and it is expressed in normal tissues at variable levels in different cell types. CDH1 plays a critical role for establishment and maintenance of polarity and differentiation of epithelium during the development period. Also, it plays an important role in signal transduction, differentiation, gene expression, cell motility and inflammations.

Method: In this study we analysed retrospective HER2/neu, TOP2A gene and Chromosome17 copy number alterations by fluorescence in situ hybridization (FISH) in primary tumor core biopsies from 100 high-risk primary breast cancer patients (tumors ≥2 cm and/or lenfatic metastase and/or distant metastases and/or under 40 years) . The methylation levels of the CDH1 and GSTP1 gene promoters were assessed Methylation Sensitive High Resolution Melting Analysis (MS-HRM).

Results: In our study, HER2/neu amplifications were identified in 22 % and TOP2A amplifications in 21 % and deletions in 4,8 % of patients. HER2/neu and TOP2A amplifications are found to be associated with IDC tumor type and high grade also HER2/neu amplifications is associated with PR(-), TOP2A amplifications is associated with ER(+). TOP2A deletions is associated with ER(-) and PR(-). Polysomy17 was present in 22 % and monosomy 11 % of patients CDH1 and GSTP1 methylation frequencies were 83,7 and 74,1 % respectively.

Conclusions:. Our study is important as being the first study that analyzes association between HER2/neu, TOP2A gene copy numbers and CDH1, GSTP1 gene promotor methylation status in Turkish population.

2.P60

SKY analysis of small marker chromosomes in acute leukemia patients

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In combination with the conventional banding and FISH, Spectral karyotyping (SKY) has become an important technique that improves the chromosome analysis. It has been shown to improve detection rate of cryptic aberrations in apparently normal samples and the accuracy in resolving complex chromosomal rearrangements. It is also an invaluable diagnostic tool for establishing the origin of small supernumerary marker chromosomes (sSMCs). SMCs characterization is of utmost importance for genetic counselling in prenatal diagnosis but also for prognostication in hematological malignancies.

Short term cultivated bone marrow cells from four acute leukemia patients were analyzed by conventional banding and FISH applying different DNA probes. G-banding revealed one or two marker chromosomes as an isolated anomaly in all patients. FISH did not resolve the origin of markers. Therefore, we decided to apply SKY (Applied Spectral Imaging, Carlsbad, CA) followed by FISH to confirm SKY findings. Several methaphases of sufficient quality were analyzed in each patient.

In one patient with two distinct markers SKY clearly showed both markers to belong to chromosome 8. In other three patients SMCs origin was not unequivocally resolved, due to insufficient chromosomal material (<6–10 Mb in size) or heterogeneity of markers.

Although SKY is an elegant technique which enables simultaneous analysis of all chromosomes, it has limitations in routine analysis of malignant diseases. Metaphase spreads of sufficient quality and quantity are needed to capture different possible clones. Albeit these conditions are fulfilled the technique can help to resolve only larger SMCs. In some patients however, SKY can contribute to a more accurate evaluation of prognostic significance of chromosomal aberration observed consequently influencing also therapeutic decisions.

2.P61

A case of TdT+ “triple-hit” lymphoma

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t(c-MYC/8q24) are the hallmark of Burkitt lymphoma (BL) but occur in subset of other mature-B-cell lymphomas as secondary aberrations. "Double-hit" or "triple-hit" lymphomas (DHL and THL) are rare subtype of lymphomas characterized by concurrent chromosomal rearrangement of c-MYC/8q24 with BCL2/18q21 and/or BCL6/3q27, complex karyotype, mature B-cell immunophenotype, Ki67~100 %, aggressive clinical behavior and poor prognosis.

We report on a case of THL with immunophenotype of B-lymphoblastic leukemia/lymphoma

(CD10+,CD38+,CD45+,PAX5+,CD79a+,CD99+,BCL-2+,BCL-6(-/+),TdT(-/+),CD34-,Ki-67-80-90 %) in 39-year-old male patient. Lymph node biopsy cells were studied at diagnosis.

Conventional cytogenetic analysis revealed complex karyotype with typical for follicular lymphoma (FL) t(14;18)(q32;q21) and presumably c-MYC and BcL6 translocations: 48,XY,dup(1)(pter→q31::q21→qter),t(3;?22)(q27;?q11),del(6)(q21),+7,+8,t(8;?22)(q24;?q11),t(14;18)(q32;q21),der(14)t(14;18)(q32;q21).

We performed molecular cytogenetic analysis that included interphase and metaphase FISH, M-FISH and M-BAND with probes: LSI c-MYC BAP, LSI c-MYC/IGH/cep8 DFTP, LSI Bcl2 B.P. LSI Bcl2/IGH DFTP, LSI Bcl6 BAP (ABBOTT), XL IGL BAP, XL IGK BAP, 24XCyte Human Multicolor ProbeKit and Human mBAND ProbeKit (MetaSystems). Molecular cytogenetic study confirmed the presence of "triple-hit"—t(BcL2/18q21) and c-MYC/8q24 and BCL6/3q27 translocations to both alleles of IgL/22q11. Furthermore, in our case it was shown that: the original clone contained a typical t(14;18)(q32;q21) involving one allele of chromosome 14, but there was a loss of normal chromosome 14 and doubling of its derivative in tumor progression at the preclinical level; Bcl6 rearrangement is represented by duplication of 3q27 region and translocation BcL6/3q27 to IgL/22q11 locus with deletion of its centromeric region on chromosome 22; c-MYC locus break point is located more telomeric than in typical Burkitt lymphoma's translocations; duplication of 1q is inverted. Detailed cytogenetic and molecular cytogenetic study is necessary for differential diagnosis of DHL and THL with BL, FL, diffuse large B-cell lymphoma as well as due to the possible aberrant "immature" immunophenotype with lymphoblastic lymphoma, to search for adequate treatment strategies for these patients.

2.P62

Optimal strategy for obtaining chromosome and fluorescence in situ hybridization results for plasma cell dyscrasias by using both positive and negative fractions of CD138 enriched plasma cells

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Plasma cell dyscrasias (PCD) comprise a genetically diverse group of diseases such as monoclonal gammopathy of undetermined significance, plasmacytoma, smoldering myeloma, indolent myeloma, and plasma cell myeloma. FISH is superior to chromosome analysis (CA) in detecting important prognostic genetic abnormalities in PCD, however, its sensitivity is hampered due to paucity of plasma cells (PC) in whole bone marrow (WBM) and false-negative results when frequency of abnormal cells is below cut-off values. Studies have shown that abnormality detection rate in EPC is greater than unselected plasma cells (UPC), but purification techniques are limiting to FISH when sample volumes are inadequate. The inability to perform CA compromises patient care since CA is equally important for detecting non-PC related abnormalities, such as secondary myelodysplastic syndrome. To optimize limited sample volumes, we designed a study where an immnuno-magnetic CD138 enriched positive selection was used for FISH while the myeloid elements in the negative selection were used for CA. FISH studies were performed on EPC, and parallel karyotyping by WBM and the negative selection. Purity of EPC was confirmed by flow-cytometry (47.3 to 96.9 %). FISH results showed that the abnormality rate of EPC (79 %) doubled compared to UPC (34 %), and CA displayed 100 % (154/154) success rate using the discarded myeloid elements. PCD abnormalities were confined to WBM while non-PCD abnormalities were found in both WBM and supernatant confirming effective removal of PC by isolation and efficient utility of the discarded myeloid elements for CA. Our results demonstrate the feasibility of using the negative fraction for CA.

3. Prenatal Diagnosis

3.P1

High resolution array-CGH screening of 5-day embryos: focus on interstitial structural CNAs with 8x60k Agilent platform

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The utilization of genome-wide screening techniques in PGD brings novel information about the incidence of copy number aberrations (CNAs). However, most of the screening platforms currently used are focused on the detection of aneuploidies, but not structural CNAs. In this study, we validated and used a high resolution microarray platform (Agilent 8x60k) to evaluate the incidence of structural CNAs in a cohort of 5-day embryos from patients and healthy donors (DEMs).

In total, 383 (56 DEMs, 327 patient's) 5-day embryos were analyzed from 86 patients and 18 DEMs. Array-CGH screening was successfully performed in 96.1 % (368/383). CNAs were detected in both patient and DEM samples in 35.3 % (128/368).

Structural CNAs were observed in 2 DEM cases (4.3 %; 2/47) and 8.4 % of patient samples (12.5 %; 40/321). Overall, we identified 42 different structural CNAs (23 regions of gain, 19 regions of loss of genetic material). Median of size was 52.7Mbp. Most frequently affected were chromosomes 5 and 8 (both 11.9 %; 5/42, respectively).

Aneuploidies were found in 12.8 % of DEMs (6/47) and in 31.8 % of all patient's samples (102/321). Overall, we detected 190 cases of CNAs affecting whole chromosome (89 cases of trisomy, 101 cases of monosomy). The most common gains of a whole chromosome were trisomies 15 and 20 (5.3 %; 10/190, respectively), while frequent loss was observed as monosomy 16 and 22 (both 5.8 %, 11/189).

Taking together, array-CGH with high resolution microarrays is a robust and cost effective technique allowing us the detection of CNAs across the whole genome in single experiment. The utilization of a novel generation of microarrays in PGD is suitable to detect structural CNAs with a resolution vastly overcoming previous generation of BAC arrays. This study was supported by OPVK-CZ.1.07/2.3.00/20.0183.

3.P2

Prenatal diagnosis and molecular characterization of a maternally inherited derivative chromosome 9 resulting in a partial deletion 9p(9p22-pter) and partial duplication 18q23.

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Simultaneous occurrence of partial 9p deletion and partial 18q duplication is very rare, with only a few cases published in the literature. This chromosomal imbalance has been associated with different patterns of dysmorphisms as well as anorectal malformations. Here, we report prenatal diagnosis and rapid characterization by subtelomere FISH of an additional case. A 39 year-old pregnant woman carrier of a balanced translocation between chromosomes 9 and 18 was referred to our laboratory for prenatal diagnosis because of an abnormal maternal serum screening result (1/141) and intrauterine growth retardation (IUGR) at 15th weeks of gestation. Conventional cytogenetic analysis of cultured amniocytes revealed a maternally inherited derivative of chromosome 9. Further FISH analysis with subtelomeric 9p and 18q probes demonstrated an unbalanced karyotype consisting on a partial 9p(9p22->pter) deletion and a partial 18q23 duplication, reformulating the conventional karyotype as 46,XX,der(9)t(9;18)(p22;q23) mat.ish der(9)t(9;18)(305J7-T7-,VIJyRM2050+). We were informed that our patient already had a previously born child with this chromosomal rearrangement who is severely affected with clinical features that could be associated with both, the 9p deletion syndrome

and the Edwards syndrome. In this work, we review the literature of prenatal diagnosis of partial 9p deletion and partial 18q duplication and we discuss the unexpected high recurrence risk in this family, as well as candidate genes that are possibly involved in the clinical features detected in our patient's born child.

3.P3

69,XXY and 92,XXXY in two consecutive pregnancies

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Triploidy is one of the most frequent chromosome aberrations in first trimester spontaneous abortions, but a few triploid conceptions survive into the second trimester. Tetraploidy is also a common finding in early abortions, although its prevalence is much lower than triploidy. Among tetraploidies, 92,XXXY karyotypes are extremely rare. To our knowledge only six cases have been reported, five of them related to hydatidiform moles. Their origin can not obviously be due to an endoreduplication of the normal diploid set of chromosomes. The patient was referred for prenatal diagnosis due to the sonographic finding of a polymalformed male fetus. An amniocentesis was performed before TOP. The autopsy confirmed the sonographic findings, and the pathological study of the placenta did not show morphological alterations. In her next pregnancy, sonographic examination disclosed a missed abortion at 6 weeks, with a visible embryo. A chorionic villi sample was obtained for cytogenetic analysis before evacuation. Macroscopic examination of the villi sample did not reveal molar vesicular appearance.

QF-PCR and cytogenetic analyses were performed on amniotic fluid (first pregnancy) and chorionic villi sample (second pregnancy). A 69,XXY and 92,XXXY karyotypes were respectively found. QF-PCR results disclosed two maternal and one single paternal alleles in the first pregnancy (digynic triploidy), and double maternal and double paternal contribution to the tetraploidy. Among the few reported cases of 92,XXXY tetraploidy, those associated to partial moles show a PPPM genotype (3 paternal and 1 maternal alleles), and the only case with PPMM genotype was found in a spontaneously aborted fetus, similar to our case. We are not aware of other cases with combination of a digynic triploidy and a tetraploidy with PPMM contribution. Our case adds evidence of the influence of the balance between paternal and maternal genomic doses on the phenotype.

3.P4

Comparison of two academic software (RAPIDR and WISECONDOR) for aneuploidies detection using semiconductor sequencing data in a NIPT process.

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Based on a statistical analysis of low coverage genome sequencing data, non-invasive prenatal testing of aneuploidies is being provided in a growing numbers of countries. It has proved a major improvement versus classic screening strategies but still requires invasive procedures when positive. Using 500 samples included in our French multicenter study, we aim to validate two different published bioinformatics tools for aneuploidy calling. All patients included had an indicated invasive sampling to achieve fetal karyotype in parallel.

WISECONDOR (WIthin SamplE COpy Number aberration DetectOR) and RAPIDR (Reliable Accurate Prenatal non-Invasive Diagnosis R package) both require a reference set of euploid samples. RAPI DR establishes a baseline for confrontation with unknown samples. WISECONDOR uses a "within sample" normalization algorithm made from the reference set and preventing from inter-individual variation. They both use a bin segmentation approach to take into account the GC-content bias. Different QC metrics are used. Fetal fraction, a well-known cause for false negative, can be estimated for male pregnancies via RAPIDR. WISECONDOR uses an inter-chromosomal concordance test to reduce technical noise. A training set of 50 samples has shown comparable performances regarding False Negative and False Positive rates for main aneuploidies (T21, T18, T13). The study will focus on the practicality for use in routine diagnosis (time of calculation, necessary resources, setting up), reliability of QC metrics and potential discrepancies.

Both applications were initially developed using data from Illumina dye sequencing technologies. In this study we will demonstrate that semiconductors sequencing data fit to these two turnkey methods.

3.P5

Non-Invasive Prenatal Testing for the most common aneuploidies (trisomies 21, 18, and 13) using a semiconductor-sequencing platform: a French multicenter pilot study

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Combined first-trimester screening has improved prenatal screening for trisomy 21. However the number of unnecessary invasive diagnostic procedures still remains high. Non-Invasive Prenatal Testing (NIPT) using massively parallel sequencing of cell-free fetal DNA from maternal plasma, which is now part of the prenatal landscape, should drastically diminish the risk associated with invasive techniques. Several publications have established NIPT effectiveness using mainly the Illumina sequencing technology.

A French consortium of seven academic hospitals collaborates to validate a common protocol and to evaluate the efficiency and reliability of NIPT of the most common chromosomal aneuploidies using a semiconductor-sequencing platform. Indeed many French laboratories are already equipped with this technology.

A total of 500 pregnant women (between 12.3 and 35 weeks of gestation) who presented a high risk of aneuploidy and underwent fetal karyotyping were included in a prospective study. 15 % of these patients presented a fetus with one of the most common aneuploidies: trisomies 21, 18 and 13. The NIPT results matched the fetal karyotyping results in

all of the cases: all trisomies were detected. The analysis of the whole genome sequencing data (including notably librairies quantification, total raw reads per sample, estimate of plasma fetal DNA fraction) enabled us to establish the quality criteria required for its use in routine diagnosis.

NIPT using a semiconductor-sequencing platform is a rapid and cost-effective alternative to invasive procedures, and represents an attractive approach for large scale population NIPT.

3.P6

Correlation between biochemical prenatal screening for trisomy 21 and the results of prenatal chromosomal analysis – retrospective study on the 11 years (2004–2014) in the „Cuza Vodă" Maternity Iasi, Romania

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We made a retrospective study concerning biochemical prenatal screenings (BPS) for trisomy 21 (T21). The study is based on the data from Genetic Department of "Cuza Vodă" Maternity Iaşi, Romania and covers the period 2004–2014. BPS was made by triple test (TT) double test (DT) and association of these analyses (TT+DT). Prenatal diagnosis (PD) was made by by FISH (all period) and/or karyotyping (in the last three years). During this period 1974 prenatal analyses were made. 1034 (52.38 %) of them were performed because of an abnormal BPS for T21: 678 TT, 319 DT, 37 cases TT+DT. In cases with abnormal BPS we found a chromosomal anomaly in 29 cases (2.8 %), including 16 T21 (1.54 %). For abnormal TT we identified 16 chromosomal anomalies: 7T21 (1.05 %), 5 trisomies 18, 3 cases of XXY and one balanced translocation t(11;22). For abnormal DT we found 11 chromosomal anomalies: 8 T21 (2.19 %) 2 monosomies X, and one triploidy (XXX). For abnormal TT+DT we identified 1 T21 (2.7 %) and one 46,XY/47,XY,+20. In the same period we found 33 other cases with T21 by PD. PD was performed because of echographic anomalies (18 cases), advanced maternal age [AMA] (6 cases), nuchal translucency [NT] (5 cases), AMA+NT (3 cases), and a previous child with T21 (1 case). Our results—1.54 % correlation between BPS and PD—could be explained by errors in storage or manipulation of blood samples, wrong interpretation of results or gestational age. All these aspects must be improved in the future for amelioration of the detection of T21 in pregnancies without high risk.

3.P7

Aneuploidy screening using circulating fetal cells in maternal blood by dual-probe FISH protocol

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Background: A long sought goal of medical genetics has been the replacement of invasive procedures for detection of chromosomal aneuploidies by isolating and analyzing fetal cells or free fetal DNA from maternal blood thereby avoiding any risk to the fetus. However, a rapid, simple, consistent and low cost procedure suitable for routine clinical practice has not yet been achieved. The purpose of this study was to assess the feasibility of predicting fetal aneuploidy by applying a dual-probe FISH protocol to fetal cells isolated from maternal blood.

Methods: 102 pregnant women received prospective testing for fetal aneuploidy through FISH analysis of fetal cells isolated from maternal blood. Results were compared with the karyotype determined by invasive procedures, or at birth.

Results: Five out of 102 samples showed a fetal aneuploidy which was confirmed by invasive prenatal diagnosis procedures. In pregnancies with an aneuploid fetus the frequency of trisomic cells (range, 0.38–0.90 %) was at least double that seen

in samples from normal pregnancies (<0.18 %). One false positive and one false negative results were obtained.

Conclusions: Non invasive prenatal genetic screening for fetal aneuploidy is feasible using fetal cells isolated from maternal blood and this could substantially reduce the need for invasive procedures.

3.P8

Fetus with two de novo chromosome aberrations: XXX and duplication 7p

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Double chromosome aberrations involving both, a de novo structural and a numerical aberration are rare.

We report on a case in which the de-novo duplication of an autosome and a sex chromosome aneuploidy were detected.

The patient was referred to our institution for fetal karyotyping due to a positive ultrasound examination at 20th week of gestation: two soft markers for aneuploidy—a single umbilical artery and an echogenic intracardiac focus were noticed. QF-PCR for the rapid prenatal diagnosis of fetal aneuploidies after amniocentesis detected a sex chromosome aneuploidy: triple X.

The karyotype revealed an extra X chromosome and in addition a derivative chromosome 7: 47,XXX,der(7), with additional genetic material on 7p.

FISH using a subtelomeric 7ptel DNA probe showed a normal FISH pattern. To define the rearranged 7p region Array-CGH was performed indicating three copies of 7p21.3p22.3, 7.65 Mb in range and confirmed an extra X chromosome.

After aCGH, the karyotype was re-written:

47,XXX,der(7).arr (X)x3,7p22.3p21.3(1,370,653-9,023,662)x3

The clinical features of triple X syndrome are subtle and can be variable: tall stature, normal sexual development and puberty. Mental retardation is rare but learning disabilities are quite common.

Chromosome 7p duplication syndrome is a rare chromosomal disorder associated with typical craniofacial anomalies: asymmetric skull, dolichocephaly, large fontanels, high forehead, micrognathia, low set malformed ears, hypertelorism. Features vary depending on the size of the duplication and can include cardiac malformations, hypermobile joints and, in most cases, intellectual disability.

The parents decided to terminate the pregnancy.

Clinical features of the fetus and the origin of the extra X chromosome and the duplicated chromosome 7 will be discussed based on clinical examination of the aborted fetus and microsatellite analysis of genomic DNA extracted from fetal tissue as well as from blood of both parents.

3.P9

Unusually large familial euchromatic variant 16p11.2 discovered at amniocentesis: a case report

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Aim. To report an unusually large familial euchromatic variant of 16p11.2 discovered in cytogenetic studies on amniotic fluid.

Case. Amniocentesis in 42 year old woman was performed due to mother's concern and complicated family and obstetric history. Cytogenetic studies by G-banding revealed an unusually large variant 16p+ chromosome (p arm twice as long as normal 16p) in the fetus. The finding was specified using FISH with chromosome 16 specific probes (Cytocell) and the genomic content of the duplicated region was clarified using chromosomal SNP microarray analysis (Illumina Inc.). Chromosomal microarray analysis could identify merely ~1,4 Mb triplication of harmless euchromatic region suggesting the presence of heterochromatic material as well. Cytogenetic studies of parental peripheral blood revealed the phenotypically normal father to be a carrier of var(16p+).

Conclusion. It is important to differentiate and exclude harmless euchromatic variants of proximal 16p11.2 from microscopically similar pathogenic 16p11.2-p12.2 duplications. In case of documented familial 16p+variation,

that is ascertained with chromosomal microarray analysis, the predicted outcome of the pregnancy can be reported as normal.

3.P10

Limitations of NIPT: a case of confined placental mosaicism

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Non-invasive prenatal testing (NIPT) is becoming increasingly popular with companies emphasizing high sensitivity and specificity as marketing tools. These statistics may be misunderstood; especially in the case of high risk results where a positive predictive value would be a more useful measure. There may be discordance between the result of the NIPT and the fetal karyotype due to feto-placental mosaicism. Mosaicism is detected in about 1 % of chorionic villus samples taken at 10 to 11 weeks.

We present a case of a discrepant NIPT result due to confined placental mosaicism. A chorionic villus sampling was performed in a 45 year old female following a high risk NIPT result for trisomy 18. The QFPCR aneuploidy test indicated mosaicism for trisomy 18; results were consistent with a trisomy 18 conception followed by trisomy rescue generating a disomic cell line, as the abnormal cell line was present in about 80 % of the cells in the sample. Karyotype analysis of multiple chorionic villus cultures confirmed the mosaicism detected by QFPC R; 24 out of 31 cells studied showed trisomy 18. An amniocentesis was performed to determine the status of the fetus. QFPCR analysis of the heavily blood stained amniotic fluid showed two genotypes consistent with MCC, however chromosome analysis of 50 cells from two independent cultures yielded a normal male karyotype. An ulta sound scan at 22 weeks was normal and the couple was counselled about the possible outcomes; they decided to continue the pregnancy. Results from postnatal testing are awaited.

We will review the different tests (NIPT, QFPCR and chromosome analysis) comparing their methodologies and potential impact on prenatal diagnosis. We highlight the importance of understanding the positive predictive values of a screening test such as NIPT.

3.P11

Chromothripsis detected by arrayCGH in a prenatal case

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The term chromothripsis accounts for an extraordinary one-step catastrophic genomic event that generates multiple complex chromosome rearrangements within one, or a handful, of genomic regions. To date, the causes and the mechanisms of formation of these events remains unclear.

We present the first prenatal case of chromothripsis on chromosome 18 identified by array-CGH. The parental origin of the restructured chromosome was investigated by QF-PCR and further characterization of the rearranged chromosome was achieved by FISH and conventional cytogenetic analyses.

A 38-years-old woman was referred for prenatal cytogenetic diagnosis at 12 weeks of gestational age because of increased nuchal translucency (4 mm) and high risk first trimester screening (1:3 for trisomy 21 and 1:422 for trisomy 18). Chorionic villus sample was obtained and QF-PCR resulted in a female fetus with normal chromosome 13, 18 and 21copy number. Cell culture failed and no cytogenetic result was available.

Nuchal translucency of 7 mm and others ultrasound markers were detected at 16 weeks of gestation. Amniotic fluid sample was collected and QF-PCR, conventional cytogenetic, FISH and array-CGH results detected a highly rearranged dicentric chromosome 18 with loss of the entire short arm and multiple gains and losses of the long arm, with at least 11 breakpoints.

The present case highlights the usefulness of performing array-CGH in prenatal samples.

3.P12

Molecular characterization of prenatally detected small supernumerary marker chromosomes: improving the genotype-phenotype correlations

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Small supernumerary marker chromosomes (sSMC) are defined as structurally abnormal chromosomes that cannot be characterized by conventional banding cytogenetics and are generally equal in size, or smaller, than the chromosome 20 of the same metaphase spread. The identification of a sSMC at prenatal diagnosis is a great challenge for genetic counseling because of missing or little clinical and prognostic information. Array-CGH (aCGH) has been shown to be a valuable tool in genetic counseling for prenatally detected sSMC allowing the identification of sSMC, its euchromatic content and the associated genes involved, contributing for a better genotype-phenotype correlation.

Three cases of mosaic de novo sSMC, detected by conventional cytogenetics, were analysed, during the last year, with Agilent whole genome array; two of them using the 180 K oligonucleotide array-CGH and the other with the 60 K oligonucleotide array-CGH.

Array-CGH analysis on DNA from cultured amniocytes characterized two mosaic alterations of chromosome 18 (one case with triplication of 14 Mb at 18(p11.32p11.21) and the other case with an amplification involving whole 18p and part of 18q of about 26 Mb and a 17 Mb amplification at 6(p12.1q13).

The majority of sSMC are derived from the acrocentric chromosomes. The sSMC derived from other autosomes, as the ones in the present study, generally have poor genotype-phenotype correlations, with phenotypes ranging from normal to severely abnormal, being their euchromatic content the most accurate predictor of phenotype.

Array-CGH analysis is an effective tool to characterize sSMC. Using this approach the origin and euchromatic content of sSMC can be identified. The measurement of the sSMC size, genetic material and associated genes allows the improvement of prenatal genetic counseling with a more accurate delineation of the genotype-phenotype correlation.

3.P13

Dealing with de novo apparently balanced rearrangements in the era of Array-CGH

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De novo apparently balanced chromosome rearrangements (dnABCRs) are detected in approximately 1:8001 to 1:10002 prenatal tests. Whenever a dnABCR is identified, the outcome for the fetus is difficult to predict because there is no evidence for a normal phenotype in a carrier parent and the rearrangement may not be truly balanced. Phenotypic abnormalities observed in the patients with dnABCRs can be due to 1) submicroscopic deletions or duplications near the breakpoints, 2) submicroscopic deletions or duplications unrelated to the ABCR, 3) gene disruption at the breakpoints,

4) a position effect, 5) uniparental disomy, or 6) another unidentified genetic or environmental factor.

We report on five recent prenatal cases carrying a dnABCRs detected with conventional karyotyping. Three of the cases had reciprocal translocations, one had a pericentric inversion and another presented both an inversion and a translocation. Array-CGH was performed to search for genomic imbalances that were revealed in two of five the cases. Only one of the imbalances identified was near one of the breakpoints and all the imbalances detected were de novo.

Interpretation of dnABCRs in a prenatal setting and the ensuing genetic counseling is challenging due to the limited resolution of routine cytogenetics. The cases presented provide further evidence that in case of prenatally detected dnABCRs, array-CGH is useful not only in identifying the genomic imbalances at the breakpoints, but also in detecting unexpectedly complex rearrangements in other chromosomes.

Seeing as the increased risk for phenotypic anomalies has been mainly attributed to the gain or loss of genetic material, the diagnosis of these imbalances is paramount and, therefore, in prenatal diagnosis, array-CGH should always be proposed to fetuses with dnABCRs, even if not associated to malformations, so that an accurate risk assessment can be provided.

3.P14

Prenatal diagnosis of unexpected de novo 5q14 deletion encompassing VCAN gene identified by array-CGH in a fetus with cystic hygroma

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A 31 year old woman was referred to our Prenatal Diagnosis Center at 11 weeks gestation for fetal cystic hygroma measuring 6.6 mm. This pregnancy occured after 2 early miscarriages and the couple was non consanguineous.

Chorionic villus sampling performed at 11 weeks showed a normal female karyotype 46,XX. Persistence of cystic hygroma in two other ultrasound examinations leads the medical team to sample amniotic fluid at 18 weeks. Conventional karyotyping confirmed an apparently normal 46,XX karyotype. An oligonucleotide based-array comparative genomic hybridization Agilent 105 K was performed in order to detect any imbalance. Array-CGH showed a de novo 2.5 Mb deletion in 5q14.2q14.3, confirmed by fluorescent in situ hybridation (FISH). This deletion included the VCAN gene.

Heterozygous mutation of the VCAN gene is associated with vitreoretinopathy (Wagner syndrome OMIM#143200 and erosive vitreoretinopathy). The penetrance of Wagner syndrome is complete and associated with variable phenotype including retinal detachment, myopia, presenile cataract and progressive chorioretinal atrophy. Wagner syndrome was associated with heterozygous mutation of the VCAN gene but no deletion was reported.

After genetic and ophthalmologic counseling, the patients decided to continue with the pregnancy. At birth, the child will be referred to an ophthalmologist for medical supervision.

3.P15

Prenatal diagnosis of Simpson-Golabi-Behmel Syndrome Type 1 by array CGH in a second trimester twin pregnancy

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Simpson-Golabi-Behmel type 1 (SGBS1) is a rare X-linked syndrome characterized by multiple congenital abnormalities, pre-postnatal overgrowth and a wide spectrum of clinical manifestations. The prevalence of the condition is unknown, since milder cases probably are underdiagnosed.

SGB1 is caused by a deletion or mutation in the GPC3 gene which maps to Xq26.2.

Here we report a case of a twin bichorionic pregnancy of a 30 years old woman who underwent CVS chromosomal analysis (13.3 week) due to an increased nucal traslucency(NT) in one fetus. Karyotype analysis revealed a 46,XY result in both fetuses. A further US control at 17 week detected in the first twin heart defects, hyperechogenic kidneys, ventricularmegalia and hypospadia. For these reasons a CGH array (Agilent, SurePrint G3 Human CGH Microarray Kit,60 k) was performed revealing a 152.8 kb deletion, involving exons 4-5-6-7 of GPC3 gene at Xq26.2 band in the malformed fetus, the second fetus was found to be normal while the mother, showing a mild phenotype, resulted to be a carrier of the same deletion. After genetic counselling, the couple opted for a selective abortion. We report a detailed description of the relation between the size of the deletion, the ultrasound findings as well as the postmortem examination.

Our case confirmed that SGBS1 could have a recognizable prenatal phenotype and could be diagnosed also in the second trimester giving the couple the basis of an appropriate genetic counselling. A better knowledge of SGBS1 phenotype could address more efficiently the molecular testing.

3.P16

Array-CGH in prenatal diagnosis—reflexions over the first 100 cases

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Introduction: Conventional cytogenetics has been the main approach for the detection of chromosomal abnormalities in prenatal diagnosis, however is being supplemented and in some cases, especially in cases with ultrasound anomalies, replaced by chromosomal microarray analysis.

Materials and Methods: Array-CGH was performed in 100 prenatal samples. The indications for the analysis were diverse, ranging from ultrasound anomalies/major abnormalities, medical interruptions due to major anomalies, carrier progenitors of genomic imbalances and even to clarify conventional cytogenetic findings. Array-CGH was performed using Agilent oligonucleotide 180 K in DNA obtained in the majority of the samples from amniotic fluid and chorionic villus, but also from fetal blood and skin biopsy of the death fetus. Each sample was hybridized against a sex-matched commercial control and the analysis was performed to detect imbalances above 400Kb in size, except in the cases where a specific familiar imbalance was being evaluated.

Results: Of the samples analyzed due to cytogenetic findings, we were able to: characterize marker and derivative chromosomes, exclude genomic imbalances in translocations and inversions, detect genomic imbalances in apparently balanced translocations and detect mosaic trisomy for chromosomes 13 and 21 in a 13;21 Robertsonian translocation. Of the samples tested due to carrier progenitors of genomic imbalances, we defined the origin of the imbalance in most of the cases. In the remaining samples, imbalances were observed: maternal, paternal, de novo and 1 imbalance resulting from a maternal balanced translocation.

Conclusions: Array-CGH is valuable not only to establish a diagnosis in samples with ultrasound anomalies but also to characterize cytogenetic findings, showing to be a good tool for genetic counseling. The use of CGH array in high-risk pregnancies in conjunction with the karyotype analysis seems to be best strategy in prenatal diagnosis.

3.P17

Routine prenatal diagnosis in a tertiary public hospital: first 3 years of experience using CGH- Array

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CGH-Array has had a major impact on genetic diagnosis over the last years. As a public tertiary hospital our Service routinely offers QF-PCR and karyotype to all prenatal invasive tests. The use of CGH-array technology has been progressively introduced from 2012 onward. Data collected between January 2012 and December 2014 account for 324 CGH-array tests out of 1675 invasive procedures (19.42 %).

Referral indications (CGH-array group) included fetal structural abnormalities detected on ultrasound (83.6 %), genetic family history (14.4 %) and others (2 %)

60 K custom oligonucleotide-based CGH array (qChip Pre v1.1 Complete), that allows an average resolution of 100–125 Kb in regions causing genomic disorders, was used over fresh/cultured AF(n=287) or CVS(n=37) samples. Results were obtained at an average time of 7 days.

Pathologic results including QF-PCR+karyotype and CGH-array were found in 48 cases in 2012 (8.05 %) and 7 of them were exclusively diagnosed by CGH-array (increasing rate 1.17 %).

52 positive cases in 2013 (9.98 %) showed only 4 of diagnosed by CGH-array (increasing rate of 0.77 %). Finally, from 60 positive cases in 2014 (10.58 %) only 4 were diagnosed by CGH-array (increasing rate of 0.71 %).

Global results give us a 0.9 % incremental yield of pathological detection by CGH-array within the context of all prenatal diagnosis procedures and a 4.6 % considering the 324 CGH-arrays. Among pathological results with CGH-array there was a significant incidence of cases with cardiac and neural tube defects (46.7 %). It was also very useful for familial reciprocal translocations carriers given faster results in comparison with karyotype.

We conclude that although CGH-array represents a valuable diagnostic tool, indications for testing in the context of prenatal diagnosis should be carefully evaluated with reliable and debated protocols.

3.P18

Prenatal diagnosis of trisomy 16: Difficult counselling and poor pregnancy outcome

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Complete fetal trisomy 16 results in early miscarriage. Only fetal mosaic trisomy 16 is compatible with surviving beyond the first trimester of pregnancy. Trisomy 16 has recently been included in noninvasive prenatal tests.

Due to trisomy rescue events maternal uniparental disomy (UPD) is relatively common und must be investigated. There is a high risk of abnormal outcome even in cases with confined placental mosaicism without UPD 16. Intrauterine growth retardation, fetal death, preeclampsia, preterm delivery, neonatal death, developmental delay, congenital heart defects and additional minor anomalies can be observed. Abnormal serum parameters in first trimester screening with a very low level of PAPP A are correlated with trisomy 16.

We present detailed ultrasound, cytogenetic and UPD test results together with outcome data of 17 pregnancies with trisomy 16 seen in a single center over the last 20 years. There were only 6 live births, all with severe intrauterine growth retardation and other pregnancy complications. Intrauterine death happened in three cases. In the remaining 8 cases pregnancies were terminated. Detailed pathology reports will be presented and results will be compared with reported cases.

Pregnancies with prenatally diagnosed trisomy 16 are at high risk of pregnancy complications especially severe growth retardation and adequately monitoring is difficult. Extensive counselling has to be provided to help the patients understand the many implications this diagnosis has for the ongoing pregnancy.

3.P19

Deletion 19p13.3 and duplication 19q13.3qter in Prenatal Diagnosis—a case report

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Introduction: The presence of, concomitantly, duplication and deletion of different regions in the same chromosome is considered a complex chromosomal rearrangement. Usually this type of anomaly results from a meiotic crossing-over of a parental chromosomal inversion and is defined as a recombinant chromosome. When it is "de novo" the abnormal chromosome is considered a derivative. The authors present a rare case of a derivative chromosome 19 observed in prenatal diagnosis.

Material and methods: A pregnant woman was referred for karyotyping due to positive biochemical screening, neural tube defects and fetal malformations. Chromosome analysis with high resolution GTL-banding was performed on metaphases obtained from cultured amniocytes. FISH studies included subtelomeric and whole chromosome painting probes, both specific for chromosome 19. MLPA techniques were performed using kits P036 and P070 (MRC-Holland) for the subtelomeric regions.

Results: The fetal karyotype revealed a structurally abnormal chromosome 19 with additional material in the terminal band of the short arm. FISH using subtelomeric probes (Vysis) and whole chromosome painting (Cytocell), both for chromosome 19, showed a 19pter deletion and a 19q13.3qter duplication, confirmed by MLPA. Since parental karyotypes were normal, this anomaly was considered "de novo".

Discussion: We compare the clinical features in this fetus with the previously reported cases with either terminal deletion and/or a duplication of distal 19q. The authors enhance the importance of the combination of high resolution banding with appropriate molecular cytogenetic techniques in the characterization of complex chromosomal rearrangements.

3.P20

Preimplantation Genetic Diagnosis: one assay that fits all.

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Current single-cell PCR or FISH based PGD-assays require family-specific designs and labor-intensive workup. Array comparative genomic hybridization (aCGH)-based methods enable genome-wide aneuploidy detection of large fragments, but do not allow diagnosing single gene disorders or submicroscopic imbalances.

Here, we present the transition in the clinic from traditional cytogenetic and molecular cytogenetic methods to a novel generic SNP-array-based method that allows us to detect in single blastomeres not only the inheritance of monogenic disorders (recessive and dominant) genome wide, but also chromosomal rearrangements and aneuploidies, including their parental origin as well as the meiotic or mitotic nature of chromosomal trisomies. The developed analysis method called "haplarithmisis" is embedded in a broad computational pipeline for single-cell haplotyping and imputation of linked disease variants (siCHILD).

We will discuss results from clinical cases for different indications including Mendelian disorders, chromosomal imbalances and complex rearrangements.

This novel generic method not only facilitates the genetic selection of unaffected embryos, but also broadens the range of classic PGD, enabling us to help couples that could not be assisted so far.

The ability to characterize the entire genome of a single human cell for SNPs and copy number variants in a single assay is a great improvement in PGD practice.

3.P21

Placental mesenchymal dysplasia: important differential diagnosis for partial moles and frequently associated with Beckwith-Wiedemann syndrome

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Placental mesenchymal dysplasia (PMD) is a rare placental anomaly. Prenatal ultrasound indicates placentomegaly and cystic structures resembling a partial mole, but with apparently normal fetal morphology. PMD is associated with intra-uterine growth restriction (IUGR), intra-uterine mortality, prematurity and in about 20 % of the cases with Beckwith-Wiedemann syndrome (BWS), respectively.

The underlying cause is unusual and interesting as PMD is a special type of mosaicism. One part of the placenta is normal with a biparental diploid genotype (preponderance of females). The dysplastic part is also diploid, but of paternal uniparental origin.

We present a case of a prematurely born female with typical signs of BWS. PMD was suspected prenatally (confirmed pathologically). Molecular studies confirmed the diagnosis of BWS (UPD11pat) and showed a genome wide paternal uniparental disomy in the majority of cells, with a low degree of biparental cells in blood.

PMD should be considered in cases with partial mole—especially if there are no major anomalies indicating triploidy.

Gynaecologists, cytogeneticists and pathologists should be aware of this rare differential diagnosis and initiate respective studies.

3.P22

Subtelomeric deletion of chromosome 3: what is it's significance?

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Subtelomeric deletions are responsible for birth defects and mental retardation and several syndromes have already been described. The 3p deletion syndrome is one of these disorders with a variable spectrum of defects depending upon the overall size of the deleted segment. Characteristic features of distal 3p- syndrome include low birth weight, microcephaly, trigonocephaly, hypotonia, psychomotor and growth retardation. To date, more than 40 cases with 3p25-p26 deletions have been described and only two, a mother and daughter, were reported without phenotypic effect.

We presented a family with a 3p deletion in the mother and two children.

The 3p deletion was detected in a prenatal diagnosis of the third child. Cytogenetic analysis showed a female karyotype with a subtelomeric deletion on the short arm of chromosome 3, confirmed by FISH technique (subtelomeric probes for chromosome 3 - Vysis). Cytogenetic and FISH studies were carried out on the couple and another child. The same subtelomeric deletion was found in the mother and her son. Array analyses revealed a loss of 7.4 Mb in 3p26.3p26.1.

The son is now followed in a genetic counseling consultation and a mild mental retardation was observed. The daughter and the mother show a normal development.

The presence of the same chromosome deletion in normal as well as abnormal phenotypes has been investigated. Several models could explain the different phenotypes. These include regions of low gene density, absence of dosage sensitivity, and functional redundancy of genes that have additional or related copies outside of the deleted region.

The authors present a literature review of subtelomeric deletion of chromosome 3 and its implications.

3.P23

Prenatal cytogenetic diagnosis by CGH array: our 2014 experience

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Since 2014, karyotyping has been replaced by the high resolution CGH array method for cytogenetic analysis of amniotic fluid. CGH array was performed on the 60 K ISCA bluegnome slides. The resolution of the analysis was 400 kb in the backbone of the genome and higher in the ISCA loci.

544 cytogenetic analyses were performed on amniotic fluid samples in our laboratory in 2014. Rapid FISH aneuploidy screening test detected 29 viable autosomal aneuploidies and 5 gonosomal aneuploidies. Cases with

autosomal aneuploidies were confirmed by karyotype and cases with gonosome aneuploidies were further analyzed by CGH array that didn't detect any other genetic abnormality.

Among the 510 remaining cases, 414 cases (81.2 %) were not reported: 404 cases were normal (only benign CNVs), and 10 cases were consisted as susceptibility CNVs not to be communicated in accordance with the Belgian guidelines and 2 cases (0.4 %) were not contributory.

For the remaining 94 cases (18.4 %) the pathogenicity of the CNVs was evaluated by at least 2 geneticists. 74 cases (14.5 %) were not reported because of lack of evidence for pathogenicity. 20 cases (3.9 %) were considered abnormal or possibly pathogenic and were reported. Among the reported cases: 6 cases corresponded to definitely pathologic CNVs (deletions of/in genes of known autosomal dominant or X linked diseases and large deletions), 2 cases corresponded to susceptibility CNVs to be communicated in accordance with the Belgian guidelines, 9 cases corresponded to unknown CNVs with possible pathologic effects (duplications of part of genes of known autosomal dominant or X linked diseases, high OMIM genes content) and 3 cases corresponded to carriers of CNVs with possible pathologic effects (duplication of part of genes of known X linked diseases in females). In some cases, pathogenicity was re-evaluated after familial investigation.

3.P24

Incidental findings of genome wide non-invasive fetal aneuploidy detection: presymptomatic identification of maternal cancers

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Non-invasive prenatal testing (NIPT) for fetal aneuploidy detection is increasingly being offered in the clinical setting following studies demonstrating high sensitivities and specificities for trisomies 21, 18 and 13 detection. However, a baseline false positive and false negative rate remains. We introduced an analysis pipeline which addresses some of the technical and biologically-derived causes of error. Importantly, it differentiates high z-scores due to fetal trisomies from those due to local maternal CNVs causing false positives.

Following routine clinical analysis of over 5000 prospective pregnancies, several other genomic imbalances were found in addition to detection of the common autosomal aneuploidies. These findings include (i) other (segmental) aneuploidies (0,3 % of cases), 4 of which could be confirmed to be fetal (1 mosaic trisomy 16 and 1 mosaic trisomy 15 which contained a uniparental disomy cell line, 2 partial duplications 18 and 1 terminal 5p deletion) and (ii) maternal imbalances. Importantly, three aberrant genome representation profiles were observed that could not be attributed to the maternal nor the fetal genomic constitution. Whole body diffusion-weighted magnetic resonance imaging and subsequent pathologic and genetic investigation uncovered the presence of respectively an ovarian carcinoma, a follicular lymphoma and a nodular sclerosis classical Hodgkin lymphoma. The copy number variations in those tumors were concordant with the NIPT plasma profile. NIPT thus enables the accurate presymptomatic detection of maternal tumors. These incidental findings are an unsuspected added benefit of NIPT.

3.P25

Prenatal finding of pericentric inversion of chromosome 3

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Every prenatal chromosomal finding of uncertain pathogenicity is a very difficult situation in genetic counselling. Even postnatally, it is often impossible to clearly correlate the cytogenetic finding and eventual phenotypic abnormality.

We present a case of Czech patient with prenatal finding of de novo pericentric inversion 46,XX,inv(3)(p11.2q21) in the amniotic cells. Amniocentesis was performed due to the advanced maternal age—there were no other risk indicators (apart from lower free ß-hCG level which was analogous to previous pregnancy resulting in a cytogenetically normal healthy daughter).

There is only one similar case presented by Spedicato FS et al.: Pericentric inversion inv(3)(p11q21). [J Med Genet. 1984 Oct;21(5):396.] The inversion was detected in a newborn with a prominent forehead and receding chin, a left preauricular tag, a long philtrum, and pendulous cheeks. Its familial occurrence in other relatives with normal phenotype (brother, father, paternal aunt and her daughter) emerged later with examination of the family.

In addition to the case from 1984, we got also the result of array comparative genomic hybridization which was normal, without any deletion in breakpoints or anywhere else. The pregnancy proceeds without any complication and apparently healthy daughter was born in term in the middle of February. The prenatal cytogenetic finding was verified at the moment and it will be extended by further molecular cytogenetic analysis of the peripheral blood. The child will be clinically examined thoroughly again before the conference (at her age of 4 months). All of these results will be presented on the poster.

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3.P26

Unbalanced translocation in a fetus with normal nuchal translucency in first trimester screening and normal prenatal karyotype

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We describe the case of a pregnant woman with normal nuchal translucency (NT) (<3 mm) in the first trimester and a triple screening risk of 1/547. The secondary markers at 14 weeks of gestation (NT: 3,1 mm (>p99), hypoplastic nasal bone, significant tricuspid regurgitation and the "a" wave absent/reversed in the ductus venosus) identified a high risk pregnancy and an invasive test was proposed and accepted. Rapid test (FISH) of amniotic fluid showed two signals for chromosomes 7 (7q11.23), 13 (13q14), 18 (18p11.1q11.1), 21 (21q22.13q22.2), 22 (22q11.2) and cell culture and subsequent cytogenetic analysis, established a normal female karyotype (approx. 400 bphs).

At 18w, after information of the echocardiographic findings (dysplastic pulmonary valve, pulmonary stenosis, moderate pulmonary insufficiency and pulmonary trunk dilation, cervical lymphangiomas, persistence of nuchal translucency, and cerebral ventriculomegaly) and their clinical significance, the couple decided to abort.

An aCGH (Agilent Technologies 8x60K) performed on the fetal tissue and analyzed with CytoGenomics 2.9 software revealed the presence of a 19,3 Mb deletion in 5p15.33-5p14.3 and a 14,5 Mb duplication in 11p15.5-11p15.2. This result was confirmed by FISH on metaphase chromosomes, using MD Cri-du-Chat CTNND 5p15 (red)/5q31 (green) and ST 11pter (blue) probes, and the formule was stablished as: 46,XX.ish der(5)t(5;11)(p14.3;p15.2)pat(CTNND-,D11S1363+).

The cardiopathy, lymphangiomas, and persistence of nuchal translucency may suggest a Noonan syndrome. However, the exclusion of chromosome aberrations continues to be necessary in order to provide suitable genetic counseling.

3.P27

Conflicting results QF-PCR and karyotyping due to structural aberration of the Y-chromosome

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Quantitative fluorescent polymerase chain reaction (QF-PCR) is an accurate and efficient technique for rapid prenatal diagnosis of trisomy 13, 18 and 21 and aneuploidies of the sex chromosomes. Discordant results between QF-PCR and karyotyping have occasionally been reported, mostly due to mosaicism.

A 20-year old female underwent chorionic villus sampling at 13 weeks' gestation because of an increased

risk of a chromosomal aberration after first trimester screening. Ultrasound investigation demonstrated no abnormalities. The results from the QF-PCR analysis were interpreted as a possible mosaic 45,X/46,XY because of the low contribution of the Y chromosome. Conventional karyotyping of the LTC demonstrated a non-mosaic 45,X. Since no abnormal findings were detected on ultrasound, a confined placental mosaicism was suggested as a possible explanation for the discordant findings. A subsequent amniocentesis revealed a normal male genotype with QF-PCR. Karyotyping, on the contrary, demonstrated a mosaic pattern with 45,X and a structural rearranged Y chromosome, probably an i(Yp). Additional FISH confirmed the presence of an isochromosome of the short arm of the Y chromosome. The case presented here demonstrates that caution should be taken when conflicting results are observed in CVS—even if a normal profile is obtained with QF-PCR in a follow-up amniocentesis. This case further illustrate that mosaicism for an abnormal cell line can result in a normal QF-PCR profile.

3.P28

Derivative chromosome 18 in prenatal diagnosis: a case report

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We report a twin pregnancy obtained by in vitro fertilization (IVF) techniques referred to our center for prenatal cytogenetic studies. Fetus 1 presented with a positive biochemical screening. The conventional cytogenetic analysis with GTL banding revealed a female fetus with a structurally abnormal chromosome 18. Fetus 2 had a normal karyotype, as did the parents. Further testing was done using MLPA and FISH techniques, in order to clarify the abnormal chromosome 18. MLPA studies revealed a deletion of the subtelomeric region of the long arm of chromosome 18 and a duplication of the subtelomeric region of the long arm of chromosome 17; FISH analysis confirmed that the duplicated (17q) sequence was translocated into the long arm of the abnormal chromosome 18. In conclusion, this fetus had a der(18), due to an initial translocation t(17;18)(q24;q21.3), resulting in partial deletion of the long arm of chromosome 18 (18q21.3→qter) and a partial duplication of the long arm of chromosome 17 (17q24→qter).

A review of the literature indicated a recent and similar case, where the phenotype associated to the derivative chromosome 18 included mental retardation, several dysmorphic features and congenital anomalies. Parents were offered adequate genetic counselling and decided not to terminate the pregnancy.

3.P29

Prenatal diagnosis of 16p duplication presenting with ultrasound abnormalities

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Duplication of the short arm of chromosome 16 is a rarely seen genomic instability with well described symptoms including mental and growth retardation, craniofacial, cardiac, urogenital abnormalities, finger anomalies and pulmonary vascular diseases. Most of the cases are derived from a chromosomal translocation, usually due to maternal rseciprocal translocation.

A 19-year-old woman, gravida 2, abortus 1, was referred to our clinic at 13 weeks of gestation because of abnormal USG findings including cystic hygroma, significantly increased nuchal translucency (NT) (10 mm) and short limbs. Chorionic villi sampling (CVS) was performed after genetic counseling. The results of the QF-PCR rapid aneuploidy screening were

normal. Array CGH (Agilent, 60 K) analysis demonstrated a 26 Mb duplication of 16p13.3- p12.1 (1100695-27109354) and the findings were confirmed by follow-up FISH analysis. Subsequent parental karyotyping revealed 46,XY and 46,XX,t(6;16)(q27q12.1) for father and mother, respectively. The 6qter deletion could not be determined by array-CGH but demonstrated by subtelomeric FISH analysis. The fetal karyotype was 46,XY,der(6)t(6;16)(q27q12.1)mat The pregnancy was terminated at 17 weeks. The phenotypic assessment could not be performed due to extensive edema in abortion material.

To our knowledge, coexistence of 16p13.3p12.2 duplication and subtelomeric 6q deletion has not been described previously in the literature. Besides, the case also emphasizes the importance of a detailed 11–14 week ultrasound assessment in diagnosing fetal chromosomal aberrations in combination with the modern aspects of array CGH, thus providing more precise and rapid prenatal diagnosis.

3.P30

A fetus with a 417 kb de novo 17p13 microduplication.

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A 28 year old woman underwent amniocentesis because fetal ultrasound revealed single umbilical artery at 12 weeks. At 16 weeks a cardiopathy including aortic valve stenosis, cardiomegaly and fibroelastosis and hyperechogenic bowel was diagnosed. The pregnancy was obtained by intracytoplasmatic sperm injection after in vitro maturation of oocytes.

Using 44 K Agilent oligo array, we detected a 417 kb microduplication 17p13.3. This duplication concerns a de novo phenomenon and encompasses 6 genes, namely YWHAE, ABR, CRK, TUSC5, MYO1C and BHLHA9 . Bruno et al. (2010) proposed two classes of 17p13.3 microduplications. Class I involves YWHAE gene but not PAFAH1B1, while class II involves PAFAH1B1 gene, with or without YWHAE or CRK genes. The current case, according to Bruno's classification, belongs to the Class I 17p13.3 microduplications.

After genetic counselling the parents decided to terminate the pregnancy. Even though the most typical characteristics for this syndrome such as developmental and psychomotor delay appear after birth, some of the phenotypic features of the syndrome could be identified in the fetus. These were similar to some previously described patients, including broad nasal bridge, hypertelorism, micrognathia, fleshy earlobes, thin upper lip, cardiopathy. We could not confirm the hand/foot anomalies (CRK is known to interact with signal pathways involved in limb development) nor cleft lip and palate (YWHAE has been implicated in the etiology of orofacial clefts) often seen in Class I duplications.

We report a fetus with a de novo 417 kb microduplication 17p13.3. We made a genotype-phenotype correlation between our case and patients described in literature. The present case illustrates the importance of micro-array analysis in prenatal genetic diagnosis. Additional reports are needed for the complete delineation of the clinical spectrum of 17p13.3 microduplications and the genotype-phenotype correlation concerning Class I and II patients.

3.P31

Cell-free fetal DNA extraction: evaluation of two methods based on silica-columns and magnetic beads.

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Introduction: The presence of cell-free fetal DNA (cffDNA) in the maternal circulation offers a non-invasive source of fetal genetic material for prenatal diagnosis. This includes fetal sex determination by specific Y chromosome sequence detection, such as SRY or DYS14. This determination is particularly important when a fetus may be affected by an X-linked disorder, such as haemophilia. Due to the low percentage of fetal DNA present in maternal plasma (<10 %) during early gestation, efficient extraction processes are required for

successful downstream detection applications in non-invasive prenatal testing (NIPT).

Objective: Evaluate, using qPCR, the cffDNA extraction efficiency of 2 different methods: QIAamp® DNA MIDI kit (method A) and Chemagic circulating NA kit (method B).

Material & Methods: Circulating DNA was extracted from 800 μL-plasma samples from 70 pregnant women carrying a male fetus. Protocols used followed manufacturer's instructions for method A and a modified protocol for method B. qPCR targeting GAPDH and SRY genes was carried out in order to determine the total cell-free DNA (cfDNA) and the fetal fraction extracted, respectively. Ct values and standard deviation were evaluated.

Results: In terms of cfDNA, GAPDH assays showed Ct and SD values of 33.3 (0.76) by method A and 29.3 (0.22) by method B. SRY assays yields a Ct and SD values of 34.3 (1.33) for method A and 32.5 (0.30) and method B. No SRY amplification was obtained for 4 samples processed by method A.

Conclusion: Modified-Chemagic circulating NA protocol is the best approach for cffDNA from maternal plasma. This modified protocol results robust and accurate for both cffDNA and cfDNA extraction.

3.P32

Prenatal screening for aneuploidy in maternal blood: an unexpected finding

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Noninvasive prenatal test (NIPT) for the most frequent foetal chromosomal abnormalities in maternal blood is becoming the most demanded laboratory tool for pregnant women, replacing the classical prenatal invasive test. The cell-free fetal DNA in the pregnant woman's plasma originates from the placenta. An abnormal NIPT result could therefore be due to confined placental mosaicism. This is one of the limitations of the test. All abnormal NIPT results must be confirmed by a conventional prenatal invasive test.

We report here a case of discordant results between NIPT and fetal karyotyping due to confined placental mosaicism of sex chromosomes. In addition, in this case, an unexpected finding in the fetal karyotype required a second amniocentesis to reach the final conclusion about the prenatal result.

3.P33

False positive and false negative results of cell free DNA testing

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The most exciting development in prenatal practice is the introduction of cell free DNA testing (cf-DNA) for the screening of common aneuploidies, which offers highest sensibility and specificity. Since cf-DNA originates from the cytotrophoblasts of the placenta, it reflects the genotype of the cytotrophoblasts, which might differ from the fetus itself. Therefore, it's mandatory to confirm the results by invasive approach. The percentages of false positive and negative results of cf-DNA are still not clear.

Here we present our confirmation results of 33 investigations with nine false positive and one false negative case.

18/19 cases with a risk for trisomy 21 obtained by cf-DNA test were confirmed, one was normal in amniocytes (1/19 false positive for trisomy 21). Another case, with pathological USG and normal cf-DNA, had mosaic trisomy 21 (70 %) by karyotyping (one false negative for trisomy 21).

2/3 cases with a risk for trisomy 18 were confirmed, but one was not (1/3 false positive for trisomy 18).

Two cases with a risk for trisomy 13 could not be confirmed (2/2 false positive for trisomy 13).

2/5 cases with a risk for monosomy X were confirmed. The other three cases were normal in amniocytes by karyotyping and interphase-FISH (3/5 false positive for monosomy X).

1/3 case with Klinefelter syndrome was confirmed in amniocytes (2/3 false positive for 47,XXY).

Discordant findings between cf-DNA and karyotyping can be explained by confined placental mosaicism etc. We believe that these cases and their clinical findings should be reported to understand the biological mechanism of discrepancy and also to determine the percentages of false positive and negative results, which are important in genetic counselling regarding cf-DNA testing.

4. Animal and Plant Cytogenetics

4.P1

Distinct DNA methylation and chromatin dynamics of spermatogenic cell-specific genes in mouse

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To understand the role of chromatin structure in the expression of protamine 1, protamine 2 genes during mouse spermatogenesis, we have examined the epigenetic profile of these specific genes correlated with global epigenetic changes during sperm differentiation.

Spermatogenic cells were obtained from 9- to 11-week-old males by centrifugation on a BSA gradient.

The dynamics of H4 hypercetylation and DNA methylation during murine spermatogenesis have been investigated.

The transmission electron microscopy analysis, immunohistochemistry technique and specific molecular investigations were employed for deciphering some peculiar aspects of chromatin organization and evolution in mouse spermatogenesis.

Spermatogonia, preleptotene spermatocytes and spermatids contained global hyperacetylated H4, whereas no acetylated H4 was noticed in leptotene or pachytene spermatocytes.

Using the bisulfite mutagenesis technique, we found that in the 5′ coding region, both Prm1 and 2 were fully methylated in pachytene spermatocytes and spermatozoa and are unmethylated when they are expressed in round spermatids.

ChIP assay using H4 hyperacetylated antibody for the 5′coding region of Prm1 and Prm2 genes, suggests a crosstalk between histone acetylation and DNA methylation. The regulation of histone deacetylase activity appears to play a major role in histone replacement during spermiogenesis and testes specific genes expression in round spermatids.

4.P2

Microdissection of chicken lampbrush chromosome regions for FISH-probes generation and high-throughput sequencing

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Microdissection represents a reliable approach for investigation of chromosomes and their regions. Microdissection technique allows to get broad panels of locus-specific and whole-chromosome painting FISH-probes that are widely used for chromosome identification, mapping of evolutionary and clinical breakpoints, revealing the orthologous regions in karyotypes of related species, and for investigation of spatial genome organization.

At the same time, keeping in mind relatively small physical size of mitotic chromosomes of higher vertebrates, the standard microdissection procedure proves to be inappropriate for analysis of tiny chromosomal regions and functional domains. In this regard, it is quite reasonable to use extended meiotic lampbrush chromosomes (LBCs) as initial material for microdissection. LBCs are highly decondensed because of their transcriptional activity that makes the microdissection procedure much simpler. In present work we developed an approach for LBCs mechanical microdissection, amplification of isolated material followed by highly specific FISH-probes preparation and high-throughput sequencing procedure. In particular, the chicken (Gallus g. domesticus) chromosome regions as small as a single chromomere (~2 Mb in size) were successfully dissected and applied for FISH and next-generation sequencing (NGS), with only one chromosomal copy being used as input material.

LBCs are enriched with unusual marker structures including so-called «complex loops», whose organization, precise genomic location, molecular composition and functions are still obscure. Here, we dissected the material of the marker structures from chicken lampbrush macrochromosomes 2 and 3, prepared specific FISH-probes and deciphered the samples using NGS. Such a complex comprehensive approach allows to assign unambiguously the position of individual chromomeres and cytological markers of lampbrush chromosomes to genomic coordinates.

The work was partially performed using experimental equipment of the Research Resource Centers 'Chromas' and 'Molecular and cell technologies' of Saint-Petersburg State University.

4.P3

Histone modifications during cytomixis in tobacco

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Cytomixis is migration of the nuclei between plant cells through intercellular channels of a special type (cytomictic channels), differing from the plasmodesmata in their structure and size. This unique phenomenon was discovered over a century ago and to date cytomixis has been observed in hundreds of various higher plant species, mostly in microsporogenesis.

It is so far unknown what drives cytomixis and what is the functional state of the chromatin migrating between cells. Using immunostaining, we have analyzed the distribution of main histone modification types (methylation, acetylation, and phosphorylation) that reflect the functional state of chromatin in the tobacco microsporocytes involved in cytomixis. We have pioneered in demonstrating that the chromatin in the cytomictic cells does not differ from the chromatin in intact microsporocytes according to all 14 analyzed histone modification types. We have also for the first time demonstrated that the migrating chromatin contains normal structures of the synaptonemal complex and lacks any signs of apoptosis. As has been shown, the chromatin migrating between cells in cytomixis is neither selectively heterochromatized nor degraded both before its migration to another cell and after it enters a recipient cell as micronuclei. We have also demonstrated that the cytomictic chromatin retains a normal transcription activity level as well as the normal processes of condensation, conjugation, and recombination corresponding to the current meiotic stage. The results admit the possibility that cytomixis may be the reason underlying a change in the karyotype of produced pollen.

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4.P4

Characterization of four species of Bovidae using satellite I DNA probes

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Taxonomy of the Bovidae family is difficult and the evolutionary relationship among most bovid subfamilies remains uncertain. We used a molecular cytogenetic approach which allows the investigation of peculiar DNA fractions, belonging to the category of highly repeated DNA sequences, often located at or near the centromere. Such DNAs are known to be reliable evolutionary markers, as changes in satellite DNA composition may coincide with speciation and are useful as phylogenetic markers. In this studies we isolated two clones, Bt1 from Bos taurus and Om1 from Ovis orientalis musimon, and used them as probes for hybridization on both genomic DNA and chromosomes in members of Bovini and Caprini tribes, respectively Bos taurus, Bubalus bubalis, Ovis orientalis musimon and Capra hircus. The two sequences were analyzed and compared to those present in the database, and turned out to be almost identical to DNA families 1.714 and 1.715, which belong to satellite I DNA. Om1 is homologous to family 1.714, while Bt1 is homologous to

family 1.715. Probes showed completely coherent results in Southern blots as well as in FISH: Bt1 sequence hybridized to Bos taurus and Bubalus bubalis chromosomes and genomic DNAs, while no signal was detected in Capra hircus and Ovis orientalis musimon. Om1 sequence hybridized to Capra hircus and Ovis orientalis musimon chromosomes and genomic DNAs, while no hybridization was observed in Bos taurus and Bubalus bubalis. Moreover, no signal has been detected on sex chromosomes and, likewise, a lack of positive FISH signal was observed in metacentric/submetacentric autosomes. The results obtained clearly show that Bovini and Caprini belong to two very distant subfamilies of Bovidae, still retaining some degree of homology at the level

4.P5

Cytogenetic characterization of an hybrid goat-sheep by GTG-banding

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In mammals interspecies hybridization occurs rarely under natural conditions. Sheep (Ovis aries, 2n=54) and goats (Capra hircus, 2n=60) are genetically different and do not readily interbreed, although information of such interspecies matings exists. Cytogenetic incompatibility is one of the causes of embryo death due to incorrect chromosome paring during the zygote formation and/or aneuploidy occurrence during the zygote division. In this study we report a preliminary cytogenetic characterization of an healthy female hybrid goat-sheep born in a flock under natural conditions.

Peripheral blood sample cultures were performed to get normal lymphocyte cell cultures. After the fixation, a part of the obtained metaphases was stained with a 5 % Giemsa solution, whereas a part was treated with 0.05 % of trypsin solution and Giemsa staining to obtain the GTG-banding. Conventional and G-banded karyotypes were arranged respectively.

All cells that were observed showed an intermediate karyotype between sheep and goat with 57 chromosomes in total. The G-banding karyotype revealed the presence of 3 metacentric and 54 acrocentric chromosomes. The autosomes involved in the hybrid combination were CHI1,3, CHI2,8 and CHI5,11 corresponding to the metacentric chromosomes OAR1, OAR2 and OAR3. The sex chromosomes were correctly arranged and no further morphological differences were evidenced by classical cytogenetic investigation. Further molecular cytogenetic and genetic analysis are in progress to clarify the recombination events occurred in this rare interspecies hybrid.

4.P6

Non-coding RNA derived from a conservative subtelomeric tandem repeat in embryos and adult tissues of Galliformes species revealed by fluorescent in situ hybridization approach

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Transcription of tandemly organized DNA at terminal chromosomal regions is poorly investigated. Previously we demonstrated transcription of the conservative subtelomeric tandem PO41 ("pattern of 41 bp") repeat in chicken malignant cells. PO41 RNAs distribution and secondary structure in interphase and through the mitosis were characterized. In this study transcription of PO41 repeat in chicken (Gallus gallus domesticus) and Japanese quail (Coturnix coturnix japonica) somatic tissues and chicken embryos was examined in detail.

Using RNA-FISH we demonstrated that both strands of PO41 repeat are transcribed in identical pattern in somatic cells of chicken and quail tissues (brain, muscles, oviduct, intestine). Chicken embryos were staged according to Hamburger and Hamilton (1951) and collected from 4-6 to 20 stages. Sense and antisense strands of PO41 repeat were found to be transcribed at each stage. Transcripts were distributed in all nuclei of developing embryos in pattern being identical to somatic and malignant cells: one-two or

dispersed foci were detected in euchromatin or close to chromocenters. In embryo dividing cells PO41 RNAs distribute between condensed chromosomes till anaphase, when they concentrate at the cleavage plane. At telophase, transcripts envelop terminal regions of chromosomes, the results being similar to those obtained in malignant cells. RNase A treatment before or RNase H after whole-mount FISH (WFISH) and DNA-WFISH were performed as control experiments.

Taken together extremely uniform character of PO41 repeat transcription and transcripts distribution at different stages of development and cell cycle, among species and cell types shown here, we conclude that PO41 ncRNAs have universal mediator or regulatory functions.

The work was partially performed using equipment of the Research Resource Centers 'Chromas' and 'Molecular and cell technologies' of Saint-Petersburg State University.

4.P7

The A, B, C and D genomes in endosperm of oat amphiploids.

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In situ hybridization of total genomic DNA was used to analyse the free nuclear endosperm of six oat amphiploids derived from crosses of 2x, 4x, and 6x species in various combinations. The parental species were included in the study. Total genomic DNA probes derived from A. nuda L. and A. eriantha Dur. were used to distinguish the A and C genomes, respectively. The study was focused on frequency of endosperm cytogenetic anomalies, the number and arrangement of chromosomes and relationships between the A, B, C and D genomes. The results showed frequent cytogenetic disorders in endosperm of amphiploids as well as their parental species, including anaphase and telophase bridges, multipolar nuclei divisions, merging of nuclei, ring and frying-pan chromosomes, laggards, hiperploidy and numerous translocations. The increased frequency of micronuclei was demonstrated in amphiploids, suggesting elimination of fragments or whole chromosomes. We reported different ploidy levels in the endosperm nuclei. The expected 3n endosperm was observed in diploid and tetraploid oats and sporadically in hexaploids. The formation of 2n endosperm was documented in all studied oats. This indicates that endosperm may be formed without the participation of sperm cell. Another possibility is the degeneration of one of the central cell nuclei during the early stages of embryo sac development. Moreover, a number of intermediate ploidy levels, typical for aneuploidy, were found. We documented the loss of chromosomes of different genomes so it seems that the elimination of chromosomes in endosperm is random.

4.P8

The monitoring of the environmental pollution in Romanian bovine farms by cytogenetic investigations

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The monitoring of the environmental pollution by using the cytogenetic investigation in Romanian cattle and buffalo farms has been developed in the last 4 years. The main goal of our study was to assess the pollutants effects on the genetic material integrity of farm animals like cattle and buffalo. The cytogenetic investigation of 350 heads (287 cattle and 63 buffaloes) reared in different farms from all over the country carried out. We identified chromosomal instability for 42 animals (31 cattle and 11 buffaloes), represented by a large number of mono-and bi-chromatidic breakages on autosomes and heterosomes, loss of chromosome fragments and gaps. All females with chromosomal instability presented reproductive disorders. SCEs-test, which is a specific test for identifying the effects of toxic agents on the genetic material integrity, has been applied. For animals with many chromosomal breakages the number of sister chromatid exchanges (SCEs) was very high for cattle (8–16 SCEs/cell) and buffaloes (10–17 SCEs/cell) compared to the normal animals. The identification and quantification of the total aflatoxin and heavy metals present in the

environment and, especially, in animal feed revealed high level then maximum permitted by European and Romanian law in force.

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4.P9

M-FISH in a rare hybrid foaled by a donkey and a zebra sire

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M-FISH (Multicolor fluorescence in situ hybridization) is one of the newest and powerful molecular cytogenetic techniques. It can be used as a source of information in many ways and for a precise description of chromosomal rearrangements in particular. In the present study, for the first time in a hybrid, we report a new way to study chromosome rearrangements on a male zonkey foaled by a donkey (Equus asinus, 2n=62, XX) and a zebra sire (Equus burchelli, 2n=44, XY) using M-FISH. We have established that the somatic cells of the hybrid possess 53 chromosomes, making very difficult pairing of homologous chromosomes. For this reason, we have applied the multi-colour Zoo-FISH using a total of 29 whole chromosome painting probes (wcps) obtained through fow-sorted zebra and horse chromosomes and, later DOP-PCR amplified and labelled. In this case, five different pools of six wcps each were prepared, making possible five sequential FISH performed on the same slide for the identification of almost all chromosomes. The acquired images were then superimposed enabling individual chromosomes to be classified based on the fluor composition, in accordance with the combinatorial labeling scheme of the M-FISH probe pool used. Finally, we have analyzed in detail these digitally acquired and processed images to make the identification of all chromosomes for the first time in this rare hybrid. We show that the zonkey has received a haploid set of chromosomes from each parent.

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4.P10

Molecular karyotyping the avian genome in a single fluorescence in situ hybridisation experiment

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The avian karyotype is extremely complex, consisting mostly of large numbers of microchromosomes that confound characterisation by classical means. Particularly, in most birds, chromosomes from number 7 onwards are extremely difficult to distinguish unless molecular cytogenetics is used. To circumvent this problem, we developed fluorescence in situ hybridisation based chromosome macro-array, which we have called the "Chicken Multiprobe System", in order to identify all of the chromosomes in the Chicken karyotype. This device has a number of utilities to address a series of scientific questions. The design of the device consists of 24 squares, with either 3 colour macro chromosome paints (generated by flow cytometry) or, for the microchromosomes, subtelomeric BAC probes in red and green fluorophores, or (the third colour for macrochromosomal paints a blue fluorescent dye). The probes are reversibly dried on to the device, then rehydrated in hybridisation buffer before being exposed to a corresponding glass slide with 24 equivalent squares, each spotted with the fixed cell sample containing metaphase spreads.

Applications of this multi-hybridisation system include detection of translocations in individual birds; characterisation of DT40 cell lines; and comparative genomics on the metaphases of other species. In recent experiments we have established that the system is effective on the chromosomes of other Galliform species. The devices can be customised to include bespoke probes and we have used it to map the chromosomal locations of viral

integration sites by co-hybridising the viral sequences on the Chicken Multiprobe System.

4.P11

Chromosomal mapping using microsatellite probes in two stingless bees species of region Amazon

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The Amazon has a great diversity with a high number of species, including bees of the tribe Meliponini. These bees are known as indigenous stingless bees and are important pollinators of crops and natural vegetation. The construction of hydropower plants, deforestation and expansion of agriculture represent threats to local diversity. So knowledge about their biology and genetics are important to assist in management plans and conservation. This work aimed to increase the cytogenetic knowledge of Amazonian species, through the physical mapping of repetitive DNA sequences in Nannotrigona punctata and Scaptotrigona bipunctata bees. The metaphase chromosomes were obtained from the cerebral ganglia in larvae defecation stage according to a specific protocol. For chromosomal mapping were used probes 18S rDNA and microsatellite GA(15) for fluorescence in situ hybridization (FISH). Approximately 10 metaphases were analyzed per individual to determine the diploid number of chromosomes and karyotype structure. The chromosome number of 2n=34 in both species. The microsatellite GA showed signs in all chromosomes, marking regions euchromatic of chromosome. The same pattern was found for the two species, also similar to that seen in other species of stingless bees, which seems to be a feature retained for this group. The 18S rDNA scored only one pair of chromosomes in the terminal portion of the short arm of Nannotrigona punctata. On the other hand, showed two labeled Scaptotrigona bipunctata pairs. Despite the great diversity of species found in Brazil, only a small number was analyzed using techniques of molecular cytogenetics. Thus, this study increase the known about bees, providing important information on the cytogenetic characteristics of species in the Amazon region.

4.P12

Intraspecific variation revealed by chromosomal mapping of microsatellite in stingless bee Trigona spinipes

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The stingless bee Trigona spinipes is widely distributed in South America. Populations of this specie have been reduced by agricultural landscapes because this species damages flowers of some crops. The aim of study was to characterize T. spinipes by classical and molecular (chromosomal mapping of 18S gene and of repetitive DNA sequence ((GA)15, (CGG)10 and (CAA) 10) cytogenetic. Metaphase chromosomes were obtained from cerebral ganglia of T. spnipes larvae from three cities of Southeastern Brazil. The chromosome classification was based on distribution of heterochromatin according to Minimum Interaction Theory proposed by Imai (1988). Chromosome number of T. spinipes was 2n=34, one euchromatic acrocentric pair (Ae) and 16 pseudoacrocentric pairs (Am). Signal of 18 s probe was distributed in three pairs in terminal region of heterochromatic arm and one chromosomal pair in interstitial region just below telomere in all samples. Only the probe (GA)15 presented signal. This probe marked euchromatic regions, being distributed (i) the long arm of chromosome Ae and (ii) in each eucromatic arm in chromosomes Am and (iii) in some heterochromatic arm in Am pairs. The colonies revealed similar pattern in chromosome number, morphology, the distribution of heterochromatin and regions of the 18S rDNA. Only the location of microsatellite probes revealed differences among T. spinipes colonies, an important highlight. This is first chromosomal mapping of microsatellite for genus Trigona. The colonies are located along the Atlantic Forest, however environmental changes, altitude and temperature, over this biome may have originated variations detected. Although we do not have sufficient information to state that observed variations reflect local adaptations. Ours data demonstrate that a new tool, chromosomal mapping of microsatellites, may be used for cytogenetic and population studies.

4.P13

Molecular cytogenetic demonstrate variation in the repetitive DNA of stingless bees of the genus Melipona

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The bees Melipona genus are social insects belonging to the tribe Meliponini. Distributed in the Neotropical region, they have economic importance and ecology because they are pollinators of native plants and grown in their areas of occurrence. Cytogenetic studies indicate diploid number 2n=18 chromosomes conserved for the species of the genus Melipona, except M. seminigra (2n=20). However the pattern of distribution and quantity of heterochromatin allowed a division the genus into two groups: the first group characterized by species with low heterochromatic content while the second would present species with high heterochromatin content. The objective of this study was to evaluate the distribution of microsatellite loci GA15 using the fluorescent in situ hybridization in species with high (M. fuliginosa and M. flavolineata) and low heterochromatic content (M. bicolor and M. puncticollis) and compare the results with the heterochromatic pattern in order to contribute to evolutionary studies of the group. The distribution of microsatellites GA15 confirms the division of Meliponidae: the species with high heterochromatin content, M. fuliginosa and M. flavolineata, marked the subtelomeric region in almost all chromosome arms, coinciding with euchromatin; already the species with low content M. punctiollis and M. bicolor, plus some subtelomeric markings, have submitted a pair of chromosomes with stronger and bigger bands in the pericentromeric region and absence of markings on some chromosomes. This indicates that the euchromatin appears in both groups have a pattern of distribution of different microsatellites, probably reflecting different evolutionary histories. Thus, the distribution of microsatellite patterns were important cytogenetic markers that allowed genetic differentiation among species of high and low DNA content. It is expected that this tool can contribute to a better understanding of the evolution pattern between species of the genus Melipona.

4.P14

Sequence characterization and genomic distribution of Panax ginseng tandem repeats (PgTR) reveal utility in chromosome identification and possible role in heterochromatin maintenance

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Repetitive elements make up a large portion of plant genomes and play major roles in molecular regulatory functions. In particular, tandem repeats have been associated with heterochromatin maintenance and gene expression. Here, we characterized the sequence and genomic distribution of a 167-bp Panax ginseng tandem repeat (PgTR), consisting of 9.6 repeat units (1,603 bp), from bacterial artificial chromosome (BAC) PgH005J07. This locus is flanked by nested fragments of DNA transposons in a Ty3/Gypsy LTR retrotransposons, implying a retrotransposon derivative of this repeat. Nine PgTR variants were identified and categorized into two subclasses; the most diverged variant was PgTR1, which comprised its own subclass and had at most 86 % sequence identity to the other variants. All repeat unit variants possessed high AT content between nt 100 and nt 125, with curvature propensity peaks corresponding to this region, implying that PgTRs play a role in heterochromatin folding. Whole-genome sequence (WGS) read mapping revealed that the PgTR units varied in genomic abundance. Additionally, all units possessed cis-acting regulatory elements associated with biotic and abiotic stress response. WGS read mapping covered only <1 % of the genomic PgTR while FISH analysis covered about 8 % and revealed a genomic distribution unique to individual chromosomes. Thus, PgTRs are reliable cytogenetic markers and can be used to discriminate between chromosomes. As such, PgTRs will contribute to comparative cytogenetics among Panax spp., increasing our understanding of genome evolutionary dynamics. Furthermore, our results should provide a foundation for future research on the role of tandem repeats in gene expression, as well as in the shaping and maintenance of the ginseng genome.

4.P15

Effects of chromosomal translocations on DNA methylation and consequences on spermatogenesis in the germline of male pigs

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Infertility is a major concern for both human and livestock. Epigenetic analyses have highlighted abnormalities in the methylation of several genes in the germline of infertile subjects, providing new informations on genetic causes of infertility. Furthermore, it has been recently hypothetized that one of the mechanisms through which balanced chromosomal translocations affect fertility is the induction in germ cells of epigenetic modifications on genes critical for meiosis. Our team previously found an abnormal methylation in an imprinted locus in three out of eight infertile boars, including the two boars carrying a chromosomal translocation. We thus aim to explore the impact of chromosomal translocations on DNA methylation during male meiosis, in a context of altered spermatogenesis. We will study genome-wide DNA methylation in the germline of these three boars and local methylation around the breakpoints in the translocation carriers, compared to a fertile non-translocated control. For these purposes, we will confirm high-throughput sequencing data using pyrosequencing on bisulfite converted DNA extracted from sperm and from meiocytes and spermatogonies sorted by FACS. We will then study their transcriptome and focus on the expression of genes with methylation alterations and genes located at the translocations breakpoints. This work should provide insights on a newly discovered epigenetic mechanism through which chromosomal translocations affect spermatogenesis and fertility.

4.P16

Preparation of chromosome-specific paints by flow-sorting of Xenopus tropicalis

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The african western clawed frog (Xenopus tropicalis, XTR), also known as the tropical clawed frog, is an important model organism for genetic and developmental studies. X. tropicalis, has a small genome, about 1.7 Gbp, with 10 chromosome pairs (2n=20). We have generated chromosome specific painting probes for 8 of these chromosome pairs from flow-sorted chromosomes, amplified and labelled by DOP-PCR, and assigned by hybridization to XTR chromosomes 1, 2, 3, 5, 7, 8, 9 and 10. A comparative genome study is underway using cross-species painting with chromosome-specific XTR probes on X. laevis (XLA), whose allotetraploid genome (3.1 Gbp, 2n=36) is separated from XTR by 50–65 million years, as part of a project on karyotype evolution in amphibians. Cross-species painting using microdissected XTR probes on XLA has been reported previously, but this seems to be the first study using amphibian whole chromosome-specific DNA from flow-sorted chromosomes.

4.P17

Genomic in situ hybridization identifies genomic relationships of Sorghum bicolor and closely related species (Poaceae)

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Cultivated sorghum [Sorghum bicolor (L.) Moench] ranks fifth in both production and planted area of cereal crops worldwide, only behind wheat, rice, maize, and barley, but the degree of relatedness of S. bicolor with closely related species in the genus is unverified. Multicolor genomic in situ hybridization (McGISH) was used to study the genome organization and evolution of S. bicolor. Total genomic DNA from closely-related species (two probe pairs from S. arundinaceum and S. × almum, and S. arundinaceum and S. propinquum) were labeled and hybridized in the

presence of blocking DNA onto metaphase spreads of S. bicolor. The probe pair of S. arundinaceum and S. × almum painted sharp signals and peak appeared in the centromere region of 16 chromosomes from S. bicolor, whereas the probe pair of S. arundinaceum and S. propinquum delineated uniform signals without peak located on 16 chromosomes of S. bicolor. The McGISH evidence support that 1) the diploid S. arundinaceum and S. propinquum are the primary ancestor of S. bicolor, 2) probe of S. × almum (progenitor) produced strong hybridization signals on 16 chromosomes of S. bicolor (ancestor), and 3) recurrent hybridization between diploid ancestors formed the diploidization S. bicolor.

5. Molecular Mechanisms of Chromosome Rearrangements

5.P1

Genomic organization and evolution of double minutes/homogeneously staining regions with MYC amplification in human cancer

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The mechanism for generating double minutes chromosomes (dmin) and homogeneously staining regions (hsr) in cancer is still poorly understood. Through an integrated approach combining next generation sequencing, single nucleotide polymorphism array, fluorescent in situ hybridization and polymerase chain reaction-based techniques, we inferred the fine structure of MYC- containing dmin/hsr amplicons harboring sequences from several different chromosomes in seven tumor cell lines, and characterized an unprecedented number of hsr insertion sites. Local chromosome shattering involving a single-step catastrophic event (chromothripsis) was recently proposed to explain clustered chromosomal rearrangements and genomic amplifications in cancer. Our bioinformatics analyses based on the listed criteria to define chromothripsis led us to exclude it as the driving force underlying amplicon genesis in our samples. Instead, the finding of coexisting heterogeneous amplicons, differing in their complexity and chromosome content, in cell lines derived from the same tumor indicated the occurrence of a multistep evolutionary process in the genesis of dmin/hsr. Our integrated approach allowed us to gather a complet view of the complex chromosome rearrangements occurring within MYC amplicons, suggesting that more than one model may be invoked to explain the origin of dmin/hsr in cancer. Finally, we identified PVT1 as a target of fusion events, confirming its role as breakpoint hotspot in MYC amplification.

5.P2

Molecular mechanisms related to abnormal phenotype of balanced chromosome rearrangements carriers

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Balanced chromosomal rearrangements (BCR) occur in approximately 1:2000 newborns and are associated with abnormal phenotypes in around 6 % of cases. Abnormal phenotypes can be caused by disruption of genes at the breakpoints, small duplications or deletions, positional effects. Conventional cytogenetic techniques have limited resolution and enables complete clinical investigation. Molecular techniques applied to BCR carriers can contribute to characterization of this type of chromosome rearrangement and, also to phenotype-genotype correlation. From last 10 years (from 2004 to 2014), 35 individuals with abnormal phenotype and BCR were registered in our service and 15 were selected to investigation by molecular techniques. Chromosomes

rearrangements involved 11 reciprocal translocation, 3 inversions and one balanced insertion. First, array genomic hybridization (AGH) was performed and genomic imbalances were detected in 20 % of cases, one at rearrangement breakpoint while other two in unrelated chromosomes. Alterations were further confirmed by fluorescence in situ hybridization and were associated to the phenotype of carriers. In patients that AGH did not show any genomic imbalance, second step was investigate chromosome breakpoints at base pair level by next generation sequence. Chromosomes involved in rearrangement were directly microdissected from metaphase slides, amplified by a commercial kit, and sufficient material for sequencing was obtained. Genomic libraries were prepared following the Illumina Nextera XT protocol and paired-end sequence was carried out in Illumina HiSEQ 2000. Currently, bioinformatics tools of alignment are analyzing sequence reads and will be presented in detail. The strategy applied in this study seems to be efficient to elucidate the genomic mechanisms responsible for abnormal phenotype of BCR carriers. This is the first study in our laboratory using this approach, which probably will improve the management of patients carrying BCR.

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5.P3

Chromothripsis: a plausible mechanism for reproductive failures and complexe chromosomal rearrangement genesis.

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Over the last 4 years, the discovery of an unexpected type of massive chromosomal rearrangements, called chromothripsis, came revolutionized our view of the genesis of complex chromosomal rearrangement in cancers and congenital disorders.

Chromothripsis is characterized by the shattering of one (or a few) chromosome segment(s) followed by a chaotic reassembly of the fragments generated through a single initial catastrophic event.

Various mechanisms, involving abortive apoptosis, telomere erosion, mitotic errors, micronuclei formation and p53 inactivation might cause chromothripsis. The remarkable point is that all these plausible mechanisms have been identified in the field of human reproduction as causal factors for reproductive failures and chromosomal abnormalities genesis. Specific features of gametogenesis and early embryonic development such as the weakness of cell cycle and mitosis checkpoints and the rapid kinetics of division in germ cells and early cleavage embryos, may contribute to the emergency of chromothripsis. All the data support the assumption that this unanticipated catastrophic phenomenon may arise more frequently than previously thought in both gametogenesis and human embryogenesis, but also confirmed its compatibility with viability and transmission to subsequent generations.

The complexity of chromothripsis and the diversity of its causative mechanisms raises questions about the origin of this phenomenon, its ties to chromosome instability and its implication in human reproduction and congenital disorders formation. Further in-depth analyses on large samples of patients and cells (gametes and blastomeres) are needed to appreciate the contribution of this cataclysmic process to altered human development and the generation of congenital diseases. This constitutes a great challenge and implies further efforts to develop and adapt new techniques, especially next-generation sequencing, to the precise identification of massive genomic rearrangements.

5.P4

Use of 5-ethynyl-2′-deoxyuridine (EdU) incorporation for identification of the late replicating X-chromosome in balanced and unbalanced alterations

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X-chromosome inactivation usually occurs randomly in normal female cells, being either maternal or paternal X-chromosome methylated. However, in women with X- chromosome alterations, the inactivation is not random: in X-autosome balanced translocations, normal X is preferentially inactivated, while in unbalanced X chromosome alterations, the aberrant X is usually

inactivated. X inactivation pattern has been identified by different approaches, such as: cytogenetically, using 5-bromo-2′deoxyuridine (BrdU) or antibodies to the acetylated histones; and molecularly, using HUMARA assay or methylation analyses. Here we present X-chromosome late replication data from seven patients: four with X-autosome balanced translocations; two with Xp deletion and one with Xq isochromosome. Lymphocyte cultures were exposed to 5-ethynyl-2′-deoxyuridine (EdU) for 2 h before preparation, using Alexa Fluor® 488 and DAPI staining, according to modifications of Click-iT® EdU Assay kit (Invitrogen). Because EdU is a labeled nucleoside analog of thymidine, its incorporation during active DNA synthesis can reveal late replication regions and the inactive X-chromosome. In order to better distinguish the derivative X chromosomes, fluorescent in situ hybridization, with centromere probes and BAC probes (red labeled) for different parts of the X chromosome, was used simultaneously with EdU procedure. As expected, we found that the normal X-chromosome was preferentially inactivated in women with X-autosome balanced translocations; while preferential inactivation occurred in the aberrant X-chromosome in unbalanced alterations. The inactivated X presented a strong green fluorescence. This EdU assay permitted an accurate and efficient cytogenetic detection of the late replication regions, being faster and more sensitive in the evaluation of X inactivation than BrdU incorporation methods.

5.P5

A novel recurrent breakpoint in the Williams–Beuren region as a cause of FISH pitfalls in the diagnosis of Williams syndrome

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Copy number variants (CNVs) of the Williams–Beuren syndrome (WBS) 7q11.23 region are responsible for neurodevelopmental disorders with multi-system involvement and variable expressivity. Here we describe three patients with structural reorganizations of the 7q11.23 region sharing a common breakpoint between LIMK1 and EIF4H/WBSCR1 genes indicating the existence of a previously unrecognized recurrent breackpoint responsible for rearrangements in the WBS region. One patient had WBS phenotype and although testing with a commercially available FISH assay was negative for deletion, aCGH showed an atypical deletion in the WBS region with atypical breakpoints. Our findings point to the existence of a previously unrecognized recurrent breakpoint responsible for rearrangements in the WBS region. Most commercial FISH assays include probes flanking this novel breakpoint and, as consequence, fail to detect reorganizations with this breakpoint. Further testing with aCGH array should be performed in patients with WBS and negative FISH results.

5.P6

Chromosomal integrity associated with different approaches for in vitro stem cells derivation

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Stem cells have brought great promises for disease modelling and cell-based therapies where special place have been taken by induced pluripotent stem cells (iPSCs). This technique allows to derive individualised stem cell lines in vitro by reprograming differentiated cells e.g. fibroblasts to pluripotent cells. One concern related to the use of reprogrammed somatic cells is the loss of genomic integrity and chromosome stability, that is a hallmark for cancer and many other human disorders.

We investigated 16 human iPSC lines reprogrammed by non-integrative Sendai virus (SeV) and another 16 iPSC lines generated by integrative lentivirus for genetic changes. At early passages the cytogenetic rearrangements were detected in 44 % (7/16) and 6 % (1/16) of iPSC lines generated by lentiviral and SeV integration correspondingly. We found that these numerical and/or structural rearrangements were associated with

chromosomes 5 and 12 known to be as the most frequently involved chromosomes. We present herein the karyotypic aberrations in the iPSC lines including a duplication on chromosome 5q13-q33 that restricts a candidate region for growth advantage.

We conclude that the use of integrative lentivirus confers a higher risk for cytogenetic abnormalities at early passages when compared to SeV based reprogramming. Thus, SeV based generation of iPSC maintains genomic integrity better than integrative lentivirus. Therefore our results suggest that SeV has better perspectives to be used for stem iPSCs derivation especially for purposes related to cell-based therapies. Additionally our findings expand the knowledge on acquired cytogenetic aberrations in iPSC after reprogramming and during culture.

5.P7

Evaluation of genotoxic effects of antihypertensive drugs: Angiotensin II receptor blockers

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Arterial Hypertension is commonly treated with antihypertensive drugs combined with appropriate changes in life-style. The large majority of hypertensive patients need long-term therapy and documentation of safety and efficiency is required, including sensitive indices of genotoxic damage.

In this study, the potential genotoxic effect of five Angiotensin II receptor blockers (ARBs: Candesartan, Telmisartan, Eprosartan, Valsartan, Olmesartan) was analyzed in vivo and in vitro in human peripheral blood lymphocytes (PBLs) by means of the cytokinesis-block micronucleous (CBMN)assay in combination with fluorescence in situ hybridization (FISH) with a centromeric probe, and by the analysis of structural chromosomal aberrations (sCAs) and expression of fragile sites (FS). The in vivo study was carried out in 55 hypertensive patients while the in vitro study was performed in 10 control individuals by adding the drugs to the culture medium at a final concentration similar to the levels found in plasma in patients. In relation to the CBMN assay, our results showed a significant increase in the frequencies of MN and binucleated cells with MN (BNMN) in vivo and especially in vitro. FISH analysis revealed no significant differences between patients and control individuals in the frequency of centromeric signals. Regarding the incidence of sCA, the data revealed again a significantly higher rate of sCA in vivo, and mostly in vitro. Chomosomal location analysis pointed at chromosomes 2 and 17 as preferential targets for the clastogenic effect of ARBs drugs. Several FS emerged as specific; among them, the locus 17q21, recently proposed as a new FS, was only expressed in ARBs-treated patients, supporting the findings for a linkage between hypertension and a telomeric locus on chromosome 17.

These results provide evidence for an association among the antihypertensive therapy, arterial hypertension and DNA damage in human lymphocytes.

5.P8

Derivative from de novo unbalanced translocations: overall frequency, parental origin and mechanism of formation

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Derivative chromosome from de novo unbalanced translocation (UT) is defined as a chromosome comprising terminal duplication and deletion of a terminal segment of another chromosome. Few studies investigated the breakpoint sequences of de novo UTs and the parental origin of the unbalanced segments. Previous studies hypothesized a mechanism of formation

different from a parental germline or low-level somatic mosaicism for balanced translocations. We present six new cases, five of them detected during prenatal diagnosis, with de novo UTs with the aim to: i) calculate their frequency in prenatal samples; ii) determine the parental origin of the unbalanced segments and iii) discuss the mechanism of formation.

Karyotyping were performed following the standard methods and the European Guidelines. After detecting an UT, parental metaphases were analyzed by karyotyping and/or FISH using specific probes to define the origin and the recurrence risk. The de novo derivative chromosomes were characterized by chromosomal microarrays and the origin of the unbalances were investigated by segregation microsatellite analysis.

The incidence of UTs in prenatal samples is 0.053 % (95 %CI: 0.032–0.085 %); in agreement with previous studies ~30 % of them are de novo and have an incidence of 0.016 % (1:6094) (95 %CI: 0.007–0.038). As expected, in patients with developmental disabilities the reported frequency is 0.23 %, higher than in general prenatal population ($p<0.01$). Four out of six reported cases showed a cryptic aberration, not detectable by conventional karyotyping due to the small size of the deleted or duplicated segment. We'll discuss the possible mechanisms of derivative formation (meiotic or mitotic) in relation to the parental origin and a literature review.

5.P9

Copy Number Variants In Premature Ovarian Failure

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Premature ovarian failure (POF) is a complex heterogeneous clinical disease that may be affected by the multiple genes that control follicle formation and is characterized by an early loss of the ovarian function. The etiology of POF generates 20 % of familial genetic causes and 5 % of chromosome X abnormalities. Autosomal chromosome abnormalities may also play a role, as well. Recently, array-CGH was found to be a very useful tool in the identification of candidate genes in different conditions. Therefore, we performed array-CGH analysis in a total of 55 POF (30,4±6,36 years) patients with normal chromosome constitutions and Fragile X specific CGG repeats. Totally, 16 different copy number variations (62.5 % deletions) were detected in 42 % of the patients. The deletion of the ASMTL, P2RY8,SHOX (chrXp22.33) and MSX1 (chr4p16.2) genes whose involvement in POF have been reported previously were also mostly seen CNVs in the study. Besides, it was striking to note that the chromosome 15q26.3 deletion was detected in 5 cases. The gene PCSK6, located in this locus, is a member of proprotein convertase (PC) family that is expressed in numerous ovarian cell types including granulosa cells and oocytes. Previous studies have reported its role in maintaining normal cellular and tissue homeostasis in the ovary. We suggested that PCSK6 activity should be examined in human ovarian pathology to ascertain further the clinical role that altered PC activity may contribute to human ovarian function.

5.P10

Genetic and epigenetic biomarkers of folate metabolism and trisomy 21

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At the end of the last Century it was hypothesized that impaiments in folate metabolism, resulting from the presence of polymorphisms in metabolic genes, could impair DNA methylation at centromeric regions, thus fovouring chromosome 21 malsegregation and the birth of child with Down Syndrome (DS). We subsequently screened peripheral blood lymphocytes of women who had a child with DS (MDS) in young age (<35 years) observing an increased tendency to chromosome malsegregation also in somatic cells (evaluated by means of the micronucleus assay coupled with FISH technique). In order to shed light on the contribution of folate metabolism to the observed increased frequency of micronucleated lymphocytes in MDS we applied artificial neural networks (ANNs) that revealed important connections between the cytogenetic damage and

common polymorphisms in genes of the folate metabolic pathway, namely MTHFR 677C>T, MTRR 66A>G, RFC1 80G>A, MTR 2756A>G, TYMS 28 bp repeats and 1494 6 bp deletion polymorphisms. Subsequent case–control studies and/or literature meta-analyses by us confirmed a contribution of both RFC1 80G>A and MTRR 66A>G polymorphisms to the maternal risk of birth of a child with DS. Moreover, we recently observed association of promoter polymorphisms in the DNMT3B gene, coding for a DNA methyltransferase protein, with the risk of birth of a child with DS, as well as increased MTHFR promoter methylation in blood DNA of MDS with respect to control mothers. Overall, our data suggest that both genetic and epigenetic biomarkers of the folate metabolic pathway could represent a maternal risk factor for trisomy 21.

5.P11

Copy number variation and expression of hTERT and hTERC genes in amniocyte cultured samples with normal and abnormal karyotype

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Telomeres are located at the ends of chromosomes and protect them from fusion and degradation. Telomerase is a ribonucleoprotein enzyme complex that adds telomeric repeats, TTAGGG, to the ends of chromosomes. The telomerase components are the telomerase reverse transcriptase (TERT) catalytic subunit, located at chromosome band 5p15.33, and the telomerase RNA (TERC) template subunit, at 3q26.3. Functional telomeres are essential for normal maintenance of chromosomes during mitotic and meiotic division. Clonal evolution of chromosome rearranged cells with increased TERT or TERC copy number has been observed, suggesting a growth advantage in cells with that increased gene dosage. The aim of our work is to study if there is any relationship between the hTERT and hTERC copy number variation in cultured amniocyte samples with normal and abnormal karyotype, to investigate whether there is correlation between a change in the number copies of the two genes together and the second step was check and compare TERT and TERC mRNA expressions, detected by relative quantifications with RT-PCR. Basic research study, interphase fluorescence in situ hybridization (FISH), to determine the TERT and TERC copy number genes, is applied to cultured amniocytes retrieved from nine samples with various chromosome abnormalities and from a control group of 20 samples with a normal karyotype. The rearranged amniocytes showed increased TERC gene copy number and enhanced gathering of the signal compared to the normal amniocytes. Gene expression analysis performed on all cultures revealed that hTERT is not expressed in 1/3 of the samples while the gene hTERC is always expressed. The expression of hTERC is higher than that of hTERT. However there was a greater expression of the amniotic samples with normal karyotype, than those with

5.P12

Unusual chromosomal rearrangement resulted in interstitial monosomy 9p: case report

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Chromosomal rearrangement is a chromosome abnormality in the structure of the chromosome that encompasses several different classes of events, like translocations, inversions, deletions and duplications. Structural chromosomal abnormalities occur in approximately 0.5 % of newborns. On the other hand, complex chromosomal rearrangements are much more rare structural anomalies. At least three break points, including genetic material exchange between two or more chromosomes, are required for complex chromosomal rearrangements (CCRs). Genomic gains and losses frequently accompany complex chromosomal rearrangements. Whether the amount of genetic material changes or not, clinical results may occur due to break points. Overall, CCRs are often associated with mental retardation, congenital abnormalities and recurrent abortions. Here, we report a case with interstitial monosomy 9p in a complex de novo rearrangement. The patient presented with craniofacial dysmorphism, delayed psychomotor development and various congenital malformations. He had also bilateral nephrolithiasis and cryptorchidism.

We combined cytogenetic studies and FISH analyses to delineate the deletion. The result of our cytogenetic studies was 46,XY,der(9)(p22→pter). In order to confirm deletion we also performed FISH analysis and these analyses showed that 9p subtelemeric region has inserted into 13th chromosome. Molecular karyotyping was performed to describe the exact genomic points of rearrangement. We confirmed that the arrangement occurred in this case is an insertion/deletion complex abnormality which has not been reported earlier.

Insertional translocations (ITs) are rare chromosomal rearrangements and at least three breaks are required for formation of ITs. The exact cytogenetic mechanisms underlying the origin of ITs and CCRs are unclear but several mechanisms have been proposed to explain the reasons of them, such as non-allelic homologous recombination, non-homologous end joining, fork stalling and template switching micro homology mediated break-induced replication and chromothripsis. Elucidation of these mechanisms will facilitate our understanding of genomic rearrangement.

5.P13

A mosaic of two cell lines carrying different terminal 22q13 deletions: a case report

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Phelan-McDermid Syndrome (PMS) is a neurodevelopmental disordercaused by the deletion or disruption of a 22q13 region. The clinical features ofPMS are highly variable and include developmental delay, expressivespeech/language delay, neonatal hypotonia, autistic traits and mild dysmorphic features.

22q13 monosomy can result from simple terminal deletions (70 %), ring chromosomes (14 %), unbalanced translocations (7 %) or interstitial deletions (17–74 kb) in 9 % of the patients. Although 22q terminal deletions are well documented, very few patients with mosaicism have been reported so far.

We describe a new case of mosaic for at least two cell lines carrying de novo different terminal 22q13 deletions detected by CGH-array (Agilent 180 K(Hg19)). All the cells have a 2, 7 Mb terminal deletion (from 48 388 703 to 51,178,264 bp), while only 40 % as confirmed by FISH with clone CTA-268H5 have a larger deletion (6,3 Mb) (from 44 864 865 to 48 31 5 347 bp).

This mosaicism may be due to distinct stabilizing events, occurring in different cells of the early embryo, of the same unstable terminal deletion. Previous studies in subjects with 22q13 deletion unraveled variety of mechanisms for chromosome repair of terminal deletions, including de novo telomere synthesis and telomere capture. In this case, we hypothesize that a terminal deletion happened in meiosis, which was imperfectly repaired by telomere capture or synthesis, leading to an unstable chromosome which subsequently broke again in a subset of blastomeres.

While these cryptic mosaics may lead to a more complex than expected correlation between deletion size and severity of phenotype, SHANK3 is up to now the only candidate gene responsible for the neurological features of the PMS phenotype which in this case was not confounding.

6. Impact of Next Generation Sequencing on Clinical Diagnosis

6.P1

Impact of Array Painting and Whole Genome Sequencing on Cytogenetic Diagnosis: from Complex Chromosome Rearrangements to chromothripsis

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Complex chromosomal rearrangements (CCR) are defined as balanced or unbalanced structural abnormalities of chromosomes with 3 breakpoints on 2 different chromosomes. CCR are rarely reported in constitutional cytogenetics and mainly include associations of translocations.

We report the case of a patient with early intellectual deficiency associated with absence of language, hypotonia and postural delay despite a good understanding and a good motor autonomy. Karyotype revealed a de novo complex translocation to chromosomes 3:

46,XY,t(3;15;5)(p11;q2?5;q23)dn.

To better characterize this RCC, we implemented various techniques such as FISH, Array Painting and Whole Genome Sequencing. These different approaches led to the identification of a much complex and massive genomic rearrangement which may be classified as a chromothripsis. This constitutes a new class of complex chromosomal rearrangements based on the shattering of one or a few chromosomal segment(s) followed by a chaotic reassembly of the chromosomal segments.

The study of this patient confirms the complexity of the genesis mechanisms of CCR and then subsequent diagnosis. The implementation of new techniques has upset our knowledge with the discovery of the unanticipated phenomena coined chromothripsis. We emphasize the difficulties of interpretation of these new techniques, particularly Whole Genome Sequencing and therefore the importance of the role of FISH at present in everyday life.

6.P2

Characterization of complex chromosomal rearrangements through new cytogenetic technologies: from discovery to discovery.

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Complex chromosomal rearrangements (CCRs) are structural abnormalities involving at least three breakpoints with the exchange of chromosomal segments. The great majority of CCRs are associations of translocations.

We report the case of a female child in intensive care for intrauterine growth retardation, neonatal respiratory distress, sucking and swallowing difficulties, global hypertonia and malformation syndrome (patent malformative ductus arteriosus, cortical giration and white matter abnormalities, ocular abnormalities, bilateral renal cortical microcysts, abnormalities of hands and feet). The child died at 3 months of life in a context of seizures, respiratory distress and heart failure.

The initial karyotype suggested a de novo unbalanced CCR with what resembled a 5p isochromosome, a chromosome 1 derivative from a t(1p36;5q13) and a 1p36.3 terminal deletion.

Chromosomal microarray (CMA) studies revealed additional de novo imbalances with a 547-kb deletion in 2p11.2, a 2509-kb duplication in 5q11.1q11.2 and 250-kb and 1190-kb deletions in 5q31.1. The 2p11.2 deletion includes the REEP1 gene responsible for autosomal dominant distal hereditary motor neuropathy type VB (HMNVB, omim: #614751).

Whole genome sequencing (WGS) clarified the breakpoints of the chromosomal rearrangements and revealed new ones, further complicating the cytogenetics of the CCR. The identification of these numerous breakpoints on two chromosomes suggests chromothripsis or chromoanasynthesis, two unanticipated phenomenon recently evidenced in constitutional chromosomal disorders.

The use of recent technologies in cytogenetics (CMA, WGS) has brought a new dynamic to this discipline by both increasing the number of etiological diagnoses made and also by apprehending the mechanisms leading to chromosomal aberrations. Ultimately a lot of CCRs are more complex than initially suggested by conventional cytogenetic analysis.

6.P3

Genetic non invasive prenatal testing: a clinical and technical experience of 3.000 cases with follow-up.

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Non Invasive Prenatal Testing (NIPT) is a fetal cell-free DNA genomic based screening for an accurate detection of chromosomal abnormalities, including chromosome 21, 18 and 13 trisomies, X and Y aneuploidies. Because NIPT is very accurate (>99 % accuracy in 21, 18 and 13 trisomies) and is not life threatening for the fetus, has been recently introduced in prenatal care as a powerful tool in cases where invasive techniques do not seem to be necessary (i.e. advanced maternal age with normal ultrasound). Here we show a NIPT protocol, called TrisoNIM®, which has been partially performed in our laboratory, based in massive parallel sequencing.

A total of 3000 samples were included. DNA from maternal plasma was extracted and sequenced using Ion Proton® protocols, optimized for the detection of trisomies 21, 18, 13. In collaboration with BGI Europe, sequenced data were uploaded to the BGI cloud and analyzed using the NIFTY algorithm. Clinical information at birth of all cases was available for follow-up.

High risk results for aneuploidies were detected in 36 cases out of 3000 (1.2 %) including: 25 trisomy 21 (0.83 %), 7 trisomy 18 (0.23 %) and 8 sexual aneuploidies (0.27 %). Confirmation results was performed in all instances by amniocentesis with prenatal array-CGH. 24/25 trisomy 21, 5/7 trisomy 18 and 5/8 sexual aneuploidies were confirmed. False positive in trisomy 21 was explained due to a vanishing twin effect; false positives in T18 and sexual aneuploidies were suggested to be caused by placental mosaicism (0.16 % of general population). No false negative were found.

TrisoNIM is a highly accurate technique for testing prenatal chromosomal aneuploidies. However, the presence of false positives due to placental biological processes such as placental mosaicism highlights the need for an invasive confirmation in every high

7. Other

7.P1

Clinical, immunological and cytogenetic findings in children with chromosome instability syndromes from Latvia.

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Introduction. Chromosome instability is a hallmark of some rare inherited monogenic disorders, which are characterized by failure to thrive, developmental delay, congenital anomalies, immunodeficiency, and predisposition to malignancy.

The aim of the study is to reveal the chromosome aberrations, clinical features and disturbances of the immune system of Nijmegen breakage syndrome (NBS), ataxia—telangiectasia syndrome (A-T) and Fanconi anemia (FA) patients from Latvia diagnosed in a single tertiary referral hospital (2006–2014).

Methods. NBS was diagnosed in two girls and two boys (average age 6.83 years, range 0.75–17.75 years) from three families by standard cytogenetic analysis. A-T was detected in two girls (7.33 and 9.50 years). Four girls and ten boys (average age 10 years, range 4.25–15.9 years) with various hematological disorders (bone marrow failure, anemia, thrombocytopenia, leukopenia and/or malignancy) were evaluated by chromosome breakage test using diepoxybutane (DEB).

Results. Characteristic chromosomal rearrangements involving breakpoints at 7p13, 7q35, 14q11.2 and 14q32 in standard blood cultures were observed in 13.6–30.0 % and 6.3 % of analysed cells in patients with NBS and A-T, respectively. NBS and A-T patients had typical clinical features. There were different changes in immunoglobulin level (mostly IgA, IgG) and cell immunity in NBS and A-T patients. FA was diagnosed in two girls and two boys (average age 9.17 years, range 8.17–10.17 years). Spontaneous chromosome aberrations were observed in 27.00±9.31 % (16–38 %) of analysed cells from FA patients compared to 2.00±2.71 % (0–8 %) in normal control population. DEB-induced chromosome aberrations were seen in 85.00±6.83 % (76–92 %) of analysed cells from FA patients compared to 7.31±3.35 % (4–12 %) in normal control population.

Conclusions. Detection of underlying genetic defect provides the appropriate family genetic counseling and subsequent management.

7.P2

Characterization by molecular cytogenetics, array-CGH and whole transcriptome analysis of different batches of HeLa cells: genomic variability and divergent gene expression

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The HeLa cell line is one of the best-characterized and used cell line in biomedical research. We compared the genomic profile of different HeLa batches by chromosome analysis, FISH and array-CGH with the aim to verify their genomic variation. The batches under study were four, and they have been obtained from four different Italian laboratories: two of them derived from the same batch but then they were cultured in two different laboratories for about 8 years, as well as the two other batches, that derived from the same source, but were then cultured in two different laboratories for about 12 years.

The results demonstrate that the four different batches analyzed exhibit a time-dependent divergence in chromosome anomalies and rearrangements, with substantial differences in DNA content. Since genomic gains or losses or whole-chromosome aneuploidy can imply drastic consequences in gene expression, we performed whole transcriptome analysis in order to verify the transcriptional effect of the different aneuploidies and rearrangements of our clones. We concluded that the genomic difference is paralleled by a severe alteration of the gene expression profile. Our results highlight that the use of HeLa cells might lead to faulty conclusions and to unreproducible results.

7.P3

Chromosome hydroxymethylation patterns in human zygotes and preimplantation embryos

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We have studied chromosome hydroxymethylation and methylation patterns in metaphase chromosomes from IVF-produced human triploid zygotes and blastomeres of cleavage-stage embryos. To obtain metaphase chromosomes, zygotes and embryos were treated with 0.1 % colchicines, 0.9 % sodium citrate and fixed with freshly prepared 3:1 methanol:acetic acid. Using indirect immunofluorescence, we have analyzed the localization of 5-hydroxymethylcytosine (5hmC) and its co-distribution with 5-methylcytosine (5mC) on the QFH-banded metaphase chromosomes.

We observed the sequential changes in 5hmC and 5mC patterns in the genome of human preimplantation embryos. In zygotes, 5hmC accumulates in both parental chromosome sets, but hydroxymethylation is more intensive in the poorly methylated paternal set. In the maternal set, chromosomes are highly methylated, but contain little 5hmC. Hydroxymethylation is highly region specific in both parental chromosome sets: hydroxymethylated loci correspond to R-bands, but not G-bands, and have well-defined borders, which coincide with the R/G-band boundaries. The centromeric regions and heterochromatin at 1q12, 9q12, 16q11.2, and Yq12 contain little 5mC and no 5hmC. We hypothesize that 5hmC may mark structural/functional genome 'units' corresponding to chromosome bands in the newly formed zygotic genome. Also, we suggest that the hydroxymethylation of R-bands in zygotes can be treated as a new characteristic distinguishing them from G-bands. At cleavages, chromosomes with asymmetrical hydroxymethylation of sister chromatids appear. They decrease in number during cleavages, whereas totally non-hydroxymethylated chromosomes become numerous.

Our findings suggest that, in the zygotic genome, 5hmC is distributed selectively and its pattern is

determined by both parental origin of chromosomes and type of chromosome bands—R, G, or C. At cleavages, chromosome hydroxymethylation pattern is dynamically changed due to passive and non-selective overall loss of 5hmC, which coincides with that of 5mC.

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7.P4

The DNA Integrity Number: A novel approach for objective integrity classification of genomic DNA samples

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Genomic DNA (gDNA) is used as starting material in the experimental workflow of many applications in molecular biology. The integrity of the DNA critically affects the success of many downstream experiments like array CGH or sequencing. Initial electrophoretic analysis of the sample is highly recommended as the respective downstream applications can be expensive and time consuming. The Agilent Genomic DNA ScreenTape Assay has been primarily developed for the electrophoretic analysis of genomic DNA samples. A ScreenTape is a pre-packaged microfluidic device designed for performing electrophoretic applications in a microscale format. It is used in combination with the Agilent 2200 TapeStation instrument. Degradation of gDNA is typically a gradual process in which high-molecular weight DNA is fragmented into smaller species. It can occur either enzymatically, chemically or mechanically. Judging the integrity of DNA by visual evaluation of the electropherogram trace is subjective and can be error-prone. In order to standardize this a novel algorithm was developed to score gDNA samples on the 2200 TapeStation. The DNA integrity number (DIN) is calculated from several features obtained from the electrophoretic trace and ranges from 1 to 10. Here we show data demonstrating the reproducibility, scalability and linearity of the DIN. The DIN is independent from instrument, reagent and sample concentration variability and can be used as objective measure for determining the integrity of gDNA.

7.P5

Clonal chromosomal alterations during the mesenchymal stem cell cultivation- case report

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The use of human mesenchymal stem cells (MSC) has been shown to be a promising strategy for cell therapy in injured tissues recovery. However, it has been described that MSCs can acquire genetic changes when cultivated in vitro and they would be more susceptible to undergo neoplastic transformation. Thus, the application of cytogenetic techniques is needed to evaluate the integrity of MSC genetic material used in cell therapy. As quality control of the cells, at the Center for Cell Technology (CTC-PUCPR), we performed regularly cytogenetic analyses (G-banding karyotyping). We report on a 67-year-old patient, male, selected to join the project: Autologous transplantation of mesenchymal cells for treatment of severe and refractory isquemic cardiomyopathy. To evaluated chromosomal alterations, one bone marrow and five MSC in different passages (P) were used. The bone marrow sample showed a normal karyotype: 46,XY[20], however, the MSC at P2 showed a hyperdiploid clone, with composite karyotype: 43~52,XY,+X,+4,+5,+8[cp4] and non-clonal trissomies: +2, +10, +12, +20. Following pre-established criteria in CTC-PUCPR, this sample was not approved for infusion. After that, the cells showed normal karyotype at P4 and P5. A mononuclear cells sample of the same patient, which had been frozen for backup, was cultivated, and at P3 showed a clonal translocation t(9;18)(p24;q11)[8]. At P5 the karyotype was normal. At this moment, from all cytogenetic analysis performed in CTC-PUCPR (152 samples), this was the only case to show a clonal cytogenetic abnormality, and it was discarded. There is controversy about the use of cells with cytogenetic abnormality for therapy, because of their tumorigenic doubtful capacity. Despite this, we decided, like Muntion et al. (2012), not to use these cells for regenerative medicine purposes.

7.P6

Cytogenetic analysis in Fanconi anemia

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Fanconi Anemia (FA) is a rare autosomal recessive or X-linked disease characterized by chromosomal instability, congenital abnormalities, progressive bone marrow failure and predisposition to developing myelodysplastic syndrome, leukemias or solid tumors. The diepoxybutane (DEB) test is the recommended method to confirm the diagnosis of FA. The DEB test was performed in patients with bone marrow failure syndromes and/or the possibility of having FA (clinical examination/family history). From January 1996 to August 2014 we analyzed peripheral blood (DEB test) and bone marrow samples (G-banding karyotyping) from patients that were referred to the Cytogenetic Laboratory at the Clinical Hospital of the Federal University of Parana. For this test, we calculated the rate of breaks per cell analysed as described by Auerbach (1989). Bone marrow samples were studied by G-banding karyotyping and metaphases were analyzed and interpreted according to ISCN—International System for Human Cytogenetic Nomenclature (2013). A total of 1839 patients were referred for the DEB test. Of these, 451 (25 %) were positive and 1388 (75 %) were negative. The bone marrow karyotypes was obtained in 471 samples of patients who tested positive for the DEB test (some patients have more than one sample). In 129 (27 %) we detected clonal chromosomal aberrations and most of them were observed on chromosomes: 1, 5, 7, 17, 21, 22, 8, 9, 11, 15 and X. Our frequency of positive DEB tests (25 %) was similar to the one reported in the literature. Cytogenetic analysis of bone marrow cells in FA patients is important to understand the progression to malignant hematological diseases although the role of these chromosomal aberrations in tumoral progression remains controversial. Our data indicates that aberrations in chromosomes 1,5 and 7 are among the most frequently described in agreement with literature.

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