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E.C.A. on Facebook

As mentioned in the earlier Newsletters, E.C.A. is on Facebook.
Each week you will find announcements of interesting articles, related to cytogenomics or to biology in general, and also pictures and stories from social events related to E.C.A. and its members. Also our E.C.A. conferences will be covered on Social Media.
You can see the weekly posts and announcements via the direct link

https://www.facebook.com/Cytogenetic/ or on the updated E.C.A. website **http://www.e-c-a.eu/**

You will find a selection of interesting Facebook posts in this Newsletter starting at page 85.
Please contact us (mariano.rocchi@uniba.it) if you wish to share an interesting news item or a pertinent article.

13th EUROPEAN CYTOGENENOMICS CONFERENCE 2021

Programme

3-5 July 2021

Saturday 3 July

CEST **Permanent Working Groups and Satellite Meetings**

09:00-09:05 Introduction by the President

09:05-10:00 **Permanent Working Group Animal & Plant Genomics**
Session-Coordinator: Pat Heslop-Harrison

Poster presentations:
Alla Krasikova: Spatial organization of topologically associated chromatin domains in chicken somatic cell types and meiotic lampbrush chromosomes revealed by FISH

Alsu Saifitdinova: Avian lampbrush chromosomes reflect the complexity of their genome organization at meiotic reprogramming stage

Tatiana Bikchurina: Hybrid sterility in gray voles

Alessandra Iannuzzi: Bovine telomere association between age and breed

Alexandr Sember: Cytogenomics of annual killifish: sex determination and repetitive DNA dynamics in closely related Nothobranchius furzeri and N. kadleci

Sebastian Pita: Satellitome analysis unravels the karyotypic evolution in Triatoma kissing bugs (Hemiptera: Reduviidae)

Pablo Mora: Comparative analysis of the satellitome in ladybirds (Coleoptera, Coccinellidae)

10:05-10:35 **Permanent Working Group Cytogenetic Toxicology and Mutagenesis**
Session-Coordinator: Emanuela Volpi

Poster presentations:
Sophia Zachaki: Chromosome instability and gene rearrangements in ectopic endometriotic tissue of women with endometriosis

Moonisah Usman: Predictors of genomic instability in childhood obesity: a study of multiple variables

Roberto Valli: Interstitial deletion of chromosome 20 and isochromosome i(7)(q10) in the bone marrow of patients with Shwachman-Diamond syndrome: novel evidence of karyotype instability related to somatic rescue mechanisms

10:40-11:35 **Industry-sponsored Satellite Symposium**

11:40-12:15 **Permanent Working Group Prenatal diagnosis**
Session-Coordinator: Jean-Michel Dupont

Tommaso Cavazza: Parental genome unification is highly error-prone in mammalian embryos

Poster presentation:
Laurence Lohmann: Whole genome NIPT: confirmation of the high performance as a screening test

12:20-12:50 **Permanent Working Group Quality Issues, Training and Cytogenomics**
Session-Coordinator: Ron Hochstenbach

Rosalind Hastings: ISCN 2020 - What's new?

Poster presentation:
Thomas Liehr: An International Collaboration for Human Ring chromosomes (ICHRC) towards developing standards and guidelines for best practice in laboratory diagnosis, clinical management and collaborative research

12:55-13:50 **Industry-sponsored Satellite Symposium**

13:55-14:25 **Permanent Working Group Cytogenomics of Hematological Malignancy**
Session-Coordinator Harald Rieder

Poster presentations:
Dolors Costa: Balanced and unbalanced translocations in a multicentric series of 2,843 patients with chronic lymphocytic leukemia

Catherine Menten: The use of optical genome mapping (Bionano) to understand a complex mechanism of KMT2A rearrangement in an AML case.

Emanuela Volpi: Analysis of chromosomal instability in Chronic Lymphocytic Leukaemia

14:30-15:25 **Permanent Working Group Cytogenomics**
Session-Coordinator: Joris Vermeesch

Poster presentations:
Flavia Maggiolini: Single-cell strand sequencing of a macaque genome reveals multiple nested inversions and breakpoint reuse during primate evolution

Vaniamin Fishman: Precise detection of balanced chromosomal rearrangements and single nucleotide variations using chromosome conformation capture

Tine De Coster: Non-canonical zygotic divisions are characterized by parental genome segregation errors

Thomas Guignard: AnnotSV and knotAnnotSV: a web server for human structural variations annotations, ranking and analysis

Jakob Schuy: A case of ring chromosome 21 with hemifacial microsomia, developmental and speech delay analysed with WGS and patient-derived iPS

15:30-16:25 **Industry-sponsored Satellite Symposium**

16:30-17:00 **Permanent Working Group Cancer Cytogenomics and Solid Tumor Studies**
Session-Coordinator: David Gisselsson Nord

Paola dal Cin: Next-Generation Technologies in Old Generation Laboratories

Opening Ceremony

Chair: Mariano Rocchi

17:05 President's address

Opening lecture: Terry Hassold: Aneuploidy: Where do we go from here?

Sunday 4 July

ONLINE POSTER AREA

10:00-12.30 **Session 1 Prenatal and preimplantation diagnosis**
Chair: Jean-Michel Dupont

10:00-10:35 Lyn Chitty: The impact of sequencing on prenatal diagnosis

10:35-11:10 Erik Sistermans: Non-invasive fetal aneuploidy screening

11:10-11:45 Carmen Rubio: Embryo aneuploidy testing, past, present and future

11:45-12:30 Selected abstracts

Stanislav Vasilyev: Aneuploidy is associated with increased methylation of different LINE 1 retrotransposon families in placenta of first trimester miscarriages

Kris Van Den Bogaert: Outcome of publicly funded nationwide first tier noninvasive prenatal screening

Radhia M'Kacher: Direct inheritance of telomere shortening and aberrations detected during prenatal period

12:30–14:00 ONLINE EXHIBITION & BREAK

14:00-16:15 **Session 2 Cancer Cytogenomics**
Chair: Roberta Vanni

14:00-14:35 Roland Schwarz: Pervasive chromosomal instability and karyotype order in tumour evolution

14:35-15:10 Florent Mouliere: Early detection of cancer by liquid biopsy

15:10-15:45 Lindsey Montefiori: Enhancer hijacking of BCL11B defines a subtype of lineage ambiguous acute leukemia

15:45-16:15 Selected abstracts

Ming Chen: primary tumor and circulating tumor cells captured by microfluidics in epithelial ovarian cancer tumor heterogeneity or allele dropout?

Barbara Dewaele: Opportunities of Optical Genome Mapping for Genetic Diagnosis in Acute Lymphoblastic Leukemia

16:15 – 16:30 ONLINE EXHIBITION & BREAK

16:30-18:45 **Session 3 Clinical cytogenomics**
Chair: Elisabeth Syk-Lundberg

16:30-17:05 Chelsea Lowther: Computational and molecular dissection of long-range positional effects in human developmental disorders

17:05-17:40 Gulio Genovese: Mosaic variants and clonal selection in health and disease

17:40-18:15 Sébastien Jaquemont: Predicting CNV effects on brain structure, function and risk for psychiatric conditions

18:15-18:45 Selected abstracts

Heleen Masset: Chimerism and mixoploidy in human preimplantation embryos disclosed by comprehensive PGT

Romain Nicolle: 16p13.11p11.2 triplication syndrome a new recognizable genomic disorder characterized by optical mapping

Monday 5 July

	ONLINE poster AREA
10:00-12.15	**Session 4 Animal and plant cytogenomics** Chair: Pat Heslop-Harrison
10:00-10:35	Tony Heitkam: Repetitive DNA, epigenetic variability, and genomic innovation in higher plants
10:35-11:10	Marta Farré-Belmonte: Genome evolution and genetic changes associated with individual and species differences
11:10-11:45	Aurora Ruiz-Herrera: Genome integrity and mechanisms driving karyotype evolution
11:45-12:15	Selected abstracts
	Lyubov P. Malinovskaya: The meiotic behavior and transmission of germline restricted chromosome (GRC) in songbirds
	Cibele Sotero-Caio: Trends of chromosome rearrangements and evolution in bats highlights from comparative chromosome painting analyses
12:15–13:45	ONLINE EXHIBITION & BREAK
13:45-15:30	**Session 5 Widening perspectives on genome variation** Chair: Joris Vermeesch
13:45-14:20	Tom Mistelli: Genome Organization: from basics to disease
14:20-14:55	Thierry Voet: Single-cell multi-omics to study genetic heterogeneity in health and disease
14.55-15:30	Segun Fatumo: The global genome/genomes sans frontiers
15:30–16:00	ONLINE EXHIBITION & BREAK
16:00-17:45	**Session 6 Disease prevalence and sex differences** Chair: Kamlesh Madan
16:00-16:35	Joris Veltman: (Cyto)genetics of male infertility
16:35-17:10	Darío Lupiáñez: Non-coding regulation of sexual features
17.10-17:45	Christine M Disteche: X-chromosome regulation and sex differences in brain anatomy
17:50-19:00	**Farewell session** Chair: Mariano Rocchi
17:50-18:25	Closing keynote:
	Evan E. Eichler: Long-read sequencing, disease variants and genome evolution
18:25-19:00	Closing ceremony

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Abstracts - Invited Lectures

L1 Aneuploidy Where do we go from here

Terry Hassold
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Trisomic and monosomic embryos account for at least 10% of human pregnancies and, for women nearing the end of their reproductive lifespan, the majority of fertilized eggs may be aneuploid. The origin of human aneuploidy usually involves errors in maternal meiosis, but despite exhaustive investigation the underlying molecular mechanisms have remained obscure. However, recent studies of human gametes, assisted reproductive technology (ART) - derived embryos and model organisms have shed new light on the complexity of meiotic defects. In this presentation we will focus on our own work on aneuploidy and recombination errors, derived from analyses of human fetal oocytes. These studies provide compelling evidence that a proportion of human oocytes are pre-destined to nondisjoin right from "the get-go"; i.e., from abnormalities in the formation or resolution of meiotic double strand breaks in the fetal stages of meiosis. We will discuss these observations in light of recent analyses of aneuploidy indicating the importance of errors in cell cycle control and maintenance of chromatid cohesion. Taken together, these analyses make it clear that errors in human female meiosis are not attributable to a single factor but to an interplay between unique features of oogenesis and a host of endogenous and exogenous factors.

L2 Non-invasive Fetal Aneuploidy Screening

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The Netherlands are the first country where NIPT is incorporated into a governmentally supported and health care funded prenatal Down syndrome screening program. April 1st 2014, the Dutch NIPT consortium, consisting of all relevant stakeholders, started a nationwide NIPT implementation study for pregnant women at increased risk of having an affected child. This study was called the TRIDENT study (Trial by Dutch laboratories for evaluation of non-invasive prenatal testing). Inclusion criteria are a risk of >1:200 for trisomy (T) 21, 18 or 13 based on

the first trimester combined test, or medical history. A follow-up study called TRIDENT-2, where NIPT is offered to all pregnant women, started April 1st 2017. During the presentation the results from TRIDENT-2 will be presented, including data on clinical follow up, and information on findings other than trisomy 21, 13 or 18.
The sequencing data generated for NIPT can be repurposed for other goals, including the detection of viral DNA. Specific viral infections such as Herpesvirus 5 (Cytomegalovirus), Parvovirus B19 and Hepatitis B, when occurring during pregnancy, are a major health concern to both mother and fetus. Based on an anonymized cohort of 108.349 pregnant women for whom the NIPT analysis was performed in Amsterdam, we characterized the DNA virome. These data will enable future implementations for the early detection of specific viral infections during pregnancy.

L3 Embryo Aneuploidy Testing, Past, Present and Future

Carmen Rubio
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Preimplantation Genetic Testing for aneuploidies (PGT-A), was introduced in clinical practice to improve pregnancy rates based on the assumption that the high rates of embryo aneuploidy found mostly in women of advanced age were responsible of the low pregnancy rates observed after IVF. The main goals for most of the indications for PGT-A are not only to increase implantation and pregnancy rates, but also to decrease miscarriages, and the risk of aneuploid offspring, as well as to decrease the time to conceive. In addition, the usefulness of PGT-A to grade embryos ahead of single embryo transfer is a great leap forward for IVF, allowing for safer pregnancies while maintaining high implantation and pregnancy rates across all patient populations. The efficiency of PGT for different indications has been assessed in several randomized controlled studies (RCT). Most of the early RCT studies were focused on the advanced maternal age group, but retrospective studies showed the potential value for other indications such us recurrent miscarriage, repetitive implantation failure, previous trisomic pregnancies and male factor. In our group, we have conducted two RCTs, in advanced maternal age and, in severe male infertility with significantly higher ongoing implantation and pregnancy rates in the PGT-A groups. In the last five years, Next Generation Sequencing (NGS) was applied to the field of embryo genetic assessment allowing more cost-efficient analysis of aneuploidies and incorporating the possibility of diagnosing mosaicism. NGS also allows for automation. The application of this new semiautomated NGS protocol has decreased the cost considerably and simplify the protocol, allowing the spread of PGT to a broader population mainly using blastocyst biopsy to increase success rates in IVF programs. The new challenges are how to evaluate the clinical impact of mosaic embryos and if transfer should be performed in some cases.
More recently, there have been different attempts to overcome trophectoderm biopsy to diagnose the chromosomal content of the embryos. A non-invasive approach has been proposed that consist in the study of the embryo cell-free DNA (cfDNA) released by the embryo to the culture media during the latest stages of preimplantation development. After the first publications, several studies have compared the results of preimplantation genetic testing of aneuploidies (PGT-A) in trophectoderm (TE) biopsies with the results of the spent blastocyst media (SBM), to establish the concordance rates among both approaches. Our team has recently published an interim analysis including 1,301 SBM and the corresponding biopsies. Global concordance rates of the SBM with the corresponding TE biopsies were 78.2%. No significant differences were detected among centers ranging from 72.5% to 86.3%. A prioritization algorithm has been developed according to the concordance results observed and we suggest that an embryo prioritization system considering the euploidy rates in the SBM compared to the TE biopsy could offer a different perspective for patients and clinicians that do not want to undergo PGT-A, avoiding invasive embryo biopsy and decreased cost, potentially increasing accessibility for a wider patient population.

L4 Pervasive chromosomal instability and karyotype order in tumour evolution

Roland F Schwarz
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Evolutionary and Cancer Genomics, Max Delbrück Centrum for Molecular Medicine, Berlin

Intra-tumour heterogeneity (ITH) is the main driver of resistance development in the clinic and the greatest impediment to targeted cancer therapies. Large-scale consortia such as PCAWG and the TRACERx have assembled vast amounts of genetic, transcriptomic, and epigenetic data to map out ITH across human cancers. Machine learning and data science approaches now start to provide in-depth insights into the evolution of many human malignancies with the prospect of predicting and preventing the evolution of resistant subclones on a per patient level.
Chromosomal instability (CIN), a hallmark of many tumours and a key characteristic separating healthy from cancerous tissue, thereby plays a central role in generating ITH. CIN describes the ability of tumours to generate and tolerate extensive somatic copy-number alterations (SCNA) and genomic rearrange-

ments. Besides its immediate clinical relevance, SCNA variability also forms a rich source of genetic variation that can be exploited for reconstructing tumour evolution in the patient in retrospective studies.
I will give an account of the recent developments in the field of CIN and cancer evolution. I will detail principles that enable us to reconstruct the evolutionary history of cancer in the patient, detect convergent structural evolution and derive haplotype-specific copy-number profiles with unprecedented resolution. I will highlight potential applications of CIN in predicting patient outcome and detecting early resistance-providing mutations or cancer driver events.

L5 Decoding the biological patterns of cell-free DNA to improve cancer detection with liquid biopsy.

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Cell-free DNA (cfDNA) in blood, urine, and other biofluids provides a unique window into human health. cfDNA exhibit the genetic and epigenetic alterations from their cell of origin and can be considered as a “liquid biopsy” and biomarker of choice in oncology. Tumor-derived plasma cfDNA is in general analyzed via cancer-related mutations. Circulating tumor DNA (ctDNA) has been used to track the evolutionary dynamics and heterogeneity of tumors. Furthermore, ctDNA analyses can detect emergence of therapy resistance, residual disease, and recurrence. But such mutation-based approaches are facing technical and biological limitations for the most challenging liquid biopsy applications (early detection of cancer and brain tumors).
Beyond the genetic alterations, a large number of epigenetic modifications could be leveraged by genome-wide sequencing methods to improve the detection of tumor signal in cfDNA and liquid biopsy. Identifying the biological patterns of tumor signal on cfDNA molecules has the potential to boost liquid biopsy for multiple applications. Recovering the cfDNA fragmentation features from genome-wide sequencing data has the potential to be compatible with high-throughput and cheap sequencing pipeline currently used for other application (e.g. non-invasive prenatal testing). Here we show that integrating different layers of cfDNA fragmentation with the end and genomic position of cfDNA fragments could improve the minimally invasive detection and monitoring of cancer in various clinical scenarios.

L6 Enhancer Hijacking Defines a Subtype of Lineage Ambiguous Leukemia

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Acute leukemias of ambiguous lineage (ALAL) pose significant diagnostic and therapeutic challenges due to lack of clarity surrounding their cellular origins and driving genomic alterations, as well as poor long-term response to conventional chemotherapy. Most ALAL cases are diagnosed as mixed phenotype acute leukemia (MPAL) or acute undifferentiated leukemia (AUL); however, early T-cell precursor acute lymphoblastic leukemias (ETP-ALL, a subset of T-ALL) often express myeloid markers, suggesting a degree of lineage ambiguity. Biological distinctions between these diseases remain vague, as T/myeloid MPAL and ETP-ALL exhibit similar mutational and immunophenotypic profiles. Critically, the myeloid lineage marker MPO is often the only diagnostic marker used to distinguish these diseases, highlighting the need for improved understanding of ALAL etiology. To resolve biological heterogeneity of ALAL, we performed a large-scale genomic analysis, including whole transcriptome and whole exome/genome sequencing, of 1,114 primary leukemia samples spanning the stem, myeloid and T lineages. Transcriptional profiling identified a group of cases (N=61) with a distinct gene expression profile that included one-third of all T/myeloid MPAL and ETP-ALL cases examined, as well as subsets of acute myeloid leukemia (AML) and AUL. Whole genome sequencing identified structural variants targeting the T-cell transcription factor gene *BCL11B* in 100% of cases with available data, and *FLT3* mutations in 80% of cases. Most structural variants resulted in chromosomal translocations that placed the *BCL11B* gene in proximity to at least 7 different super-enhancers active in hematopoietic stem or early progenitor cells, a cell type where *BCL11B* is normally repressed. Additionally, 20% of cases harbored high-copy tandem amplification of a 2.5 kb evolutionarily conserved non-coding element downstream of *BCL11B* that we term *BCL11B* Enhancer Tandem Amplification, or BETA. Analysis of 3D chromatin structure in primary leukemia samples demonstrated ectopic chromatin interactions between *BCL11B* and rearranged hematopoietic stem/progenitor cell super-enhancers, or between *BCL11B* and BETA, thereby demonstrating enhancer hijacking or de novo super-enhancer formation as the mechanism of aberrant *BCL11B* expression in this subtype. In vitro modeling in human CD34+ stem/progenitor cells demonstrated that ectopic *BCL11B* expression was sufficient to upregulate T-lineage gene expression programs and block myeloid differentiation, which was exacerbated by constitutive FLT3 signaling. These data identify *BCL11B* deregulation in primitive hematopoietic cells as an

oncogenic driver of a subset of ALAL that transcends conventional immunophenotypic distinctions.

L7 Computational and molecular dissection of long-range positional effects in human developmental disorders

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Balanced chromosomal rearrangements (BCRs) alter genome organization and confer significant risk for developmental disorders (DDs), yet the direct disruption of a known disease gene explains only a minority of cases. To evaluate a range of mechanisms by which BCRs may confer risk for DDs, we sequenced and analyzed 1,420 BCR breakpoints in 710 individuals, including 406 DD cases as well as the first large-scale sequence-resolved cohort of 304 healthy BCR carriers. Using this dataset we observed no enrichment of cases compared to controls with a BCR breakpoint directly disrupting protein-coding genes (67.6% of cases vs. 69.2% of controls; P = 0.924). However, after categorizing all protein-coding genes into four tiers based on evidence for association with disease (e.g., Tier 1 = known dominant DD gene; Tier 4 = no association), we find that 21.3% of DD cases harbored a BCR disrupting a Tier 1 gene compared to just 3.4% of controls (OR=7.6, $p=1.6E10^{-12}$). We observed no such enrichment for Tiers 2-4. We next explored evidence for pathogenic long-range positional effects (LRPEs) by performing a series of analyses against genome-wide expectations for breakpoint localization and direct comparisons of cases and controls across annotations of genomic features. After removing BCRs that directly disrupted Tier 1 genes, we observed no difference in the average linear distance of BCR breakpoints to the nearest Tier 1 gene between cases and controls (p=0.159), indicating that proximity to known disease genes alone is not predictive of pathogenic LRPEs. Given that genomes are organized into hierarchical structures, we hypothesized that disruption of topological associating domains (TADs), which are critical for cis-regulatory interactions, could act as a source of non-coding risk. We found a significant enrichment of case BCR breakpoints within TADs containing Tier 1 genes (OR=1.43, p=0.036) and identified three TADs enriched for noncoding BCRs in DD cases at a Bonferroni adjusted genome-wide significance threshold of $P<2.2x10^{-5}$, each of which encompassed a known DD gene (*MEF2C*, *FOXG1*, and *SOX9*). We performed phased HiC analyses on six DD cases with noncoding BCRs disrupting the TAD containing *MEF2C* and observed significant changes in chromatin contact domains for all cases when compared to the non-rearranged allele. We also explored additional TADs with suggestive evidence for association with pathogenic LRPEs based on a Benjamini-Hochberg false discovery rate <10% and identified three additional candidate LRPE loci that contained known dominant DD genes (*BCL11A*, *KIDINS220*, and *BCL11B)* that were disrupted by cases with compelling gene-phenotype correlations. Approximately 7.4% of cases in our DD cohort harbored a noncoding BCR that disrupted one of these six TADs compared to only 0.3% of the controls, suggesting that these loci likely represent highly penetrant LRPEs. In order to predict other LRPEs across the genome we statistically prioritized six genomic features that were enriched in TADs preferentially disrupted by BCRs in DD cases versus controls, including ultraconserved noncoding elements, enhancers and DD-associated genes. Given these results, we performed a subsequent mechanistic dissection of the long-range regulatory network associated with our top genome-wide significant peak (the locus containing *MEF2C*) and its constituent 3D functional elements using Cas9-based genome editing in iPS-derived neural models. We demonstrated that alteration to these 3D structures result in diverse and unpredictable chromatin contact changes and gene expression patterns. Collectively, these data suggest a rich landscape of complex pathogenic mechanisms associated with BCRs in human developmental disorders.

L8 Mosaic Variants and Clonal Selection in Health and Disease

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Mosaic loss of the X chromosome (mLOX) is the most frequently occurring mosaic chromosomal alteration detected in peripheral leukocyte DNA of females, with a prevalence far higher than similar events on the autosomes. We studied mLOX in 426,923 women of European ancestry from large biobanks in the United Kingdom, Finland, and Massachusetts to characterize the germline genetic architecture of mLOX.
Using a highly sensitive methodology to detect mosaic chromosomal alterations based on detection of haplotypic imbalance across long DNA segments, we detected mLOX even when present in only 1% of blood-derived DNA analyzed with DNA microarrays. As observed for other mosaic events, detected mLOX was age-related with a prevalence of less than 4% in women younger than 50 and reaching a prevalence of one in three after age 70. To explore the possible contribution of risk for chronic diseases, we evaluated mLOX presence against Finnish national medical registries. In 52,450 Finnish women without any cancer history at DNA acquisition we identified a strong association between incident cases of endometriosis of intestine (HR 4.8; 95% CI 2.3 to

9.9; P=1.9x10-5) and presence of mLOX at DNA sampling.
We performed a GWAS meta-analysis of mLOX across individuals from multiple biobanks and identified 8 germline susceptibility loci across the autosomes. We further analyzed whether alleles at heterozygous sites on chromosome X were preferentially on the lost chromosome X and we identified several such variants across the whole chromosome, including the pseudoautosomal regions. Many of these variants have been previously reported to be significantly associated with blood cell counts.
In conclusion, by leveraging genotype data from more than 400,000 female individuals, we were able to detect germline variants associated with mLOX and chromosome X alleles preferentially lost in the presence of mLOX, providing the most comprehensive understanding for the genetic architecture of mLOX.

L9 Genome evolution associated with individual and species differences

Marta Farré-Belmonte
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The role of chromosome rearrangements in driving evolution has been a long-standing question of evolutionary biology. Whole-genome comparisons among species point to regions in genomes where the order of orthologous sequences can be maintained for millions of years of evolution, demarcated by evolutionary breakpoint regions (EBRs). The genomic content of both regions differs, with EBRs being clustered in regions with a high number of repetitive elements and genes. However, the reason behind these differences is unknown. By reconstructing ancestral genome karyotypes using new methods and chromosome-level genome assemblies, we can now pinpoint the genomic changes occurred between and within species at very high resolution. Combined with transcriptomics and epigenomics data we are now starting to uncover the potential functional role of chromosome rearrangements in genome evolution. In this talk I will focus on three mammalian clades characterised by large karyotypic differences (ruminants, rodents, and marsupials). Using 21 ruminant, 15 rodent and 6 marsupial genome assemblies we established the ancestral karyotypes of 15 phylogenetic nodes. We then traced their genomic changes during evolution and combined it with differences in gene expression to finally characterise the impact of chromosome rearrangements in evolution.

L10 Genome Integrity and Mechanisms Driving Karyotype Evolution

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Unlocking the genomic basis of speciation is a research priority in biology fueled by the ongoing debate on species concepts and facilitated by the availability of an unprecedented large number of genomic resources. Through comparative genomics of both closely and distantly related mammalian species our research group, along with others, has contributed to models that explain genome structure and evolution. Such reconstructions have revealed that the genomic regions implicated in structural evolutionary changes, disrupt genomic synteny, and are clustered in regions more prone to break and reorganize. In searching for the origin (and consequences) of this evolutionary instability, we have provided insights on the genomic features that characterize evolutionary regions. These include repetitive elements and changes in gene expression that are caused by genome reshuffling may have a selective advantage through the development of new adaptive characters specific to mammalian lineages. Given the diversity of factors associated with genome reshuffling it is most unlikely that the sequence composition of genomes is solely responsible for genomic instability during evolution. This view represents a new interpretative evolutionary hypothesis that has recently been unified by our research group as the 'Integrative Breakage Model', which postulates that the permissiveness of some genomic regions to undergo chromosomal breakage and genomic rearrangements could be influenced by chromatin conformation and chromosome nuclear occupancy. Here I will provide evidence that suggest that certain properties of local DNA sequences, together with the epigenetic state of the chromatin and the three-dimensional genome architecture, are key elements in determining karyotype evolution and genome re-shuffling in mammals.

L11 Genome Organization: From Basics to Disease

Tom Misteli
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The genome is a major physical and functional component of every cell. The hereditary information, which is encoded in the one-dimensional sequence of the DNA, assumes complex three-dimensional architecture in living organisms. Several fundamental principles of genome organization, such as the

presence of chromatin loops and domains, non-random positions of genomic loci, the presence of sub-nuclear compartments, and the highly dynamic nature of genome architecture, are now recognized as well-established features of genomes. The convergence of genetic, biochemical, biophysical, and cell biological methods is beginning to uncover some of the molecular mechanisms that shape the organization of genomes in space and time and recent findings, based on genome-wide mapping and single-cell analysis, suggest that genomes are self-organizing systems driven by the interplay of structure and function. Disruption of spatial genome organization is associated with many disease states including developmental disorders, cancer and aging. The exploration of basic principles of genome organization in the context of disease promises to provide novel insight into fundamental mechanisms of genome function and has the potential to lead to novel diagnostic and therapeutic applications in a variety of diseases.

L 12 Single-cell multi-omics to study genetic heterogeneity in health and disease

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A leading cause of pregnancy loss is the frequent acquisition of genomic abnormalities in human pre-implantation embryos. However, the impact of genomic abnormalities on cellular phenotypic states and development of the early human embryo remains largely unclear. We combined single-cell genome-plus-transcriptome sequencing (G&T-seq) and integrative computational approaches to comprehensively identify aneuploidies, construct the gene regulatory networks of human pre-implantation embryo development, and investigate the transcriptional and developmental effects of aneuploidies. On the genomic level, we show that whole-chromosome mis-segregation as well as segmental rearrangements are frequent at all stages of early embryogenesis and in all cell lineages. At the transcriptome level, we disclose a network of transcription factors constituting major gene regulatory modules that characterize the distinct lineages of human pre-implantation embryos, and identify critical regulators of cell identity in human embryos. By integrating single-cell DNA-plus-RNA information, we unveil how transcriptional dosage effects of genes within DNA losses or gains alter during human pre-implantation development. Furthermore, our analyses provide evidence of cell competition selecting against aneuploid cells in the embryo. Finally, the application of G&T-seq to other human tissues in health and disease to study genetic mosaicism will be introduced as well. In summary, our multi-modal analyses provide new insights in the first cell divisions of human life, and beyond.

L13 Global Genome: Harnessing the Potential of African Diversity for Genomic Medicine

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Genomics medicine promises treatments, diagnoses and interventions that are tailored towards an individuals or group of individuals at highest risk of disease by considering their genetic predisposition. A polygenic risk score (PRS) provides opportunity to assess an individual's risk for a complex disease by summing the contribution of genetic variants into a single score to estimate the individual's genetic risk for disease compares to others. However, the underrepresentation of African populations in genomics research limits the accuracy of PRS and implementation of genomic in Africa.

To demonstrate the prospective of genomics in Africans as a great resource for genomic medicine, we collected the largest ever genotyped and whole genome sequence from Africa and evaluated if African American or multi-ethnic derived PRSs would improve polygenic prediction in continental Africans.

The African American derived PRS was more predictive of type 2 diabetes compared to the European and multi-ethnic derived scores. Notably, participants in the 10^{th} decile of this PRS had a 3.19-fold greater risk (OR 3.19; 95%CI (1.94-5.29), p = 5.33×10^{-6}) of developing diabetes and were diagnosed 2.6 years earlier compared to those in the first decile. Similarly, the African Americans derived lipid traits PRSs were more predictive compared to the Europeans and multi-ancestry approach. For low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) the African Americans PRSs accounted for 8.49%, 6.93%, and 2.26% variance of the respective traits. The multivariate derived African Americans PRSs was slightly more predictive than the univariate approach.

In conclusion, the African American derived PRS enhances polygenic prediction of type 2 diabetes and lipids traits in continental Africans. Improved representation of non-Europeans populations (including Africans) in GWAS, promises to provide better tools for precision medicine interventions.

L14 (Cyto)genetics of Male Infertility

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Severe forms of male infertility are mostly genetics in origin and having a genetic diagnosis is important to guide reproductive approaches and identify preventable co-morbidities. Well-known cytogenetics causes include Klinefelter syndrome and AZF microdeletions on the Y chromosome. Unfortunately, the genomics revolution has largely bypassed this field and diagnostic guidelines have not been updated to include next generation sequencing approaches.
In this presentation I will provide an overview of the genetics of male infertility and highlight some of the recent advances in the field. In addition, I will discuss our current unpublished research which is focused on studying the role of de novo mutations (DNMs) and structural variations in severe forms of male infertility. We recently performed a unique trio-based exome sequencing study in a cohort of 185 males with azoospermia or severe oligozoospermia and their unaffected parents. We identified a total of 145 protein altering DNMs and two de novo Copy Number Variations. A total of 29 DNMs were classified as possibly causative for the male infertility phenotype based on mutation impact and gene function. We observed a significant enrichment of Loss of function (LoF) DNMs in LoF intolerant genes as well as predicted pathogenic missense DNMs in missense intolerant genes. We also noted a significant enrichment in protein interactions amongst the genes affected by predicted pathogenic DNMs, in particular involving proteins involved in RNA binding and mRNA splicing. Our results provide the first support for an important role of autosomal and X-linked DNMs in the origin of severe male infertility.
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L15 Non-coding Regulation of Sexual Features

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Up to 99% of the human genome does not encode for proteins. Instead, it is densely populated by non-coding regulatory elements, such as promoters, insulators or enhancers, which instruct gene expression with spatiotemporal precision. Recent studies have highlighted the key role of 3D chromatin organization in mediating such regulatory interplay. In particular, topologically associating domains (TADs) emerge as fundamental units of 3D chromatin organization, which constrain the functional interactions between enhancers and their putative genes (*1*, *2*).
Structural and quantitative chromosomal rearrangements, collectively referred to as Structural Variation (SV), contribute to a large extent to the genetic diversity of the human genome and thus are of high relevance for cancer, rare diseases and evolutionary genetics. Recent studies have shown that SVs can not only affect gene dosage but also modulate basic mechanisms of non-coding gene regulation (*3*–*5*). SVs can alter the copy number of enhancer elements or modify the 3D genome by disrupting higher-order chromatin organization such as TADs. As a result of these position effects, SVs can influence the expression of genes distant from the breakpoints, thus causing the appearance of phenotypes. Therefore, the impact of SVs on the 3D genome and on gene expression regulation has to be considered when interpreting the phenotypical consequences of these variant types (*6*).
In this talk, I will highlight how alterations on 3D chromatin organization can induce developmental phenotypes. I will specifically discuss about the iberian mole *Talpa occidentalis,* an emerging model to study mammalian intersexuality, and a paradigmatic example of how SVs can influence sexual features (*7*).

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L 16 X chromosome regulation and sex differences in brain anatomy

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Sex-differences in cognition and psychopathology are common in human, and may be contributed to by influences of gonadal hormones and/or sex-chromosomes on brain development. Gonadal hormones play a major role in sexual differentiation of the brain, but less is known regarding the role of sex-chromosomes. The X chromosome, which is enriched in genes expressed in brain, is regulated by mechanisms that alleviate the fundamental genetic difference between XY and XX individuals. To reduce imbalance between the sexes X inactivation evolved to randomly shut down most of one X chromosome in each XX cell. However, a significant number of genes escape X inactivation, which results in sex differences in X-linked gene expression. We will discuss the impact of these processes on health and disease in normal males and females and in individuals with X aneuploidy. Our single-cell based analyses of gene expression show sex differences to be present even at early stages of differentiation. Analyses of specific human cell types including neural precursors derived from induced pluripotent stem cells reveal sex differences in gene expression contributed not only by X- and Y-linked genes, but also by autosomal genes. Characterization of the transcriptome and methylome shows distinct signatures depending on the sex chromosome makeup in neural precursor cells, which may contribute to sex differences in brain development.

(For abstracts added in proof, please see page 97)

L17 Long-read sequencing, disease variation and genome evolution.

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The discovery and resolution of genetic variation is critical to understanding disease and evolution. I will present our most recent work sequencing diverse human and nonhuman primate genomes using both ultra-long and high-fidelity long-read sequencing technologies. We have developed multiplatform methods to fully phase and assemble diploid genomes without parental data. This allows us to detect and sequence resolve most inversions and copy number variants from several bases up to 50 kbp—the vast majority of which are not routinely characterized by short-read sequencing. We are now developing approaches to characterize some of the largest (>50 kbp) and most complex forms of structural variation mapping to segmental duplications, acrocentric regions and centromeres. Advances in this area have made possible the first telomere-to-telomere assemblies of the human genome providing new biological insights into regions typically excluded from human genetic studies but well known for decades from cytogenetics. The recovery of Mbp of duplicated sequence and structurally variant sequence absent from the reference genome has led to new genetic associations and the identification of new genes missing from the human reference genome including the discovery of regions both introgressed and under selection in specific human populations. Long-read phased genome assemblies have provided the long-awaited bridge linking cytogenetics to sequencing-based genetics research. Assembly-based variant discovery has the potential to provide a complete understand of human genetic variation at every level and, we predict, will be the future of genetic and clinical based research.

Abstracts - Oral Presentations

O1 Aneuploidy is associated with increased methylation of different LINE 1 retrotransposon families in placenta of first trimester miscarriages

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The incidence of aneuploidy and the level of DNA methylation seem to be mirrored features of the human genome. However, the data about their relationship in early human embryo development are limited. Therefore, this study aimed to analyze the

chromosome-specific DNA methylation of the most common repetitive element in the human genome, LINE-1 retrotransposon, in the placenta of first-trimester embryos with aneuploidy. LINE-1 methylation was assessed in chorionic villus samples from miscarriages with trisomy of autosomes (2, 6, 8-10, 13-15, 16, 18, and 20-22) (n=74), monosomy X (n=16), normal karyotype (n=51) and 31 induced abortions by targeted bisulfite massive parallel sequencing. LINE-1 methylation index was analyzed as an average across the genome and separately for 32 chromosome-specific loci with specific SNPs. The level of LINE-1 methylation was significantly elevated in chorionic villi of miscarriages with both trisomy of autosomes (45.2±4.3%) and monosomy X (46.9±4.2%) compared to miscarriages with a normal karyotype (42.6 ± 5.6%) and induced abortions (40.0±2.4%) ($p<0.00001$). The level of methylation varied significantly for chromosome-specific LINE-1 loci. It was increased not only in the elements located on the chromosome for which aneuploidy was observed but also in the whole genome. The LINE-1 elements from the hominid-specific L1PA2 subfamily had a significantly lower methylation index (18.3± 11.1%) compared to the younger elements from the L1HS subfamily (27.0±9.8%, $p=0.025$) in induced abortions. This may result from the usage of antisense L1PA2 promoters for genes significant for placental development. However, DNA methylation of both LINE-1 families was equally increased in miscarriages with aneuploidy. This may indicate that an aberrant LINE-1 methylation profile was established before the differentiation of placental tissues, and possibly even before fertilization.

This study was supported by the Russian Science Foundation (project 19-74-10026).

O2 Outcome of publicly funded nationwide first tier noninvasive prenatal screening

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Purpose

Noninvasive prenatal screening (NIPS) using cell-free DNA has transformed prenatal care. Belgium was the first country to implement and fully reimburse NIPS as a first-tier screening test offered to all pregnant women. A consortium consisting of all Belgian genetic centers report the outcome of two years genome-wide NIPS implementation.

Methods

The performance for the common trisomies and for secondary findings was evaluated based on 153,575 genome-wide NIP tests. Furthermore, the evolution of the number of invasive tests and the incidence of Down syndrome live births was registered.Results

Trisomies 21, 18, and 13 were detected in respectively 0.32%, 0.07%, and 0.06% of cases, with overall positive predictive values (PPVs) of 92.4%, 84.6%, and 43.9%. Rare autosomal trisomies and fetal segmental imbalances were detected in respectively 0.23% and 0.07% of cases with PPVs of 4.1% and 47%. The number of invasive obstetric procedures decreased by 52%. The number of trisomy 21 live births dropped to 0.04%.

Conclusion

Expanding the scope of NIPS beyond trisomy 21 fetal screening allows the implementation of personalized genomic medicine for the obstetric population. This genome-wide NIPS approach has been embedded successfully in prenatal genetic care in Belgium and might serve as a framework for other countries offering NIPS.

O3 Direct inheritance of telomere shortening and aberrations detected during prenatal period

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Background:
Telomeres play vital roles in maintaining chromosomal integrity throughout life. However, the heritability of telomere dysfunction, e.g. short telomeres and telomere aberrations, is unknown. Here, we address this issue by assessing lengths and stability of telomeres and also the integrity of chromosomes in fetal samples taken for detailed prenatal diagnosis of their aberrant karyotypes as well as in circulating lymphocytes of the parents of the fetuses in question.
Material and methods:
Cytogenetic preparations of ten prenatal samples (five with structural aberrations and five with numerical aberrations) and of peripheral blood lymphocytes of their respective parents were analyzed. We used an automatized high-throughput technique based on fluorescence in situ hybridization (FISH) with probes specific for telomeres and centromeres as well as probes that allow identification of each chromosome. Thus, large numbers of cells can be screened for telomere aberrations including telomere loss and terminal deletions and also for telomere length variation between samples. A cohort of 100 healthy donors was used as a control.
Results:
Karyotypes of the relevant parents were normal. However, telomere shortening and high rates of telomere aberrations were identified in one or both of the parents of the ten fetuses with structural or numerical aberrations. Moreover, clonal telomere aberrations were assessed. In foetuses with numerical and structural aberrations we detected an increase of telomere loss and of telomere deletions in specific chromosomes relative to that in their parents. Our findings support the notion that unstable telomeres in the foetal cells may contribute to the formation of chromosomal aberrations. Furthermore, our data demonstrate that regulation of telomere length as well as telomere aberrations are inherited traits.
Conclusion:
Our study indicates that telomere lengths and aberrations are inherited traits, and, therefore, that analysis of telomeres should be included in prenatal diagnosis. By applying our method to a larger cohort, our analysis of telomere dysfunction will be validated as a potentially central tool in prenatal diagnosis.

O4 Comparison of genetic profiling between primary tumor and circulating tumor cells captured by microfluidics in epithelial ovarian: cancer tumor heterogeneity or allele dropout?

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Epithelial ovarian cancer (EOC) is a leading cause of cancer mortality among women but unfortunately is usually not diagnosed until advanced stage. Early detection of EOC is of paramount importance to improve outcomes. Analysis of circulating tumor cells (CTCs), also known as liquid biopsy, is emerging as one of the promising biomarkers for early detection of solid tumors. However, discrepancies among the germline, primary tumor, and liquid biopsy regarding oncogenomics are a serious concern and can adversely affect downstream cancer management. Here, we illustrate the potential and pitfalls of CTCs by presenting two cases of stage I EOC. We successfully isolated and recovered CTCs by a silicon-based nanostructured microfluidics system, the automated Cell RevealTM. We examined the genomics of CTCs as well as the primary tumor and germline control (peripheral blood mononuclear cells) by whole exome sequencing. Different signatures were then investigated by analysis of identified mutation loci distinguishing those that may only arise in the primary tumor or CTCs. A calculation model is proposed to test if the highly variable allele frequencies among loci are due to allele dropout in pooled CTCs or tumor heterogeneity. This proof-of-principle study provides a strategy to elucidate the possible cause of genomic discrepancy among the germline, primary tumor, and CTCs, which is helpful for further large-scale use of such technology in liquid biopsy to be integrated into clinical management protocols.

O5 Opportunities of Optical Genome Mapping for Genetic Diagnosis in Acute Lymphoblastic Leukemia

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Acute lymphoblastic leukemia (ALL) is a prevalent hematopoietic malignancy that requires urgent medical intervention to prevent inferior outcome. ALL is characterized by recurrent structural aberrations, whole chromosome copy number aberrations and microdeletions of prognostic value.
Currently an extensive panel of tests is required, including karyotype, FISH, arrays or Multiple Ligation-dependent Probe Amplification (MLPA) and PCR-based diagnostic methods. Testing is thus costly and cascade testing is often performed resulting in lengthy turn-around-times (TAT). This does not conform to study protocols that require rapid results to stratify patients into different treatment arms and to identify those who could benefit from "targeted" therapies, such as the Ph-like ALLs. It is therefore interesting to examine new technologies, like Optical Genome Mapping (OGM) (Bionano Genomics). OGM utilizes ultra-long linear DNA molecules, enzymatically labeled at specific sequence motifs, to detect numerical and structural aberrations (>500bp) genome wide with a TAT of less than one week.
To assess whether implementation of OGM could (partially) replace current analytical strategies, 10 diagnostic T- and 10 diagnostic B-ALL cases were included. All samples were analyzed with the Rare Variant and the De Novo Assembly Pipelines. Filters were set to detect CNAs sized >5 Mb. For submicroscopic deletions and gains, only regions encompassing clinically relevant loci were investigated.
Results:
• OGM results were concordant with MLPA.
• All recurrent translocations identified by routine strategies were correctly identified by OGM.
OGM further identified:
• A t(5;11) TCF7-SPI1 in a case with a failed karyotype.
• A t(12;22) ZNF384-EP300 fusion in a case with a normal karyotype.
In general there was an excellent concordance of chromosome gains and losses and for hyperdiploidy.
Moreover, OGM identified also hyperdiploidy in a case with a normal karyotype. Interestingly, FISH analysis of the same case suggested the presence of a hypodiploid clone. This demonstrates the limitation of DNA-based techniques to accurately determine ploidy when allele information is not available.
Conclusion:
Our data demonstrate that OGM is a promising technique to identify structural and numerical abnormalities in ALL. OGM provided an informative result in each case and results are highly concordant with other techniques.

O6 Chimerism and mixoploidy in human preimplantation embryos disclosed by comprehensive PGT

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Comprehensive preimplantation genetic testing (PGT), through haplotyping, offers the ability to select embryos free from monogenic disorders (PGT-M), while evaluating the chromosomal component and its parental origin at the same time in a presumably fertile population. Aneuploidy has been proven to be common in human preimplantation embryos. However, up to now, little is understood about the chromosomal aneuploidy burden a human embryo can bear, making aneuploidy screening (PGT-A) most valuable to decrease the time to pregnancy. Moreover, apart from single aneuploidies, genome-wide abnormalities are known to exist in human embryos as well, albeit rather rare. We dissociated 22 human day-5/day-6 embryos unsuitable for transfer based on the results of PGT-M analysis from day-3 single blastomere or day-5 trophectoderm biopsies. In this study, the analysis of the preimplantation embryo biopsies included embryos with genome-wide uniparental (n=10) and chaotic genomic profiles (n=12). Upon dissociation of the embryos to single cells, 40 % of genome-wide uniparental embryos by initial analysis were proven to actually be mixoploid and/or chimeric. Specifically, we uncovered mixtures of gynogenetic and biparental, gynogenetic and androgenetic or sampled solely gynogenetic and androgenetic cells, independently. In the group of embryos with complex chromosomal abnormalities, embryos frequently displayed an abundance of biparental balanced cell lineages, perhaps favorable for the further development of the embryos to reach day-5/day-6. Dependent on the genomic makeup of the embryo, genome-wide abnormalities such as uniparental diploidy, chimerism and mixoploidy could lead to embryonic arrest, pregnancy complications or viable offspring. Due to the technology used here, we were able to disclose the parental origin of the aberrations and provide insights into their occurrence and developmental propagation of distinct cell lineages in the preimplantation stage.

O7 16p13.11p11.2 triplication syndrome: a new recognizable genomic disorder characterized by optical mapping

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Highly identical segmental duplications (SDs) account for over 5% of the human genome and are enriched in the short arm of the chromosome 16. These SDs are susceptibility factors for recurrent chromosomal rearrangements mediated by non-allelic homologous recombination (NAHR). Chromosomal microarray analysis (CMA) has been widely used as the first-tier test for individuals with developmental disabilities and/or congenital anomalies and several genomic disorders involving the 16p-arm have been identified with this technique. However, the resolution of CMA and the limitations of short-reads whole genome sequencing (WGS) technology do not allow the full characterization of the most complex chromosomal rearrangements preventing a good understanding of the underlying mechanism. Herein, we report on two unrelated patients with a novo 16p13.11p11.2 triplication detected by CMA sharing a similar phenotype including hypotonia, severe neuro-developmental delay with profound speech impairment and hyperkinetic behavior, chronic otitis media and distinctive facial features. Patient 1 also carries the recurrent 16p11.2 duplication encompassing the SH2B1 gene and patient 2 carries a smaller 16p11.2 duplication that does not encompass the SH2B1 gene. Complete genetic characterization of these events was unreliable by whole genome sequencing because the breakpoints lie within SD. Consequently, we used Bionano optical mapping to fully characterize these chromosomal abnormalities. Thus, we propose a two-step mechanism to explain these rearrangements: a U-type exchange at a distal SD between homologous chromatids for both patients, followed by a NAHR event at BP1-BP3 region for patient 1 and a NAHR event at BP1-BP2 region for patient 2. In conclusion, Bionano Genomics is a useful technology for unravelling the origin of complex chromosomal rearrangements involving SDs.

O8 The meiotic behavior and transmission of germline restricted chromosome (GRC) in songbirds

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In songbirds, germline-restricted chromosome (GRC) was described as an additional chromosome, which is present in germ cells, absent from somatic cells, eliminated during spermatogenesis and transmitted generally via oogenesis. However, little is known about how the GRC meiotic behavior differs in the distant songbird lineages.
We examined the GRC synapsis, recombination pattern and copy number variation in sand and pale martins (Riparia riparia and R. diluta) in comparison with distantly related estrildid finches: zebra finch (Taeniopygia guttata) and Bengalese finch (Lonchura striata domesctica).
Meiotic behavior of GRC was surprisingly similar between martins and estrildid finches. In females, GRC was mostly present in two copies in the pachytene oocytes. They form the normal bivalent recombining predominantly at the terminal ends. The zebra finch and sand martin females shown the polymorphism for GRC number: around one sixth of females contains one GRC copy. In males, GRC was present in one copy in the most pachytene spermatocytes. After the first meiotic division, GRC was eliminated and visualized as a round chromatin body near the spermatocytes II. The sperm do not carry this chromosome and GRC transmission occurs via females.
We assume that the shared features of GRCs are formed under the selection pressure to provide its transmission into the egg. We suggest that the GRC transmission involves a meiotic drive and non-disjunction. During the first meiotic division GRC preferentially segregates into an oocyte. During the second meiotic division sister GRCs segregate orderly producing the eggs with one or two GRC copies. The resulted eggs are fertilized by sperm containing no GRC. Given that no songbird without GRC was found yet, we assumed that such behavioral pattern is beneficial for GRC functioning, apparently, in the early embryogenesis and gametogenesis.
This research was funded by RFBR, project number 19-34-90118 and the Ministry of Science and Higher Education of the Russian Federation, project number 2019-0546 (FSUS-2020-0040).

O9 Trends of chromosome rearrangements and evolution in bats: highlights from comparative chromosome painting analyses

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The order Chiroptera includes all bats, with over 1400 species distributed in 21 extant families. Despite their widespread occurrence and diversity, only less than 10% of the currently described species have been subjected to com-parative cytogenetic analyses using chromosome painting. Nevertheless, lineage-specific trends of chromosome evolution have begun to unravel, suggesting links between chromosome change and species diversity in several taxa. Here, we present an overview of the trends of karyotypic evolution within distinct bat lineages uncovered by comparative chromosome painting and highlight the latest examples on how chromosome data were useful to detect cryptic species and aid in integrative taxonomic studies. We will focus on three families (Rhinolophidae, Megadermatidae and Phyllostomidae) to exemplify less frequent chromosome alterations in bats, such as B chromosomes, translocation and inversion polymorphisms, as well as sex-autosome translocations, detected using distinct sets of whole chromosome paints (from the bats Macrotus californicus, Aselliscus stoliczkanus, Myotis myotis, and from human and tree shrew) and banding pattern comparison. We conclude that although Robertsonian translocations are the prevailing mode of chromosomal evolution in Chiroptera, distinct trends exist across lineages, with major implications in systematic studies of bats.

Abstracts - Poster presentations

1. Accreditation Quality Control Education

1.P1 An International Collaboration for Human Ring chromosomes (ICHRC) towards developing standards and guidelines for best practice in laboratory diagnosis clinical management and collaborative research

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Human constitutional ring chromosomes are a rare type of chromosome structural abnormality with an estimated incidence of 1 in 50,000 for an annual occurrence in 2,800 newborns. A search of PubMed since 1970's retrieved 836 cases of autosomal rings from 737 publications, which accounted for about 30% of annual cases. of these cases, chromosome microarray analysis had been performed on 199 cases and deletions, duplications or complex rearrangements in the ring chromosomes were detected in 85% (169/199) of cases. A general clinical manifestation of developmental delay, growth retardation and short stature for ring chromosome syndrome has been proposed. However, a broad spectrum of clinical findings ranging from relatively normal phenotypes to chromosome related or region-specific features for epilepsy, infertility and cancer predisposition have been reported in the literature. Efforts towards collaborative studies for patients with ring chromosome 14 and 20 have been organized. Here, we propose an International Collaboration for Human Ring Chromosomes (ICHRC) towards developing standards and guidelines for best practice in laboratory diagnosis, clinical management and collaborative research. The specific aims of ICHRC are: 1) to develop laboratory guidelines for analyzing human ring chromosome, 2) To review and register ring chromosome cases into the Human Ring Chromosome Registry as an online database, and 3) to perform further genomic characterization and functional analysis of ring chromosome structure and behavior. An organization committee for ICHRC has been initiated and task forces will be organized. We are calling for clinical cytogeneticists, molecular geneticists, clinical geneticists interested in this project to join and participate in this collaboration. Main contact persons are Li, Peining (peining.li@

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2. Animal and Plant Cytogenomics

2.P1 Liraglutide and Metformin Modulation of Gut Microbiota in Aged Obese Rats

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The gut microbiota has an impact on host metabolism, glucose homeostasis, and weight gain. Altered gut microbiota has been a notice in obesity and diabetes. The effect of antidiabetic drugs on the gut microbiome has been noticed. The relationship between the gut microbiota and the mechanism of metformin and liraglutide in elderly individuals is unclear.
In this study, the gut microbiota composition was investigated using a rat model of high-sugar-high-fat diet (HSHFD) induced obesity with and without liraglutide and metformin treatment. We aimed to determine metformin and liraglutide's influence on the gut microbiota in aged obese male rats.
Fourteen-week-old male Sprague-Dawley rats were fed with a standard diet or a high-sugar-high-fat diet (HSHFD) for 20 weeks. Age-obese male rats were injected subcutaneously with liraglutide (0.3 mg/kg/day), a metformin (50 mg/kg/day), or normal saline for 14 weeks. The gut microbiota was characterized by 16S ribosomal RNA gene sequencing of the V3-V4 region on the MiSeq platform.
Liraglutide and metformin treatments induced significant weight-loss in HSHFD rats and showed improvements in glycemic parameters (fasting glucose and glucose tolerance). The gut microbiota analysis showed that liraglutide and metformin changed the overall composition and some genera's relative abundance. The abundance of the genera Akkermansia and Ruminoclostridium was significantly increased by metformin but decreased the relative abundance of genus Lactobacillus in rats fed an HSHFD. Lachnospiraceae and Romboutsia abundances decreased, and Akkermansia increased considerably after rats' liraglutide treatment on the HSHFD.
Liraglutide and metformin treatments had a positive effect on gut microbiome dysbiosis. Our findings suggest that modulation of the gut microbiota by metformin or liraglutide results in metabolic improvements in aged obese rats.

2.P2 Spatial organization of topologically associated chromatin domains in chicken somatic cell types and meiotic lampbrush chromosomes revealed by FISH

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In the last two decades the evolution of chromatin conformation capture methods allowed to identify multiple hierarchical levels of chromatin domains from loops to megabase-sized compartments. While functional significance of these contact chromatin domains in genome regulation has been principally demonstrated, their structural arrangements are far from being characterized. Here we used FISH-based approaches to analyze 3D-organization of chromatin nanodomains in several mitotic and meiotic cell types.
Changes in architecture of chromatin domains during disappearance of topologically associated domains (TADs) in differentiating chicken erythroblasts was characterized using three-color 3D-FISH with BAC probes and laser scanning confocal microscopy. 3D distances and degree of colocalization between linearly equidistant genomic loci located in a single or in neighboring TADs were measured. We established that erythrocyte chromatin has atypical pattern of local compactization with distant interactions between genomic loci across the borders of conservative TADs revealed in other cell types by in situ Hi-C. Nevertheless in undifferentiated erythrocyte precursors, genomic loci belonging to one TAD were found to locate closer and colocalize more often than genomic loci belonging to neighboring TADs.
We further juxtaposed conservative TADs, revealed in interphase nuclei, with chromatin domains in giant transcriptionally active lampbrush chromosomes from growing chicken oocytes. Mapping of equidistant genomic regions located in neighboring somatic TADs demonstrated variety of their localization at chromomere-loop complexes. Regions of neighboring TADs can be detected within a single chromomere; while regions belonging to a single TAD can be detected in two neighboring chromomeres.
Research was supported by Russian Science Foundation (grant # 19-74-20075) and was

performed using the equipment of Resource Center 'Molecular and cell technologies' (Saint-Petersburg State University).

2.P3 LINE 1 retrotransposon massive enrichment in tamarins' genomes of the Cebidae family (Platyrrhini Primates) and its evolutionary significance

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Classic cytogenetic investigations showed that tamarins have conserved karyotypes differing only by heterochromatin and inversions; to study the differences in heterochromatin distribution among tamarins, we applied the molecular cytogenetic approach by Fluorescence In Situ Hybridization (FISH) with LINE-1 probes onto chromosomes of Saguinus mystax, Leontocebus fuscicollis and Leontopithecus rosalia; we have also stained chromosomes with DAPI/CMA3, obtained C sequential banding and compared these patterns. We analysed heterochromatic DNA distribution, including LINE-1 repeats, because they have been shown to be involved in genome architecture and in the occurrence of chromosomal rearrangements in many vertebrates.
We found bright LINE-1 probe signals at centromeric or pericentromeric areas at CMA3 positive region and at non centromeric position along chromosome arms; we analysed the pattern of LINE-1 distribution obtained in a phylogenetic perspective considering human chromosomal homologies and C banding pattern. Our data let us to hypothesised that LINE-1 centromeric expansions and accumulation presumably arose in the common tamarins' ancestor and that LINE-1 presence at the junction of human chromosome associations is presumably linked to interchromosomal rearrangements. Furthermore, other faint signals could be linked both to intrachromosomal rearrangements such as inversions or to retro-transposition events. Indeed, we find differences in LINE-1 amplification and distribution especially on chromosome pairs 19-22; this different pattern of distribution permits us to show at molecular level the occurrence of inversions responsible of the main differences between the analysed species.

2.P4 Avian lampbrush chromosomes reflect the complexity of their genome organization at meiotic reprogramming stage

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Eukaryotic chromosomes undergo a significant decrease in methylation during meiosis, which is attributed to the activation of chromatin necessary for precise synapsis of homologues. In the diplotene, bivalents are converted into lampbrush chromosomes with reduced packing and discrete structure formed by numerous linearly arranged chromomeres and lateral loops coated with transcripts. For many years, it was believed that the morphology of these loops reflected a high level of synthetic activity. In our work on avian lampbrushes, we have shown that the most developed loops are maintained on chromosomes during dictyotene with low synthetic activity. At the stage of dramatic growth in the vitellogenic phase, chromosomes pack and transcriptional activity on them is even more suppressed, so the cytoplasm of the oocyte begins to grow due to the flow of RNA and proteins from the follicular cells. The study of avian genomes, including the use of sequencing methods based on long reads, has allowed us to study the composition of lampbrush loops in details. The most conservative and meaningful elements of the genome are not included in the lateral loops, whereas heterochromatin and other sequences inactivated in somatic cells are widely represented in their composition. In Japanese quail with an increased genome size due to the increase in the proportion of repetitive elements, we observe the appearance of characteristic lateral loops in the regions corresponding to heterochromatin blocks.
The equipment we used was provided by the "Chromas" Core Facility of Saint-Petersburg State University.

2.P5 Hybrid sterility in grey voles

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Formation of reproductive isolation is the most important stage of speciation. In mammals, one of the key mechanism of reproductive isolation is hybrid sterility that occurs gradually. Recently diverged species of the grey voles with unclear taxonomic status comprising "mystacinus" group of the most speciose genus Microtus (Rodentia; Arvicolinae) provide a good model of the first speciation stages. To shed light at this taxonomic puzzle and get insight

into cytological mechanisms of the hybrid sterility we examined reproductive performance, dynamics of spermatogenesis and chromosome pairing and recombination in meiosis in male F1 hybrids between five Microtus species.
The male hybrids between M. mystacinus, M. kermanensis and M. rossiaemeridionalis of "mystacinus" group were completely sterile, therefore they should be considered as valid biological species. A severity of spermatogenic aberrations in all hybrids examined in general increased with an increase of phylogenetic distance between the parental species. However, within some groups of hybrids we observed a variety in severity of the spermatogenic abnormalities. The hybrids between closely related species M. kermanensis and M. rossiaemeridionalis showed normal synapsis and recombination of most autosomes but few the smallest ones and a wide variation of spermatogenesis arrest stage: from stage of mature although abnormal spermatozoa to spermatogonia only. The hybrids between the most distant species M. kermanensis and M. transcaspicus showed complete asynapsis of all autosomes and arrest of spermatogenesis at the very beginning of meiotic prophase. Chromosome asynapsis was apparently the main cause of meiotic arrest and apoptosis of spermatocytes.
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2.P6 Bovine telomere association between age and breed

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Telomeres consist of thousands of non-coding repetitive sequences of DNA, highly conserved, localized at the end of chromosomes, and responsible for maintaining DNA integrity during each cell division. In mammals, the majority of younger individuals show longer telomeres than older of the same species. For this reason, telomere length (TL) in blood cells could be used as a sensitive biomarker of animal welfare, being a heritable trait associated with late-life health and mortality in humans. In this study, we evaluated the association between leukocyte TL (RLTL) and age in two cattle breeds, Agerolese (AGR) and Italian Friesian (HFR), using a q-PCR. AGR cattle is an indigenous Italian breed characterized by extended productive longevity and higher genome stability due to minimal zootechnical selective pressure. HFR cattle is the most reared breed in Italy, where a severe genomic selection has always been applied for decades. Cattles were divided into three groups according to their breed and age: AGR (1-6 years), HFR (1-6 years), and AGRe (8-13 years). RLTL results significantly higher ($p < 0.01$) in AGR than in HFR, during their entire lifespan and statistically different between AGR vs AGRe ($p < 0.001$) and between HFR vs AGRe ($p < 0.05$). The RLTL shows a negative correlation with the animal's age, both in the AGR and in the HFR. We also analyzed the telomere erosion rate between AGR vs HFR, having identical results (0.079/years). We can assert that AGR has always presented a higher TL than HFR and a similar telomere erosion rate to HFR, suggesting that TL is a heritable trait influenced by the breed. Its association with a productive lifespan may be used in breeding programs aiming to enhance cow functional longevity.

2.P7 The Impact of Chromosomal Fusions on 3D Genome Folding in the Germ Line

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Chromosomal rearrangements such as Robertsonian fusions (Rb fusion) are key factors for evolution, yet their impact on spatial folding of chromosomes inside the nucleus and its regulatory effects on gene expression remains unclear. Here, we take advantage

of chromosome conformation capture (Hi-C) to study how the higher-order chromatin is affected by chromosomal fusions in the house mouse germ line. We show how chromosomal fusions alter the nuclear architecture during meiosis affecting not only chromosomes involved in the reorganization but also not-fused chromosomes. In primary spermatocytes, the presence of Rb fusions induces heterologous interactions, whereas in post-meiotic cells these heterologous interactions are reduced, causing an increase of intra-chromosomal interactions and therefore a reorganization of TADs. Moreover, new meiotic-specific inter-chromosomal interactions related with olfactory receptor family clusters were detected in post-meiotic cells. These results suggest an adaptive role of Rb fusions by rearranging nuclear occupancy. Overall, our results provide new insights into how genome reshuffling influences chromatin folding and therefore its high-order organization inside the nucleus of germ cells.

2.P8 Devil is in the details - meiotic chromosome dynamics in Australian Marsupials

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During meiotic prophase I, homologous chromosomes pair, synapse and recombine in a tightly regulated process, which ensures the generation of genetically variable haploid gametes. This process has canonical features that are highly conserved across mammals, although there are notable differences between taxa. The mechanisms underlying meiotic cell division have been thoroughly studied in many model species. However, our understanding of the dynamics of meiotic prophase I in non-traditional model vertebrates is still in its infancy, especially when viewed from a phylogenetic perspective. Here, we compare the similarities and differences in the regulation of meiosis in two previously uncharacterized Australian marsupial species: the Tammar wallaby (Macropus eugenii: family Macropodidae) and the fat-tailed dunnart (Sminthopsis crassicaudata: family Dasyuridae). We performed a cytological analysis of the meiotic prophase I, including the study of chromosome synapsis, double strand break (DSB) formation and meiotic sex chromosome inactivation (MSCI). Our results show that sex chromosomes associate forming the so-called dense plate (DP) following different strategies in both marsupial species, which correlates with differential sex chromosomes architecture and transcriptional patterns. Furthermore, we characterize a previously undescribed phenomenon in primary spermatocytes of the fat-tailed dunnart: heterologous telomeric associations accompanied with telomere transcription and elongation, which is consistent with the telomeric dimorphism characteristic of the Dasyuridae family. Overall, our results provide new insights into the regulation of meiotic prophase I in marsupials.

2.P9 Characterization of con / com satellite DNAs in Deschampsia P. Beauv (POACEAE) species

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The genus Deschampsia P. Beauv (Poaceae) involves a group of widespread polymorphic species. Many of them are highly tolerant to environmental stress conditions including extreme polar habitats, and could serve as a source of genes useful for crop breeding. However, genome diversity and chromosomal phylogeny within the genus are still insufficiently studied. Satellite DNAs, including CON/COM families, are the main components of a plant repeatome, which contribute to chromosome organization. For the first time, we obtained CON1, CON2 and COM2 DNA sequences from genomes of eleven Deschampsia accessions covering seven species (D. antarctica E. Desv., D. cespitosa (L.) P. Beauv., D. elongata (Hook.) Munro, D. flexuosa (L.) Trin., D. danthonioides (Trin.) Munro, D. parvula (Hook.f.) E. Desv. and D. sukatschewii (Popl.) Roshev) and also three species (Helictotrichon pubescens (Huds.) Pilg., Kolleria macrantha (Ledeb.) Schult., Tricetum phleoides (d'Urv.) Kunth.)from related genera. According to the BLAST analysis, the obtained CON/COM DNAs demonstrated high degree of homology though multiple sequence alignment (MSA) revealed different alteration profiles in these sequences, which indicated that they tended to diverge independently in genomes of the studied species. The performed multicolour FISH with the use of 45S rDNA, 5S rDNA, CON1, CON2 and COM2 as probes allowed us to study the specific patterns of their distribution in karyotypes, identify all homologous pairs of chromosomes and also detected chromosomal rearrangements. The obtained CON/COM repeats demonstrated clustered or dispersed localization along chromosomes, which indicated that they could change their structure and organization in genomes during the species divergence. Based on the obtained data, the studied accessions were subdivided into groups, and

schematic trees were built, which could clarify the relationships within the genus Deschampsia. Our findings can serve as a basis for further genetic and biotechnological studies.
This work was supported by the Russian Foundation for Basic Research (KOMFI No. 17-00-00336) and the Program of Fundamental Research of State Academies (No. 01201363824).

2.P10 Genome specific microsatellite markers for cytogenomics research in Brassica and Raphanus hybrids

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The genus Brassica comprise important species, and along with Raphanus sativus, are used for food, medicine, and model organisms for chromosome evolution and polyploidy studies. Most Brassica species such as, B. rapa (AA, 2n =20), B. oleracea (CC, 2n =18), and B. nigra (BB, 2n =16) are valued for their shoots whereas R. sativus (RR genome, 2n =18) is valued for its roots. Synthetic hybrids between Brassica species and Raphanus sativus have been developed to utilize both shoot and root systems in a single plant. To study genetic stability in these hybrids, it is important to trace chromosome or chromosome fragment introgression, which can be achieved by visually distinguishing subgenomes from diploid progenitors using fluorescence in situ hybridization. However, because of the close evolutionary distance between these species, developing genome-specific probes from genomic DNA has been challenging and cumbersome. Here, we address these problems by developing genome-specific microsatellite FISH probes. We mined for microsatellites in the A, C, B, and R genomes in silico using < 1× whole-genome short reads and analyzed their genome specificity by FISH in different natural and synthetic Brassica and Raphanus hybrids. Out of the 22 non-redundant microsatellites with > 6kb total bases, we validated three that show specific FISH signals to the A (ACBR_msat14), C (ACBR_msat20), and B/R (ACBR_msat01) genomes. Importantly, the C genome-specific ACBR_msat20 probe revealed an interesting dynamic from being dispersed in the C genome to being localized at the NOR site in the synthetic three-genome tetraploid hybrid with putative ACRR genome. This observation suggests the active involvement of the 45S rDNA array in genome rearrangements, as have been observed in other studies.

2.P11 DNA extraction for sex identification of monomorphic birds

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In various species of birds sex identification is difficult. More than 50% of the world-wide birds are monomorphic (Grifftits et al., 1996). The aim of this study was to identify the CHD (chromodomain helicase DNA binding protein) genes from sexual chromosomes (Z and W) from various samples colected from: Gallus gallus domesticus, Melopsittacus undulatus, Psittacus erithacus and Ara ararauna.
Samples of feathers, swabs, blood and liver were collected. For DNA extraction was used a commercial kit (Isolate Genomic DNA kit; Bioline Reagents Limited, London, U.K.). From all types of tissue, the used protocols were according to the manufacturers. The calamus of the feathers were cutted in small pieces and then were disrupts through high-speed shaking in plastic tubes with stainless steel beads (TissueLyserII, Qiagen, US). To quantify the DNA Spectrophotometer NanoDrop ND-1000 was used. The identification of CHD gene was done according to previously described protocols (Griffits et al., 1996; Ito et al., 2003), using the primers P2 and P8 (Generi-Biotech, Hradec Králové, Czech Republic). PCR was carried out in a 25 µl reaction mixture consisting of 12.5 µl of MyTaq Red HS Mix (Bioline Reagents Limited, London, U.K.) and 25 pM of each primer.
The PCR results showed in females two products corresponding to CHD-W and CHD-Z, and in male one product which correspond to CHD- Z gene. The lowest DNA concentration that was amplified was 4.4 ng/µl in feathers samples, 16.5 ng/µl in swabs samples and 21.3ng/µl in blood samples.
We can conclude that all studied tissue samples can be used to identify the sex of monomorphic birds, but for feathers we recommend the mechanical denaturation before DNA extraction.

2.P12 One of the parental genomes is eliminated in gonocytes of P. esculentus but not in non specific F1 interspecies hybrids of water frogs

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The programmed DNA elimination plays a crucial role in the development of various organisms. Moreover, programmed DNA elimination was found during gametogenesis of some interspecies animal and plant hybrids. In germ cells of such hybrids, one of the parental genomes is eliminated while the other one is endoreplicated and transmitted to clonal gametes.

To investigate the mechanism of genome elimination, we have chosen frogs from the Pelophylax esculentus complex. This complex consists of two parental species P. ridibundus and P. lessonae, and their hybrid, P. esculentus. To check whether hybridization of P. ridibundus and P. lessonae with different water frog species can cause genome elimination in the offspring, we analyzed gonads of tadpoles obtained from crosses of P. ridibundus and P. lessonae with several closely related species – P. kurtmuelleri, P. epeiroticus and P shqipericus.

In offspring from crosses between P. lessonae and P. ridibundus, we detected micronuclei as well as misaligned chromosomes during gonocyte division. Misaligned chromosomes unable to attach to the spindle and possibly lag during anaphase. To check which genome is eliminated in the micronuclei, we identified misaligned chromosomes and chromosomes enclosed in the micronuclei using FISH with species-specific probes. To confirm elimination of one of the parental genomes, we additionally established genome composition in gonocytes. Nevertheless, in non-specific crosses, elimination was not observed or was extremely rare, and both genomes were present in germ cells.

Thus, the ability to genome elimination appeared only in specific F1 hybrids between P. ridibundus and P. lessonae, namely P. esculentus, and was not detected in other F1 interspecies hybrids.

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2.P13 Chromosomal analysis in a case of foetal abnormality detected in a stillborn male calf

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In order to identify the aetiology of Schistosomus reflexus (SR), foetal abnormality, which occurred in a stillborn male calf of Romanian Black and White dairy cattle breed, a cytogenetic investigation was carried out. The study of the blood samples collected from the malformed foetus was very difficult because few good metaphases for examination were obtained. Regarding the chromosomal complement of the stillborn dam, this was severely affected, the number of mono-and bi-chromatidic breakages on autosomes and heterosomes, loss of chromosome fragments and gaps being significantly higher than in normal configurations. Furthermore, the sister chromatid exchanges(SCEs)test has been used and revealed a high number of SCEs (9-15 SCEs/cell) and particularly, the presence of double interchromatidic exchanges on the same chromosome. These results suggest that the chromosomal instability identified in the malformed calf’s mother is characterized by a high rate of SCEs and that could be related with the presence of different environmental toxic agents. Considering all of the clinical and laboratory results and knowing that the presence of a toxic chemical agent in the first months of gestation can induce foetal growth and development disorders, the aetiology of the SR congenital malformation could be attributed to a teratogenic cause.

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2.P14 Cytogenomics of annual killifish: sex determination and repetitive DNA dynamics in closely related Nothobranchius furzeri and N. kadleci

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African annual killifishes of the genus Nothobranchius (Teleostei: Nothobranchiidae) display unique adaptations to temporary water pools in African savannahs. Diapausing embryos, fast development and exceptionally short lifespan make them vital models for various biological disciplines including biomedical research. Available cytogenetic and genomic data suggest fast rate of karyotype and sex chromosome evolution in their small allopatric populations. Here we analyzed the repetitive DNA landscape and sex chromosome differentiation in several populations of turquoise killifish, N. furzeri, and its sister species N. kadleci. Besides conventional cytogenetics, fluorescent staining, synaptonemal

complex analysis and fluorescence in situ hybridization with rDNA, telomeric and whole-genomic probes, we mapped selected sex chromosome-specific bacterial artificial chromosome (BAC) clones from N. furzeri genome library and performed low-coverage genome sequencing with subsequent RepeatExplorer analysis to identify abundant repetitive DNA sequences in both species. We revealed shared XY sex chromosome system varying in extent of differentiation among populations of both species. XY-specific hybridization patterns of different repeats and BAC clones allowed us to infer probable mechanisms underlying stepwise XY differentiation. We further revealed most abundant repeats to be shared by both species and mapped three of them chromosomally. By complementing data from earlier study, we showed that two repeats are centromere-specific and the third one is scattered throughout the genome but forming Y-specific accumulation. Subsequent mapping to chromosomes of other Nothobranchius species showed, besides various types of polymorphisms, fast turnover of two centromeric repeats but the conserved presence of the third, scattered repeat in all analyzed Nothobranchius genomes. Our data collectively imply important contribution of repetitive DNA to sex chromosome evolution, karyotype dynamics and species divergence in Nothobranchius killifishes.

2.P15 Satellitome analysis unravels the karyotypic evolution in Triatoma kissing bugs (Hemiptera Reduviidae)

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Chagas disease is a neglected disease from Latin America affecting 6-7 million people worldwide and transmitted by haematophagous insects of the subfamily Triatominae. In the absence of vaccines, the reduction of disease incidence depends mainly on vector control. An accurate taxonomic identification and knowledge about the genome biology of these insects are keys to ensuring successful vector control campaigns. Triatominae comprise more than 150 species, being the Triatoma genus the most numerous with about 90 species.
We present a comparative satellitome analysis on several evolutionary closely and distant Triatoma species. Our results indicate that satellite DNA represents the most abundant fraction of repetitive DNA within the genomes. Each Triatoma species presented more than 40 satDNA families, although only 4 to 9 of them are found in frequencies higher than 1% of the total genome of each species. For these families specific probes were made to determine their chromosomal location by fluorescent in situ hybridization. These principal repeat families are located in the heterochromatic and euchromatic regions of autosomes and sex chromosomes. All Triatoma species studied shared a satDNA family, constituted by (GATA)n repeats, located on the Y chromosome. However, each species bears their own satDNA families, some of them disproportionate amplified as seen for distant T. rubrofasciata and T. infestans. On the other hand, sister species such as T. infestans and T. delpontei share several, but not all, principal satDNA families. According to our results, the satDNA differentiation is the main responsible for the karyotype evolution in Triatoma genus.

2.P16 Comparative analysis of the satellitome in ladybirds (Coleoptera Coccinellidae)

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Coleoptera is one of the biggest and most diverse group of insects. Coccinellids (commonly known as ladybirds or ladybugs) comprises about 6,000 species in 360 genera. Almost the 90% of them are carnivorous, being one of the most effective animals used in biological pest control. The 10% herbivorous species can be pest of important crops. Satellitome (whole collection of satellite DNA) analysis can be used in order to compare the organization and evolution of genomes. We study the satellitome in Hippodamia variegata, Henosepilachna argus, Epilachna paenulata and Adalia bipunctata genomes, using bioinformatics pipeline based on low-coverage sequencing data and public data deposited in databases. A very different number of satDNA families, from 19 to 51, composes the satellitome of these four species. However, the amount of satDNA in each species with respect to the total genome was somewhat more similar, ranged between 15% and 24%. We mapped the main satDNA families in each species performing the first cytogenomic study. The comparative analysis of the four satellitomes showed that only few of the satDNA families were shared between species. For example, C. argus and E. paenulata only shared three families and C. argus and A. bipunctata shared only one family. On the contrary, comparing with public data, we found that A. bipunctata shared at least 11 families with other species of coccinellids and with other coleopteran insects. The results here presented has led us to generate and amplify the existing knowledge within

this topic and has allowed us to study the evolution of satDNA across the phylogeny of the ladybugs.

2.P17 Bioinformatics characterization of DNA satellite repeats from mango genomes and related Mangifera species

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Genomic characterization of crop wild relatives is fundamentally important for food security, for guiding breeding programs, and for local conservation efforts. Across many Asian countries, mango (Mangifera indica) is an important fruit crop with many cultivars, varieties, and related wild species. Its center of origin and diversity is mainly localized on the Indian subcontinent and the Southeast Asian region, including Bangladesh. Despite the availability of a mango reference genome sequence, the Mangifera germplasm remains largely uncharacterized, especially in Bangladesh, one of the proposed centers of origin. As a result, local breeding and conservation efforts are undirected, and may likely result in loss of the natural Mangifera biodiversity. To assist the characterization of the Mangifera germplasm, we aim to develop cytogenetic probes for karyotyping and species identification, also allowing us to trace evolution of the Mangifera biodiversity. As repetitive DNAs and especially satellite DNAs (satDNAs) have a high sequence turnover and can occupy large regions along the chromosomes, these may provide ideal probes for Mangifera germplasm characterization. To comprehensively characterize mango's repeat content, we have applied RepeatExplorer-based bioinformatics on the genome of the Mangifera indica cultivar 'Alphonso'. We have focused on six satDNA candidates (SatMan1-SatMan6) with monomer sizes ranging between 119 bp- 3370 bp. Interestingly, SatMan2 and SatMan3 have a significant sequence similarity with polygalacturonase (PG22-8) gene and the microsatellite MiSHRS1 in M. indica. These satDNAs will provide the basis for a cytogenetic screen of the Mangifera germplasm, and will help to understand the genetic basis of wild species, cultivars and varieties from Bangladesh.

3. Clinical Cytogenomics

3.P1 A 15 mb interstitial duplication in band Xq27.1 q28 in a female infant with hypotonia, microcephaly, prominent forehead and retrognathia a case report

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A one month old term female baby was referred to our clinic from the newborn intensive care unit (NICU) because of hypotonia and feeding difficulty. Prenatal and natal history revealed oligohydramnios in the 3rd trimester, fetal malposition, fetal distress and meconium in the amniotic fluid.
After birth, the patient needed NICU for respiratory support. Physical examination showed hypotonia, microcephaly, prominent forehead, sparse eyebrows, full cheeks, retrognathia, long philtrum, bilateral single transverse palmar creases and clinodactyly. After 1 month of care she was discharged from hospital. Follow up examinations revealed that the patient had secundum atrial septal defect, neuromotor delay and feeding difficulties continued. Hearing tests and opthalmologic examinations were normal. EEG was normal and there was no clinical history of seizures. Imaging studies showed corpus callosum hypoplasia, ventriculomegaly and pontocerebellar hypoplasia. GTG banded chromosome analysis showed 46,XX,der(18)t(X,18)(q27.1;p11.3)dmat karyotype; the deletion 18p11.2-->pter and the duplication Xq27-->qter were confirmed with MLPA (SALSA MLPA probemix P070 Subtelomeres Mix 2B) analysis. Since the cytogenetic results are not fully compatible with the patient's symptoms, we performed array-CGH for patient and her mother to obtain more accurate results which revealed ≈15Mb duplication of Xq27.1-q28 duplication and a 207 Kb deletion of 18p11.32 in the proband. However, the mother's array-CGH was found to be normal, which confirms a balanced translocation. Because the 18p11.32 deletion did not contain any morbid OMIM gene we have attributed probands' phenotype completely to the Xq27.1-q28 duplication.
Although X-linked deletions and duplications do not usually cause major symptoms in female patients, the explanation of our patient's phenotype with X duplication indicates that this case has some unique feature for us and the literature.

3.P2 Genetic testing of miscarriages using a QF PCR / aCGH / MLPA strategy: five years experiences from North Eastern Slovenia

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Introduction Traditional testing of miscarriage samples involved culture of tissue followed by G-banded chromosome analysis; this approach has a high failure rate, is labour intensive and has a resolution of around 8-10 Mb. To improve the diagnostic yield and efficiency we have updated our testing strategy in 2016. Since then we use more comprehensive strategy: QF-PCR assay for all samples, followed by array CGH or MLPA, depends on a gestational age of the embrionic material. Here we describe the results from the last 5 years of our strategy.
Methods: Fetal tissue samples and maternal samples were tested using QF-PCR for the detection of aneuploidy for chromosomes 13, 15, 16, 18, 21, 22, X and Y. Confirmed fetal samples without aneuplody of tested chromosomes and less than 15 weeks of gestational age were then tested by MLPA while fetal samples older than 15 weeks were analyzed by aCGH.
Results: From 335 analysed samples, 266 samples were confirmed as fetal material (78,57%). In those QF-PCR analysis identified aneuploidy/triploidy in 21.42%. MLPA detected subtelomeric imbalances in a further 12.79% (11 out of 86) while aCGH analysis detected imbalance in 4.87% (6 out of 123) of samples. All detected imbalances by aCGH were submicroscopic (in range 0.19-2.6Mb) and 2 out of 6 (33.33%) were classified as causative for the spontaneous misscariage.
Conclusions: This efficient QF-PCR/aCGH/MLPA strategy has a lower failure rate and higher diagnostic yield than karyotype. It is therefore an efficient and cost-effective diagnostic testing strategy for misscarriage products.

3.P3 A novel small deletion overlapping 3q29 microdeletion syndrome in a patient with patent ductus arteriosus and normal neurodevelopment

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Individuals with 3q29 microdeletion syndrome are hemizygous for a 1.6Mb interval containing 21 protein coding genes. They are characterized by global developmental delay and/or intellectual disability, and increased risk for neuropsychiatric disorders. Other common findings are failure to thrive and feeding problems, heart defects (particularly patent ductus arteriosus, PDA), gastrointestinal disorders, dental abnormalities and slight facial dysmorphology.
We report an atypical de novo 0.6Mb deletion in 3q29 detected by array-CGH (Agilent Technologies) overlapping 3q29 microdeletion syndrome. As far as we know, it is the smallest one described to date. Our patient is a 4 years old girl, premature (36+6 weeks) with low birth weight. The first child of healthy non-consanguineous parents, without any neurocognitive abnormality, and a heart malformation (PDA) with left ventricular dilatation and unremarkable family history.
We have compared the 3q29 deletions with previously reported in an attempt to establish genotype-phenotype correlations. There are several genes of interest for the neurodevelopmental and psychiatric phenotypes for this syndrome: DLG1, FBXO45, PAK2, and RNF168. DLG1 gene is not deleted in our patient who has no neurological symptoms, therefore it would support that haploinsufficiency of this gene could play an important role in these disorders.
On the other hand, although PDA occurs more commonly in premature babies, it has been detected in about 12% of 3q29 deletion patients. C3orf43 gene, included in the critical region for this syndrome, has a high expression in heart and has been previously suggested as a candidate gene for heart defects. Deletion of this gene in our patient would support this hypothesis.
This and other similar cases nested within the recurrent microdeletion syndromes with unique breakpoints can play an important role in point out relevant genes associated to clinical manifestations of the syndrome.

3.P4 Report of a rare 7p22.1p21.2 interstitial deletion in a newborn with a polymalformative syndrome

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The increased use of chromosomal microarray analysis (CMA) has led to the identification of new microdeletion/microduplication regions, enabling better genotype-phenotype correlations.
Here we report a 2-day-old newborn, delivered at 33 week-gestation, with Apgar score of 4/6/6, and referred for genetic diagnosis due to a polymalformative syndrome. During pregnancy a risk for T18 of ¼ was assessed after combined prenatal screening at 13 week-gestation, but the parents refused invasive testing. Additionally, ultrasound at 28 weeks revealed omphalocele, hepatomegaly, and left duplex kidney.
Postnatal Affymetrix Cytoscan 750 CMA revealed a 8.95 Mb interstitial deletion at 7p22.1p21.2, in a female profile: arr[GRCh37] 7p22.1p21.2(5149391_14100949)x1.
After parental analysis this deletion was shown to be de novo.
This large deletion includes 30 OMIM genes, including ACTB whose gain of function missense mutations are causal for Baraitser-Winter syndrome.
Interstitial deletions involving the short arm of chromosome 7 are rare but recently some patients with intellectual disability, short stature and microcephaly have been reported with a non-recurrent microdeletion in 7p22.1 that includes ACTB.
The premature newborn described here showed neither short stature nor microcephaly; intellectual disability can only be assessed later on. However, in the first days after delivery multiple cardiac defects and hypothyroidism were diagnosed , whereas hepatomegaly and omphalocele detected prenatally had regressed to almost normality.
The collection of more cases with these rare deletions in the 7p22.1p21.2 region will help delineating a more detailed phenotype, hence leading to better genetic counseling for patients and families, and may also establish this region as a microdeletion syndrome in the future.

3.P5 In vitro models of chromosomal diseases based on iPSCs from patients with ring chromosomes

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Human induced pluripotent stem cell (iPSC)-based models is a promising strategy for evaluation of the functional effects of chromosomal aberrations. Although these models are generally assumed to capture the genetic variants, some exceptions are possible, especially for ring chromosomes (constitutional and supernumerary), where mosaicism has been well documented in vivo. Intellectual disability and developmental abnormality often occur in patients with ring chromosomes, but the phenotype of carriers is sometimes unpredictable due to inherent mitotic instability of the rings in somatic cells. Analysis of own and available published data about stability of patients-derived iPSCs with ring chromosomes was carried out. Currently, four studies have been published with iPSCs from patients with r(8), r(13) (3 cases), r(14), r(17), r(18), r(21) and r(22). Bershteyn et al., 2014 reported loss of r(17) during iPSC derivation followed by duplication of the wild-type homolog, giving rise to uniparental isodisomy. We found a similar phenomenon for r(8)-iPSC in all six iPSC lines after passage 10. The results of CGH+SNP analysis provided evidence of uniparental isodisomy; UPiD(8)pat. In total, spontaneous karyotype correction in iPSC was found in 4/6 clones with r(17), 6/6 clones with r(8), 7/13 clones with r(13) and 1/3 clones with r(14), but not reported for r(18) and r(22). Remarkably, ring chromosome behaviour in iPSCs varied between lines with different rings and, sometimes, between different clones with the same ring chromosome. Currently, we have an affected newborn with an unstable mosaic supernumerary ring chromosome 10 under observation. Caution is necessary when dealing with ring chromosome-iPSCs. Confirmation of the presence of the ring chromosome in source fibroblasts, iPSC clones, and differentiated cells is essential, and reprogramming and iPSCs differentiation should be carried out as early as possible.
This study was supported by the Russian Science Foundation, grants 16-15-10-231, 21-65-00017.

3.P6 Five cases of constitutional neocentric supernumerary marker chromosomes

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Neocentric small supernumerary marker chromosomes (sSMCs) occur in around 1-3% of all published cases with sSMCs. There are two types of neocentric sSMCs. The majority originate as result of U-type exchange and are small inverted duplicated chromosomes leading to an unbalanced karyotype. Another group is based on interstitial deletion and rearranged to form a ring and linear chromosomes in a balanced karyotype. Identification of the structure and origin of sSMCs in patients with multiple congenital anomalies or with reproductive failure is an important task for cytogenetic diagnostics. We present five cases of constitutional neocentric sSMCs de novo characterized by GTG-banding, chromosomal microarray and FISH analysis (multicolor FISH, multicolor banding, centromeric, subtelomeric probes).
Case 1. A 39-year-old normal male, apart from infertility. Karyotype: mos 47,XY,+mar[20]/46,XY[3]. FISH analysis showed that the sSMC was derived from interstitial deletion of chromosome 1, del(1)(pter→13.3::p11.1→qter)transformed to a ring chromosome r(1)(::p13.3→p11.1::). Interphase FISH determined a karyotype with a neocentric chromosome in 100 % of cells.
Case 2. An 8-year-old boy was referred for evaluation because of developmental and psychomotor delay. Karyotype: 47,XY,+mar. The sSMS was characterized as inv dup(8)(pter→p23::p23→pter) by FISH analysis.
Case 3. A 2-year-old girl with psychomotor delay. Karyotype: mos 47,XX,+mar[12]/46,XX[10]. FISH analysis identified inv dup(13)(qter→q32::q32→ qter).
Case 4. A 3-month-old girl with multiple congenital anomalies. Karyotype: mos 47,XX,+mar[8]/46,XX [23]. Chromosomal microarray analysis detected a mosaic copy gain of 15q25.3→q26.3. FISH analysis confirmed a neocentric inv dup(15)(qter→q25.3: :q25.3→qter) and revealed a level of mosaicism with sSMC in 35% and a normal karyotype in 65% of the cells.
Case 5. An 8-year-old boy with short stature and speech delay. Karyotype: 47,XY,+mar. Chromosomal microarray analysis was normal. FISH showed that the sSMC was derived from an interstitial deletion of chromosome chromosome 16 and was a ring chromosome r(16)(::q13→q23::).

3.P7 A familial 9q22.2q22.33 duplication involving the PTCH1 gene characterised by short stature, dysmorphic features and mild mental retardation

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The partial trisomy 9q involving the duplication of band 9q22 is manifested by a constellation of symptoms including short stature, intellectual disability, microcephaly, pyloric stenosis, facial dysmorphism, and various defects of the heart, distal extremities, eyes, thyroid, and esophagus.
We report a case of a six year old female patient with short stature, microcephaly, facial dysmorphism, delayed speech and language development, fifth finger clinodactyly of both hands and syndactyly involving second and third toes of the feet.
The father presented the similar phenotype with short stature, microcephaly, prominent nose, mild intellectual disability and fifth finger clinodactyly of hands.
Whole exome sequencing including CNV analysis performed on the DNA of the patient and his father revealed in both of them a pathogenic heterozygous 7431 kb duplication within the region 9q22.2q22.33 involving 97 genes, including PTCH1. The result was confirmed by chromosomal microarray analysis and was consistent with the diagnosis of 9q22 duplication syndrome.
On the basis of the described functions of the duplicated genes, PTCH1 represents a candidate gene that may be responsible for the phenotypic findings, although the other genes in this duplicated segment may also contribute to the phenotype.
To our best knowledge, this is the first case report of 9q22 duplication syndrome in Albania. Similar duplications have been previously reported in only a few cases. Our report provides evidence to support a specific phenotype associated with a 9q22.3 microduplication and confirms that the duplication of this chromosomal region is associated with trisomy 9q phenotype.

3.P8 Evidence for pleiotropic CNVs by enrichment analysis of human miscarriages

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Prevalence of pathogenic copy number variations (CNVs) in different pathologies indicate that the same variation can manifest as a disease of one system of organs or multiple congenital malformations, be associated with impaired embryogenesis, and also be inherited from healthy parents. With the involvement of literature data and our own results, we aimed to identify CNVs with pleiotropic effect, which manifest by both impaired prenatal development and postnatal diseases. Using chromo-

some microarray analysis, 2471 samples of human products of conception (POCs) (1-3 trimesters) with developmental delay and/or congenital malformations were examined. Altogether 468 pathogenic CNVs were identified with involvement of 5037 genes. Using the enrichment analysis with the Human Phenotype (HP) and Jensen Diseases (JD) ontologies all genes were classified into 36 and nine categories respectively ($p \leq 0.05$). Among HP categories 33 were related to abnormalities of different organs and systems; one category was linked to nuchal translucency (HP:0010880) and two were devoted to behavioural problems (obsessive-compulsive behaviour, OCB), HP:0000722; abnormal eating behaviour, HP:0100738). Importantly, six of 17 genes from OCB category (RFC2, EHMT1, CRKL, MAPK1, GTF2IRD1, GTF2I) are located at the 7q11.23 deleted region in patients with Williams-Beuren syndrome (OMIM 609757), and mice knockdown of them leads to embryo lethality (MGI Mammalian Phenotype, MP 2019). Among nine JD categories seven were devoted to pathology of one organ/system and two known syndromes - Beckwith-Wiedemann, del11p15.5 (OMIM 602631) and 1p36 deletion (OMIM 607872). The following genes located within 11p15.5 and 1p36, respectively, are associated with embryo lethality in mice also – CDKN1C, IGF2, MRPL23, ASCL2 and CASZ1, PRDM16 (MP 2019). Thus, we revealed pathogenic CNVs with pleiotropic effect that observed both in abnormal POCs and patients with microdeletion/ microduplication syndromes. This study was supported by the Russian Science Foundation, grant 21-65-00017.

3.P9 Effectivity of whole exome sequencing in copy number variant detection in children with neurodevelopmental disorders

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Copy number variants (CNVs) are a common source of genetic variation in neurodevelopmental disorders (NDDs). Chromosomal microarray analysis (CMA) is currently regarded as the gold standard for its detection. Whole-exome sequencing (WES) is widely accepted as a powerful tool for exome-wide detection of single-nucleotide variants (SNVs) and small insertions and deletions (InDels). Detection of CNVs using WES have also recently become possible through the development of special algorithms and software. Our study evaluated two WES read-depth based CNV detection pipelines using high-resolution CMA as a standard in 20 families (trios or quatros) of children with severe NDDs and associated congenital abnormalities. A total of 15 CNVs in 8 families (384–3025 kb) identified using Agilent CGH+SNP array platform were compared to CNVs identified using WES by Human Core Exome (Twist Biosciences) on Illumina NovaSeq 6000. Using two WES in-house CNV detection pipelines developed by Masaryk University and Newcastle University, respectively, we confirmed and specified all 15 CNVs previously detected by CMA. All length variabilities in findings were verified using qPCR and manually curated. Both pipelines detected an elevated proportion of small variants compared to CMA, however, no clinically relevant findings were newly discovered. Our pilot study confirmed that combined identification of SNVs, InDels, and CNVs would increase the versatility of WES in diagnostics of NDDs in children.
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3.P10 A clinical manifestation of 18p tetrasomy in a child with signs of Down syndrome.

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Introduction:
Chromosomal disorders are one of the most common problems in clinical genetics that causes early disability and mortality. They require confirmation by various genetic methods and a multidisciplinary approach.
Materials and methods:
Clinical, standard karyotyping, FISH (Down loci), NGS.
Anamnesis:
The child is from the first pregnancy as a result of in vitro fertilization. The main prenatal tests were performed: normal ultrasound at 10-11 weeks (neck space of 1 mm, nasal bones - 2.3); according to biochemical screening, the pregnant woman did not fall into the group of genetic risk. Birth at 40 weeks, 3750g/54cm. The newborn had a clinical picture of Down syndrome: palmar crease on the left hand, poor muscle tone, jaundice, heart defects - open ductus arteriosus, open oval window, pulmonary hypertension.
Discussion:
Chromosome analysis showed the presence of a regular extra marker chromosome - 47,XY,+mar. The origin of the marker chromosome from chromosome

21 is excluded by the FISH - ish 21q22.13-22.2(D21S341,D21S342)x2. The low-resolution NGS was used to identify the marker chromosome. It showed the triplication of the short arm of chromosome 18(p11.32p11.1). It follows that the extra marker chromosome is an isochromosome of the short arm of chromosome 18. The parents' chromosomes are normal.
At 3 months the microcephaly, varus installation of the right foot, the high palate were added to clinical features.
This chromosomal rearrangement is associated with the syndrome of tetrasomy 18p. The frequency is 1/180000. About 250 cases in the world have been described. Clinical features are similar to the Down syndrome.
Conclusion:
Early detection of chromosomal pathology using different genetic laboratory methods is important for the further prognosis of life and the calculation of genetic risk.

3.P11 Intronic microduplications of RBFOX1 gene and their phenotypic consequences

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Introduction:
RBFOX1, one of the largest genes, is located in chromosomal region 16p13.3 and encodes a protein that plays role as a splicing factor. This gene / RBFOX1 is responsible for positive and also negative regulation of alternative splicing of pre-mRNA and is expressed in heart, muscles and central nervous system, especially in neurons. Mutations and intragenic deletions (exonic mainly) of RBFOX1 were detected in patients with neurodevelopmental disorders (intellectual disability, autism spectrum disorder, epilepsy) and neuropsychiatric diseases (schizophrenia, bipolar disorder).
Materials, methods:
We present four patients referred to genetic testing due to variety of neurodevelopmental problems. DNA was extracted from peripheral blood and analyzed by array-CGH (aCGH) platform SurePrint CGH 8x60K G3 ISCA v.2 and the CytoGenomics software (Agilent Technologies).
Results:
aCGH analysis revealed intronic duplication of RBFOX1 gene in all cases. We identified two identical, 25 kb duplications, encompassing only intron 2, and two bigger duplications (48 kb; 80kb) localized in intron 4. In two cases, additional aberration was detected – duplication of region 3p13 and 6q26 in one patient and mutation in MEF2C (c.44G>A p.(Arg15His)) in the other patient.
Conclusion:
While the mutations and intragenic deletions of RBFOX1 are known to be associated with neurodevelopmental disorders, the intragenic or whole gene duplications are still topic of discussion. There is also an hypothesis that duplications are (thought to be) a risk factors rather than a cause of disease, especially in combination with other factors (second-hit model, environmental factors, etc.). Up to date, there is a limited number of published cases with these types of aberrations of RBFOX1. We report four new patients with neurodevelopmental disorders who carry the intronic duplication of RBFOX1.
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3.P12 Chromosomal microarray results in children referred due to language delay

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Objective and methods:
Language delay is a frequent early warning sign of abnormal development. A retrospective chart review was conducted on 919 children referred for single nucleotide polymorphism (SNP) chromosomal microarray testing over a two-year period in order to (1) estimate the detection rate of abnormalities in children with language delay as the main reason for referral and (2) find the most frequent copy number variations (CNVs). For statistical analyses X^2 tests were performed.
Results:
A third of children (32%, n=293, mean age 3,8±2,1 years) referred for chromosomal microarray testing had language delay. One-fifth (22%, n=65) of them had language delay as the only reason for referral, many children additionally had autistic features (22%, n=64) or family members with similar problems (21%, n=61). The detection rate of abnormalities in children with language delay was 15% (n=43) and was not higher in patients with positive family history or more severe symptoms. 8% (n=22) of children with language delay had pathogenic or likely pathogenic CNVs, 4% (n=12) had variants of unknown significance and 3% (n=9) had >5 Mb regions of homozygosity (ROHs). Three patients had CNVs containing regions associated with primary speech and language disorders (FOXP1, FOXP2, DYX6) and one patient had a ROH containing CMIP and SLI2. Most frequent abnormal CNVs were recurrent deletions of 16p11.2 (n=4) and 16p12.2 (n=3), known to have variable penetrance.

Following a normal array result most of the patients (57%, n=142) had additional genetic testing: mostly next generation sequencing and Fragile X syndrome exclusion.
Conclusions:
Chromosomal microarray is an essential first-tier genetic test for children with language delay.

3.P13 A complex chromosomal aberration with an insertion and a paracentric inversion in the recipient chromosome and its reproductive consequences

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In an interchromosomal insertion, a segment of one chromosome is inserted into another (recipient) chromosome. Carriers of balanced interchromosomal insertions have one of the highest risks (theoretical risk of 50%) of producing gametes with either a deletion or a duplication of the inserted segment. They, therefore, suffer from reproductive problems and have a risk of having a child with birth defects due to chromosome imbalance. Paracentric inversions, on the other hand, may cause infertility but do not usually lead to the birth of a child with chromosome imbalance. This is because the recombinants of paracentric inversions produced at meiosis are dicentric or acentric chromosomes that are usually not viable.
We present a case of a man with an inversion as well as an interchromosomal insertion into the same chromosome.
A healthy young couple was referred for infertility. The karyotype of the man showed a paracentric inversion of a segment of 9q as well as an insertion of a segment of 6q, adjacent to the distal inversion breakpoint in 9q. Both aberrations appeared to be balanced. The karyotype was studied by locus specific FISH and mband, but precise size of inserted material, about 50 Mb, was determined only after preimplantation NGS had been performed. None of the 9 embryos had a normal genomic profile.
The patient´s karyotype was determined as 46,XY,inv(9)(q21.2q32)ins(9;6)(q32;q16.1q24.3).
This is a unique chromosomal finding. Interchromosomal insertions of 6q have been published but without cytogenetically visible rearrangements of the recipient chromosome

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3.P14 Balanced Robertsonian translocation der(13;14)(q11;q11) in a woman with recurrent pregnancy loss: A rare case report

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A balanced Robertsonian translocation is one of the major chromosomal abnormalities with a prevalence rate of 0.1% of the general and 1% of the infertile population. We present a non-homologous Robertsonian translocation in a woman with a history of recurrent pregnancy loss (RPL).
A couple (Female was 26 and Male was 27 years old) with RPL were referred to our medical genetics outpatient clinic for cytogenetic evaluation. Couple were both healthy and non-consanguineous. Chromosomal analysis on Trypsin GTG-banded metaphases from peripheral blood revealed an abnormal karyotype in the female partner, 45,XX,der(13;14)(q11;q11) chromosomal constitution, while the male partner showed normal 46,XY karyotype. There was no history of recurrent miscarriages or infertility in the patient's pedigree. This is the 7th der(13;14) case that has been referred at our medical genetics outpatient clinic with RPL/infertility.
Structural and/or numerical chromosomal abnormalities may lead to miscarriages and infertility with a prevalence rate of 10-20 % of RPL and 50% of abortions has chromosomal abnormalities. Clarifying the underlying cause of every miscarriage is not possible. But one of the most important known causes of first trimester abortions is the presence of parental chromosomal aberrations. Robertsonian translocations are common chromosomal aberrations detected by karyotype analysis in individuals with recurrent miscarriages and this case shows us how important karyotype analysis is in the investigation of RPL. Results from this rare case show the importance and validity of conventional cytogenetic analysis in the detection of balanced Robertsonian translocations and that prenatal diagnosis should be offered to couples with RPL in the further pregnancies.

3.P15 De novo pericentric inversion of chromosome 1(p13q21) and male infertility A case report

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A pericentric inversion of chromosome 1(inv1) is a rare structural chromosome abnormality that, regardless of the chromosomal breakpoints, causes infertility in males who have spermatogenic failure and azoospermia. In this report, we present a patient with azoospermia due to a de novo pericentric inversion of chromosome 1.
A married couple (the female was 30 and the male was 37 years old) with of a history of sterility and azoospermia were referred to the medical genetics outpatient clinic for karyotyping. Although the woman has a healthy child from her first marriage, her first husband does not have a healthy child. The couple was non-consanguineous and there was no history of recurrent miscarriages or infertility in the pedigree.
A heparinized peripheral blood sample was used for karyotyping (GTG and C banded) and an EDTA blood sample was used for QF-PCR analyses. The female had a normal karyotype and the karyotype of the male was 46,XY,inv(1)(p13q21). No AZF microdeletions were detected in the chromosome Y with STR QF-PCR analysis. The man was diagnosed as having a de novo pericentric inversion of chromosome 1 after both his parents were found to have normal karyotypes.
Results from this case show that a pericentric inversion of chromosome 1 may cause male infertility, possibly by affecting some functional gene or genes related to male infertility. More similar cases need to be studied by multiple advanced medical genetic techniques.

3.P16 Familial case of non classical unbalanced X;Y translocation associated with POF

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Background: X;Y translocations result in various phenotypes, including disorders of sex development (DSD), infertility and intellectual disability. Rare familial cases of inherited X;Y translocations have been described in the literature.
Clinical case: We report a familial case of non-classical X;Y translocation. The proband is 25 years-old female, who was admitted for genetic examination because of infertility and secondary amenorrhea (at 24 years' age). Premature ovarian failure (POF) was diagnosed. She has healthy fertile brother (29 y.o.). The proband's mother (49 y.o.), had six natural pregnancies (childbirth, n=2, medical abortion, n=4) and developed secondary amenorrhea before the age of 40. Both patients were otherwise healthy females with normal height and intelligence, and no signs of masculinization.
Materials and methods: Chromosome analysis was done on cultured peripheral blood lymphocytes using the GTG- and C-staining. Fluorescence in situ hybridization (FISH) with the DNA probes (DXZ1, DYZ1) was performed on peripheral blood lymphocytes. Array comparative genomic hybridization (arrayCGH) was done using DNA samples from peripheral blood leucocytes.
Results: The karyotype 46,X,der(X)t(X;Y)(q26.2; q11.2). ish(DXZ1+,DYZ1+) was detected in the proband and her mother. FISH and arrayCGH revealed a derivative X-chromosome with terminal Xq deletion resulting in Xq26.2-Xq28 monosomy – [hg19](131751272_1549291279)×1, and translocation of Yq fragment, including distal heterochromatin (Yq12) and a portion of Yq11.2 locus ([hg19](16059893_28644194)×1) with the breakpoint in proximal border of AZFb region.
Conclusion: Some X;Y translocations can be maternally inherited, .at that the same translocations may be compatible with female fertility, also as secondary amenorrhea and POF.

3.P17 Genetic characteristics and clinical consequences of constitutional ring chromosomes

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In humans ring chromosomes (RCs) occur in approximately 1:50000 newborns. The presence of a RC causes mitotic instability and often results in dynamic mosaicism with cells showing chromosomal or segmental aneuploidies, leading to various more severe phenotypic consequences than due to primary deletion. We report RCs identified in 6 patients with varied abnormal phenotypes and evaluated by molecular cytogenetic techniques. RCs were detected by GTG-banded chromosome analysis: 46,XX,r(3)(p26q29), 46,XY,r(15)(p11.2q26), 46,XY, r(18)(p11.1q21), 46,XY,r(18)(p11.1q23), 46,XX,r(20)(p13q13.2), 46,XX,r(22)(p13q13). All samples were analyzed by multicolor banding FISH technique with corresponding probe sets. FISH with corresponding subtelomeric, locus-specific, centromere-specific DNA-probes as well as probes for pan-telomeric regions and for acro-p-arms was also applied. Chromosomal microarray (CMA) was performed in a case with r(3) to define the accurate boundaries of the deletion detected by FISH. Chromosomal content and breakpoints could be determined in all cases using molecular cytogenetics. In four RC cases, except cases with r(18), secondary chromosomal imbalances was detected. Double rings, interlocked and broken/open rings were found when r(3) and r(15) were present in karyotype (10% and 12% of cells respectively). In cases with r(20) and r(22) only loss

of the rings (12,5% and 11,5% of cells respectively) and double ring formation (4% and 1,5% respectively) were revealed. Clinical manifestations varied significantly in patients with RCs. Each patient presented their own phenotypic features considering the genes deleted from one or both chromosome arms except the patient with r(3) in which a 4.4 Mb deletion containing TRNT1, CRBN, SUMF1 morbid genes was detected by CMA in 3p26.3p36.1. However, the clinical features were not correlated to haploinsufficiency of these genes.
Conclusion: The relationship between RC size and chromosomal instability has been confirmed. We concluded that the severity of symptoms in the patient with r(3) is influenced by a secondary chromosomal imbalance due to RC instability.

3.P18 Contribution of chromosomal microduplications to the etiology of intellectual disability

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The contribution of chromosomal microduplications to hereditary and congenital human pathology is still underestimated due to the high variability of phenotypic manifestations and frequent inheritance from healthy parents. Using array-based comparative genomic hybridization, the molecular karyotypes of 1000 children with intellectual disability, dysmorphia and / or congenital anomalies were studied. Chromosomal microduplications with pathogenic and potentially pathogenic significance were detected in 164 patients (16%), microdeletions in 128 patients (13%), combinations of various types of CNVs were found in 83 patients (8%). The clinical significance of partial trisomies was determined by their size, inheritance, number and function of affected genes, as well as data from bioinformatics resources. Nine partial trisomies were identified as pathogenic because they were associated with known microduplication syndromes. The frequency of microduplications with a potentially pathogenic significance was 12.7%, whereas with an unknown value of 2.8%. Twenty patients had microduplications localized in regions of known microdeletions with proven pathogenic value, thus the frequency of reciprocal rearrangements to known microdeletion syndromes was estimated to 2%. An analysis of the origin showed that only 30% of the microduplications arose de novo, whereas 70% of the cases were inherited from healthy parents (24% of microduplications had a paternal origin, 46% were of maternal origin). Inherited microduplications were up to 1.5 Mb, and de novo origin was characteristic for larger rearrangements ($p = 0.04$, Fisher exact test). These data indicate the importance of molecular testing of patients with idiopathic intellectual disability, the need for careful parental examination, the development of new approaches for prenatal and preimplantation genetic testing, as well as the importance of basic research of incomplete penetrance phenomenon. This study was supported by the Russian Science Foundation, grant 21-65-00017.

3.P19 A rare Koolen de Vries syndrome caused by 17q21.31 deletion that encompassing KANSL1 gene: a case report

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Introduction: Koolen-de Vries syndrome (KdVS) is a rare genetic multisystem disorder that is characterized by neonatal/childhood hypotonia, epilepsy, developmental delay, intellectual disability, dysmorphisms, congenital malformations and behavioural disorders. A girl with Koolen-de Vries syndrome with KANSL1(KAT8 regulatory NSL complex subunit 1) gene deletion is presented in the current report
Case: A 12-year-old girl with epilepsy, ataxia and neurodevelopmental delay was referred to our department. Physical examination revealed neurodevelopmental delay, microcephaly, micrognathia, poor speech development, large prominent ears, thick eyebrows, prominent nose, drooling and friendly behavior. Brain MRI showed abnormal cortical gyration. Her parents were non-consanguineous and both healthy.
Methods and Results: Peripheral blood samples was used for DNA isolation and karyotype analysis by trypsin-GTG banding after lymphocyte cell culture. She had a 46,XX normal female karyotype. A 64.4 kb heterozygous deletion of chromosome 17 (locus 17q21.31), encompassing the KANSL1 gene was detected after MicroArray-CGH(Illumina iScan System 300K MicroArray platform) analysis.
Conclusion: The KANSL1 gene encodes a nuclear protein that plays a role in chromatin modification as a member of a histon acetyltransferase (HAT) complex. Since KdVS is inherited as autosomal dominant, male and female individuals are equally affected. The present case was diagnosed as de novo due to the presence of clinical signs and molecular findings of structural KANSL1 gene deletion. Based on the current case report, we confirm that high resolution array-CGH is a very effective tool for detecting deletions and/or duplications in cases with suitable clinical phenotype in spite of a normal karyotype.

3.P20 Characterization of a rare mosaicism of an autosomal translocation of t(6;9) underlying mental retardation by using conventional cytogenetics and MicroArray CGH methods: a case report

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Objective: Mental retardation (MR) is typically described as an overall IQ lower than 70, associated with functional deficit in adaptive behaviour, such as self-care, daily activities and social skills. Chromosomal abnormalities are one of the most important causes of mental retardation. In this report, we present cytogenetic and molecular cytogenetic results from a carrier of a balanced translocation with mental retardation.
Case: We report an 8-years-old boy, of non-consanguineous parents, with mental retardation, speech and language impairment, esotropia and long philtrum. Results of brain MRI and EEG were normal. Lymphocyte cultures were set up from heparinized blood and DNA isolation was done on EDTA blood samples. Chromosome analysis was done on trypsin-GTG banded metaphases and microarray techniques were used to identify the affected gene or genes.
Result: The karyotype was mos46,XY,t(6;9)(p23;q22,1)[16]/46,XY[4]. Both parents had normal karyotypes. MicroArray-CGH (Illumina iScan System 300K MicroArray platform) analysis detected a heterozygous deletion of 480 kb in chromosome 3 (at band 3p14.1). Although there is no morbid OMIM gene in the 3p14.1 region, larger deletions covering this region in the Decipher database have been associated with "Intellectual Disability" and reported as "VUS-Variant of Uncertain Significance".
Discussion: Genetics plays a major role in the etiology of mental retardation. This case report confirms the role of chromosomal anomalies in the etiology of MR. We recommended analysis from skin biopsy or FISH analysis from buccal sample in connection with the mosaicism and referred the patient to pediatric hematology.

3.P21 Cytogenetic and molecular analyses of balanced reciprocal translocation in an infertile female patient with 46,XX,t(11;17)pat karyotype

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Objective: Balanced and unbalanced chromosomal rearrangements have been reported in individuals with habitual abortion and infertility. Here we report cytogenetic and molecular findings in an infertile female with a balanced reciprocal translocation.
Method: a heparinized peripheral blood sample was used for lymphocyte cell cultures and an EDTA blood sample was used for total genomic DNA isolation. Trypsin-GTG banded metaphases were analysed by fluorescent in-situ hybridization (FISH) for cytogenetic profiling and genome-wide micro-array-CGH technique was used for molecular cytogenetics to identify the affected gene or genes at the chromosomal breakpoints.
Case: A 38 year-old infertile female patient was referred to our clinic for cytogenetic analysis. She had been married for eleven years and was unable to have healthy children in spite of two in vitro fertilization (IVF) attempts. On physical and spermiogram examinations, it was reported that she and her husband did not have any clinical and/or organ failure such as ovarian, tubal or uterine abnormalities. The couple had no familial history of RPL and/or infertility. Her karyotype was 46,XX,t(11;17) pat. Her father was a carrier of the same translocation. Chromosomal breakpoints were identified by specific FISH probes, which showed signals between 11q11~q13.2 on chromosome 11, and between 17q22~q23 on the chromosome 17. Microarray-CGH analysis detected a deletion of 74.5 kb in size in chromosome band 11p15.4.
Conclusion: Once again the current case results showed us the importance and crucial role of conventional cytogenetics and its confirmation by advance molecular cytogenetic techniques in the identification of a balanced reciprocal translocation.

3.P22 Recurrent 3q29 microduplication: French cohort of 16 patients

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Background: Recurrent 3q29 microduplication (3q29dup) is currently associated with developmental delay, intellectual disability, and/or behavioral

disorders. This duplication is typically 1.6 Mb in length. To date about 29 patients are described in the literature with heterogeneous phenotypes. A recent study compared clinical features in 31 patients with 3q29dup to a control group. No specific syndrome is defined yet, and the pathogenicity of this duplication remains still uncertain. In this preliminary work, we report a French ACLF and Anddi-Rares cohort of 16 patients with 3q29dup.
Methods: We included patients with 3q29dup identified by array-CGH and localized from 194 to 198 Mbp. Clinical features and cytogenetic data were collected using a form fulfilled by genetic clinicians.
Results: 16 patients from 9 French genetic centers have been included, with 9 males and 8 females, aged from 1 to 35 years of age. Duplications sizes range from 1.4 to 3 Mb. Six patients have an additional cytogenetic variation. The duplication was inherited in 7 patients. The phenotype is highly variable in our cohort, but main features are developmental delay (8/16), intellectual disability or learning disability (10/16), behavioral disorders (7/16). No other symptoms are predominant but are listed. One patient was asymptomatic.
Discussion: Results of this study are congruent with the unspecific neuropsychological syndrome described previously. This variation could be a susceptibility factor for neuropsychological disorders. Associated genetic variation may as well participate to the broad phenotype. Further studies are necessary to establish the pathogenicity of 3q29dup. In this way, we call for a collaboration to extend our cohort.

3.P23 Patterns of gene expression in iPS cell derived neurons obtained from affected and unaffected carrier of CNTN6 microduplication at 3p26.3

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Incompletely penetrance of copy number variations (CNVs) remains an urgent issue for genetic counselling. We aimed to compare the differentially expressed genes (DEGs) in neurons, derived from iPS cells of CNTN6 microduplication carriers, namely an unaffected father and his son with intellectual disability. Transcriptome analysis was performed on cortical neurons, differentiated from iPSC lines with CNTN6 microduplication (2 lines from father and son) and wild-type neurons (3 lines from 2 unrelated individuals). The microarray data were analyzed with the Limma R package (p-value < 0.05; expression levels differ more than 2 times). WebGestalt resource was used to DEGs functional enrichment analysis. Comparison with wild-type neurons revealed 3228 (1835 up- and 1393 downregulated) and 3522 (384 up- and 3138 downregulated) DEGs in the father's and son's cells, respectively, indicating a trend towards down-regulation of son's neuronal gene expression (89% of DEGs). Analysis suggested 4 types of transcriptional effects among DEGs in their neurons: common upregulated (47) and downregulated (784) genes, as well as genes with opposite direction expression changes: upregulated in the father's and downregulated in the son's cells (634), and vice versa (14). Common downregulated genes were enriched in the following GO categories: receptor localization to synapse, synapse organization, neurogenesis, behavior, etc. DEGs, changed their expression in opposite direction, were involved in cell cycle phase transition, DNA metabolic process, RNA processing, and cellular response to stress. Unique father's upregulated genes (1154) engaged in cytidine to uridine editing, regulation of interleukin-1 secretion, pyrimidine nucleoside catabolic process, regulation of viral genome replication, etc. Unique father's downregulated genes (595) were involved in trans-synaptic signaling. The study was supported by RFBR (№ 19-315-90105).

3.P24 DNA methyltransferase genes and congenital heart defects in Down syndrome

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Background: Approximately 50% of people with Down syndrome (DS) have congenital heart defects

(CHD), mostly septal defect. Better understanding of the etiology of CHD is fundamental to improve diagnosis, clinical management and counselling. The DNA methylation is proved to be related to CHD, especially during embryogenesis when cardiogenesis occur. DNA methyltransferases (DNMT) genes are essential for maintaining methylation and chromatin stability, as well as the establishment of de novo methylation patterns during gametogenesis and embryogenesis.
Aim: The aim of this study was to analyze the single nucleotide polymorphisms of DNMT genes: DNMT1 (rs2228611), DNMT3A (rs1550117) and DNMT3B (rs2424913, rs1569686) in DS participants according to CHD.
Method: A total of 190 participants with DS have been enrolled in this study. Genotyping of polymorphisms was performed using a combination of polymerase chain reaction and restriction fragment length polymorphism methods. Statistical significance was considered at $P<0.05$.
Results: CHDs was present in 45% (86/190) of participants. The frequency of alleles and genotypes (single or combinations) of DNMT gene polymorphisms was not significantly different between group with CHD and group without CHD ($P>0.05$).
Conclusion: The occurrence of CHD in the study group was in accordance with literature. The investigated polymorphisms of DNMT genes were not associated with the incidence of CHD among DS participants in our study.

3.P25 Complex 3-way balanced chromosomal translocation t(2;6;4) in a man with oligoasthenoteratozoospermia

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In this case report, we describe a 38-year-old-male with an unfulfilled desire to have children. The spermiogram of the patient showed a restriction of spermiogenesis in form of oligoasthenoteratozoospermia (OAT). It is one of the most common causes of male infertility. In order to clarify the reason for the OAT, chromosomal analysis from cultured peripheral lymphocytes was performed, and showed an unusual three-way balanced translocation t(2;6;4) in our patient. Subsequent FISH-analysis confirmed the cytogenetic result of the translocation. To narrow down and to identify possible relevant gene in the breakpoint regions, Bionano Genomics' optical mapping was used. Three-way balanced translocations are an unusual constitutional complex chromosomal rearrangements and can lead to infertility. This result is therefore compatible with the oligoasthenoteratozoospermia. It is to be expected that optical genome mapping technology provide more information about breakpoints in relevant genes; evaluation follows.

3.P26 10q26 deletion syndrome: a French cohort study

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10q26 deletion syndrome (OMIM #609625) is a rare autosomal dominant genetic disorder with about 100 patients reported. Most cases are sporadic. Global developmental delay, short stature, microcephaly and typical facial appearance with triangular face, large forehead, low-set malformed ears, hypertelorism, prominent nose and a thin vermilion of the upper lip constitute the main clinical features. The clinical spectrum is very heterogeneous and neurobehavioral manifestations, deafness, limb malformations, cardiac and urogenital abnormalities can be associated. Thus, patients with 10q26 chromosomal deletion need multidisciplinary management strategies from birth.
One of the main reasons for this heterogeneity is the variety of 10qter region chromosomal deletions summarized into the "10q26 deletion syndrome".
Various studies proposed critical regions to explain the main phenotype (Yatzenko & all. 2009; Choucair & all. 2015; Lin S & all 2016) or more specific

features (Vera-Carbonell & all. 2015; Choucair & all. 2015). In addition, these studies proposed about 20 genes of interest such as DOCK1 and FGFR2 to explain the different clinical features observed.
We report a French ACLF cohort from 10 centers presenting 10q26 complete or partial deletions (size: 64kb to 12.5Mb), complex chromosomal rearrangement and derivative chromosomes diagnosed using DNA-array. We studied cases from birth to adult ages with few prenatal cases. There were no significant discrepancies between sex distributions. Main 10q26 deletions were terminal deletions and 10q26.3 deletions.

3.P27 Single cell RNA sequencing analysis of a rare 45,X/48,XYYY syndrome highlights a function role of Y chromosome expression.

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A 14 year-old boy with height smaller than expected from mid-parental height, initially diagnosed with a primary empty sella syndrome, was incidentally found to have a mosaic triple Y syndrome (mos 45,X/48,XYYY) in standard chromosomal analysis. Further cytogenetics studies by interphase fluorescent hybridization in situ (FISH) confirmed the presence of aberration both in blood lymphocytes and fibroblasts, with the similar fraction (range: 47 - 61%) of mosaicism. Parents were reported to have normal karyotypes, however, father was diagnosed with asthenospermia. The boy was conceived through insemination procedure.
In addition to the standard cytogenetics, the molecular analysis with single-cell RNA sequencing (scRNA-seq) technique on the individual's peripheral blood mononuclear cells (PBMC) was performed. Based on the PBMC marker genes expression profiles, the subpopulations of B cells, CD4+ and CD8+ T cells, monocytes, natural killer cells and progenitors have been identified, while the expression of key Y-chromosome protein coding genes allowed us to distinguish the two types of cells: with or without chromosome Y. We demonstrate that the distribution of cells varied substantially between cell types, with the greatest frequency of cells without Y chromosome in B cells and monocytes. Differential gene expression analysis revealed 30 autosomal genes differentially expressed between cells with and without chromosome Y. The scRNA-seq results were confirmed with an independent droplet digital PCR method with AMELY and AMELX probes (Thermo Fisher).
The scRNA-seq results suggest that chromosome Y might have a function role, despite being considered as "genetic wasteland" over years.

3.P28 Cohen syndrome resulting from compound heterozygosity for a CNV and a SNV in VPS13B gene in a child with intellectual disability

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Cohen syndrome (OMIM 216550) is a rare autosomal recessive disorder with pronounced clinical heterogeneity. The VPS13B gene (OMIM 607817), which is involved in the development of ocular, hematological and central nervous systems, is so far the only gene known to be associated with the Cohen syndrome. Point mutations, large intragenic deletions and duplications have been described in this gene. We report on a 2 ½ -year-old male patient with moderate intellectual disabilities, delay in motor and speech development, autistic behavior, friendly character, joint hypermobility, muscular hypotonia and neutropenia with a history of recurrent infections. The boy is the second child of non-consanguineous and healthy parents of Albanian origin after a spontaneous uncomplicated delivery. GTG banding was initially performed and revealed a normal male karyotype. Methylation-specific PCR to rule out Angelman syndrome showed a normal methylation pattern. Microarray analysis discovered a 49 kb heterozygous deletion in the VPS13B gene on chromosome 8q22.2 encompassing exons 9 to 16 of the VPS13B gene. Subsequent next-generation-sequencing revealed a point mutation in exon 46 in the second allele of the VPS13B gene leading to a stop codon. Examination of the parents follows. Our case presents an example of a recessive disorder due to compound heterozygosity for a CNV and a SNV. It shows that rare benign copy number variants can

underly autosomal recessive disorders and lead to disease in the homozygous state or in compound heterozygosity with another mutation, and underscores the importance of detailed clinical information. These findings have important consequences for the diagnosis of patients with genetic disorders.

3.P29 Characterization of small supernumerary marker chromosomes in individuals without phenotypic abnormalities

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Introduction: The frequency of small supernumerary marker chromosomes (sSMC) is in around 0.044% of newborn children.
Materials and Methods: In total, 39 sSMC carriers, 23 women (59%) and 16 men (41%) were identified among clinically healthy patients studied cytogenetically due to fertility problems. Multicolor FISH, centromere-specific M-FISH, FISH analysis with corresponding CEP, WCP DNA probes and also Acro-P probe were used to characterize the sSMC.
Results: In 90% cases (35/39), sSMCs originated from an acrocentric chromosome. Most of them were bisatellite dicentric sSMCs in the form of inverted duplications. About half of them (17/35) were derived from chromosome 15. The rest contained pericentromeric heterochromatin of chromosomes 14 or 22 (11/35 cases) and 13 or 21 (5/35 cases). The exception was two cases of sSMC, when the derivatives of chromosomes 21 and 22 contained one centromere and were small ring or min chromosomes. Derivatives of non-acrocentric chromosomes were identified in 10% cases (4/39). Ring chromosomes originated from chromosomes 6, 8, 9 and 16.
Conclusions: Thus, sSMC are a morphologically and genetically heterogeneous group of chromosomal aberrations, the identification and characterization of which is possible only using an complex molecular cytogenetic approach.

3.P30 A rare case of 5q22.3q31.1 interstitial deletion in a boy with obesity and hypogonadism

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Background:
Constitutional 5q interstitial deletion syndrome is a rare condition with <50 cases reported in the literature. The frequent features of this syndrome are developmental delay, failure to thrive, facial dysmorphism, osteoarticular, cardiac, renal and cerebral malformations, but no consistent genotype-phenotype correlation has been established.
Aim:
To present a patient with a large interstitial deletion 5q22.3q31.1.
Case report
An 11-year-old boy was referred for karyotypic analysis due to obesity, gynecomastia, hypoplastic external genitalia and hypogonadism.
Methods/Results:
Cytogenetic analysis was performed on peripheral blood lymphocytes by GTG banding. Analysis revealed a male karyotype with an interstitial deletion of the long arm of one chromosome 5 in all metaphases examined [46,XY,del(5)(q23.1→23.3)]. Further molecular karyotypic analysis using the 8x60K G3 ISCA V2 CGH microarray platform for precise identification of the deleted segment breakpoints confirmed a 16.8Mb deletion of the 5q22.3–q31.1 chromosomal region. The most important genes included in the deleted segment are: ALDH7A1, PHAX, MARCHF3, MEGF10, PRRC1, CTXN3, SLC12A2, FBN2, SLC27A6, CHSY3, HINT1, LYRM7, RAPGEF6, FNIP1, ACSL6, IL3, CSF2.
Conclusions:
A patient with a heterozygous deletion of the 5q22.3–q31.1 region who does not exhibit the typical features of 5q interstitial deletion syndrome is described. Although the deleted region overlaps with the common deleted regions in patients with severe clinical characteristics, our proband had a milder phenotype indicating that the size of the deletion is not always associated with the severity of the phenotype. In only 3 of the reported cases, patients had hypoplastic genitalia along with other symptoms. Thus, haploinsufficiency of genes in the deleted segment does not seem to play a critical role in determining the karyotype–phenotype correlation and probably other factors (eg. modifier genes) may be responsible for the phenotypic variability of the syndrome.

3.P31 12q21 interstitial deletions: seven new cases

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Interstitial deletions in 12q21 have been described in less than one dozen patients and are all non-recurrent CNVs. The 12q21 region spans 21,2 Mb and, though with a gene density lower than the entire chromosome 12 (2.9 vs 8.0 coding genes/Mb), includes 61 genes out of which 9 with haploinsufficiency prediction score (pLI) ≥0.9 and 15 cataloged as morbid genes. Despite common features, such as cognitive impairment, congenital malformations and ectodermal defects, the clinical presentation is characterized by wide clinical expressivity depending on size and location of the deletion and involved genes. In most cases the deletions are large making difficult to establish the causative genes of individual phenotypic signs. Recently a critical region in 12q21.2 has been narrowed down to 1,6 Mb, including four genes: SYT1, PAWR, PPP1R12A and OTOGL, out of which SYT1 encoding a highly conserved synaptic vesicle protein and PPP1R12A a regulatory subunit of myosin phosphatase with a role in neurotransmitter release, appear the major candidates for developmental delay, genitourinary congenital anomalies and ectodermal anomalies. A distal critical region, in 12q21.33, including the four DCN, KERA, LUM and EPYC genes defines the contiguous gene deletion syndrome PACD (posterior amorphous corneal dystrophy). We describe seven new patients with deletions detected by array-CGH through a cooperation primed by the Cytogenomics Working Group of the Italian Society of Human Genetics. The involved regions spanning 1 to 18 Mb are differentially positioned from 12q21.1 to 12q21.33. Three deletions overlap the critical region in 12q21.2, confirming its potential causality for the aforementioned clinical features, while the others are differentially associated to clinical signs such as hearing loss and craniofacial dysmorphisms (prominent forehead, broad nose, wide mouth and ogival palate). The combined genotype-phenotype of our patients, those in the literature and DECIPHER, allows to associate the deletion of specific 12q21 genes with distinct phenotypic traits.

3.P32 Homozygosity in 22q in blood associated with mosaic trisomy 22 in skin biopsy in a female patient with puberty delay and hypergonadotropic hypogonadism - a case report

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Full non-mosaic trisomy 22 is common in spontaneous miscarriages accounting for 3% to 5% of all miscarriages. In contrast, mosaic trisomy 22 is generally compatible with life with a limited number of patients reported. The phenotype is variable and mainly characterized by pre- and post-natal growth restriction, developmental delay, microcephaly, cardiovascular abnormalities, genito-urinary tract anomalies and dysmorphic features. The diagnosis can be difficult due to the presence of abnormal cells lines only in some tissues. Normal euploid blood lymphocytes analysis is frequent and the mosaic status and rate of abnormal cells vary in different tissues.
We describe a 17-year-old girl referred for delayed puberty and hypergonadotropic hypogonadism. A mosaic Turner Syndrome was clinically suspected. Additional clinical features were: mild facial dysmorphic features, bilateral cubitus valgus, hallux valgus and genu valgum, hypoplastic 2nd right toenail and 4th left toenail, some linear pigmentary changes along the Blaschko lines especially on the upper limbs, ventricular septal defect and scoliosis due to right supernumerary hemi-vertebra L3. No cognitive or developmental delay were noted. The MRI showed infantile uterus and ovary agenesis. A SNP-array was performed and showed a loss of heterozygosity of 14Mbs in 22q12.3q13.33 due to non-allelic homologous recombination. FISH analyses on hyper- and hypo-pigmentary skin biopsy showed mosaic trisomy 22 in 22% and 26% of cells, respectively. The mosaic occurred following trisomic rescue.
In conclusion we report on a 17 YO female patient with puberty delay, hypergonadotropic hypogonadism, infant uterus and ovary agenesis without several frequent features such as pre- and post-natal growth retardation, developmental delay and microcephaly. Cytogenetic work-up allows the detection of a segmental homozygosity in 22q in blood lymphocytes and mosaic trisomy 22 in hyper and hypo-pigmentary skin regions. This case highlights the variability of the phenotype in patients with trisomy 22 and the importance of cytogenetic analysis on skin biopsy in patients with suspicion of mosaic chromosomal anomaly.

3.P33 Genomic profiling in a group of Romanian patients with autism spectrum disorders

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Autism spectrum disorders (ASDs) represent a group with high clinical and biological heterogeneity, characterized by a complex genetic architecture with a wide spectrum of genetic defects. The combined approach of high-resolution screening for copy number variants (CNVs) and targeted testing for monogenic defects increased the genetic diagnostic yield in recent years.
We report the results of array-based genomic comparative hybridization (array-CGH) and methylation specific multiplex ligation-dependent probe amplification (MS-MLPA) testing, in a group of 70 ASDs patients.
The patients, 52 males and 18 females, were referred to our laboratory for genetic testing with ASDs as main phenotypic feature accompanied by different other clinical problems, including developmental delay, epilepsy, congenital malformations. The clinical evaluation included neurological, psychiatric and psychological evaluations completed with specific ASDs tests (ADOS, ADI-R). Array-CGH with 4x180K platforms (Agilent Technologies) was performed in all patients; MS-MLPA was used for aberrant methylation detection of FMR1 and AFF2 genes (MRC Holland) in male patients only.
Five pathogenic and three likely pathogenic CNVs were detected in our group, varying in size from 28 Kb to 16.8 Mb (median of 2.37 Mb). Many of these CNVs involve well-described syndromic regions (deletions of 1q21.1, 3q13.31, 2q23.1 including MBD5 gene, 11q24). Two samples had an abnormal methylation status of FMR1 gene indicative of a full mutation for Fragile X syndrome.
Our study illustrates the utility of combined genomic profiling and FMR1 MLPA screening in the investigation of patients with ASDs, especially in cases with a complex phenotype, and brings new phenotypic and molecular data that may contribute to the delineation of rare clinical entities associated with autism.
Funding: The research leading to these results has received funding from the EEA Grant 2014-2021, under the project contract No 6/2019.

3.P34 Clinical imaging and genetic studies in a pediatric brain heterotopia cohort

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Brain heterotopia (BH) is a rare developmental disorder caused by neuronal migration anomalies. These conditions have multifactorial etiology, including genetic and environmental, and are associated with severe forms of epilepsy and intellectual disability (ID). In this study we present the results of clinical and genetic investigation in 18 patients with BH.
General clinical examination, neurologic, dysmorphologic, psychiatric/psychologic evaluations and MRI were performed for all the children. Array based comparative genomic hybridization - array-CGH (Agilent Technologies) was used for all patients; Sanger sequencing (ABI 3500 Genetic Analyzer) of DCX and FLNA was performed for two and three patients, respectively.
MRI detected subcortical band heterotopia in two patients and periventricular nodular heterotopia (isolated or associated with other structural defects) in 16 patients. Most of the patients (16 cases) were referred for epilepsy with or without ID.

A pathogenic frameshift mutation was detected in DCX gene (NM_001195553.2:c.478ins; NP_001182 482.1:p.Glu160fs) in a female patient with sub-cortical band heterotopia. No FLNA mutations were identified in our group. Two genomic imbalances with potential clinical significance were identified, dup(22q11.2) and del(7q35) including CNTNAP2 gene. We found no previous reports of heterotopia associated with these two CNVs; however, taking into account the neurodevelopmental roles of some of the genes involved, their contribution to heterotopia pathogenesis worth further analysis.
Our study brings new data on the clinical features and epilepsy phenotypes. Both genomic imbalances and gene mutations were detected in our patient group; two CNVs not reported by date in heterotopia patients were found. Brain imaging and genetic studies were instrumental in the diagnostic and patient care algorithm.
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3.P35 Dicentric Y chromosome mosaic with different cell lines in a male with sexual ambiguity

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Dicentric Y chromosomes are common structural rearrangements observed in Y chromosome. They are very unstable during mitosis due to the presence of two centromeres, being, most of the times, found in mosaic with a 45,X cell line. Depending on the location of the breakpoint and the proportions of the mosaicism, phenotypic manifestations, especially due to the process of gonadal development, range from infertile male to Turner Syndrome or individuals with ambiguous genitalia.
The present study reports a case of a male with sexual ambiguity, mild cognitive delay, dysmorphisms, gynecomastia, short stature and brachydactyly. Genetic analysis by arrayCGH 180k, cytogenetic analysis and FISH, revealed the presence of a mosaicism involving a 45,X cell line and at least four cell lines presenting different dicentric Y chromosomes with the breakpoints in terminal segment of the short arm. The break and fusion in this region result in a duplication of almost all the Y chromosome in the dicentric rearrangements found, but with different deletions and duplications in the breakpoints.
Although isodicentric and dicentric chromosomes of the Y chromosome are frequently observed, only a few reports refer to different cell lines with different derivatives of the Y-chromosome rearrangement.
The location of the break and fusion of dicentric Y are variable and determine the Y material duplicated, leading to highly variable duplications and deletions of the Y chromosome.
Sexual development variability in these cases depends on the structure of the dicentric chromosome and the distribution of the different cells lines on the various tissues.
The combination of classical and molecular cytogenetic analysis and genomic technologies is important to characterize the rearrangement, providing information to better understand this structural chromosomal abnormality and to establish the genotype-phenotype correlation for genetic counselling.

3.P36 Using FISH for characterization in detail of small supernumerary marker chromosomes in patients without phenotypic features

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Small supernumerary marker chromosomes (sSMC) occur with a frequency of 0.044% in the human population; 70% of carriers have no clinical manifestations. sSMC are often found in mosaic formClinically normal sSMC carriers can be a model for characterizing the length of the non-dose-sensitive pericentric regions of the human genome. We formed a group of patients without phenotypic features with sSMC(15) (n=5) and sSMC(22) (n=7) mainly in a mosaic state. The sSMC have been characterized as inv dup(q11.1) using FISH with CEP DNA probes for chromosomes 15 and 22, as well as M-FISH. Considering that the majority of our patients were mosaics, it is not advisable to carry out chromosome microarray analysis in order to study copy number of the pericentric euchromatic material. FISH using homemade DNA probes 30 kb in size on the chr15(hg19):22,907,175-22,935,737,chr22(hg19): 18, 351,035-18,378,305 (proximal probe) and chr22 (hg19):19,076,741-19,107,497 (distal probe) regions was performed on metaphase chromosomes. DNA probes were prepared using LR-PCR followed by nick-translation labeling. The study region of chromosome 15 was not detected in all clinically normal sSMC(15) carriers. Six out of seven

sSMC(22) carriers contained only pericentric heterochromatin. In the remaining one case, the pericentric euchromatic material with a size of 470 kb (proximal probe) was present in the sSMC(22), however, no hybridization signal was detected for the distal probe. We assume that the presence of 470 kb triplication in the genome, localized in the proximal pericentric region of 22q (17,900,000–18,378,305), is not associated with an abnormal phenotype. We hypothesize that this region does not contain potentially dose-sensitive genes or that there may be a positional effect.

3.P37 NFIB associated intellectual disability and or speech delay first report of two novel structural variant disruptions

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The nuclear factor I (NFI) genes encode a family of transcription factors essential for multi-organ development during embryogenesis. NFIA and NFIX have been implicated in abnormal clinical phenotype manifestation. NFIB haploinsufficiency, caused by microdeletions and point mutations, has only recently been reported in patients with variable intellectual disability, macrocephaly, motor and speech delay. However, NFIB disruptions caused by structural variants have not been reported.
Here we report two independent cases with NFIB disruptions that were identified through low-coverage whole-genome mate-pair sequencing (WG-MPS). Specifically, WG-MPS was applied to map break-points in a female with dysmorphic facial features, speech delay and a balanced t(4;9)(q26;p24)dn (patient 1), and a male with intellectual disability and an inv(9)(p22q21.2) inherited from his father with developmental and speech delay (patients 2,3). PCR primers flanking the predicted rearrangement junctions and Sanger sequencing were used to potentially identify clinically-relevant genes at the breakpoint sites.
The rearrangement breakpoints were refined to the base-pair level in all affected individuals. The derivative 9 breakpoint (chr9:14104379-14104385) in patient 1 directly disrupted NFIB intron 10, while the inv(9) breakpoint (chr9:14451707-14451711), identically found in patients 2,3, mapped ~138kb upstream NFIB (NM_001190737.2) (hg38). The remaining breakpoints were located in intergenic regions.
In conclusion, this study reports for the first time two cases with overlapping phenotypes carrying structural variants that disrupt directly or indirectly the NFIB gene. Future functional studies will define the underlying molecular mechanisms and further support the impact of these novel findings, thus expanding the current literature on the heterogeneous pathogenicity of NFIB variants.

3.P38 Diagnostic odyssey of a muscular dystrophy with a childhood onset in a female with an X autosome translocation

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Background: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive neuromuscular disease. DMD generally follows a severe course in boys, while girls rarely show symptoms. One of the few exceptions for this is X; autosome translocation involving dystrophin gene locus. Females carrying balanced X;autosome translocations are a clinically heterogeneous group of patients, ranging from phenotypically normal women to history of recurrent miscarriage, gonadal dysfunction, congenital abnormalities or developmental delay.
Material and methods: The patient was referred to our centre for premarital genetic counselling at age of 24 years after a long diagnostic odyssey. She presented with severe muscle weakness, gait disturbance with childhood onset at the age of 10 years and was suspected to suffer from muscular dystrophy disease. Final diagnosis was based on clinical symptoms, family history, biochemistry markers, electromyography, echocardiography, pathology, molecular genetic analysis and cytogenetic testing.
Results: Creatine kinase level and transaminases levels registered high values. EMG was consistent with a myopathic process. Two muscle biopsies of the right gastrocnemius and deltoid revealed dystrophinopathy. Echocardiogram showed mild systolic dysfunction of left ventricle. MLPA for exons of the dystrophin gene, combined with a muscle disease NGS panel, WES and WGS were applied and all results showed no mutations. Finally karyotype analysis in our genetic centre revealed a de novo balanced translocation between chromosomes 4 and X, the exchange point was located in band p21 of

the X chromosome. Patient features were attributed to the breakpoint being in the dystrophin gene.
Conclusions: When no mutation is detected in girls by Duchenne/Becker muscular dystrophy karyotype analysis can help to identify the chromosomal abnormality responsible for the muscular disease. The collaboration between molecular biologists and cytogeneticists during the diagnostic process and counselling is mandatory.

3.P39 Exceptional finding A phenotypically normal female diagnosed as natural chimera with XXY/XX

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Chimera is an organism harboring cells from two or more zygotes. Different mechanisms lead to natural chimeras such as feto-maternal placental cell trafficking, fusion of two zygotes or transfer of cells between dizygotic twins via fused placentas. We report a case of a 28-year-old phenotypically normal female who was referred together with her husband, the latter diagnosed with asthenoteratozoospermia, for routine karyotyping prior to artificial reproduction. Vaginal ultrasound showed normal ovaries and uterus. Hormonal profile was within normal limits (LH, FSH, testosterone, androstendion, estradiol, progesterone, AMH, SHBG). Conventional cytogenetic analysis from cultured lymphocytes revealed a mosaic karyotype 47,XXY/46,XX. Targeted FISH confirmed structurally normal Y- and two X-chromosomes in XXY-cells (LSI-SRY (sex determining region of Y, Vysis) and CEP X (DXZ1, Vysis). Another blood sample was taken and FISH was performed with the centromeric probes DYZ3 and DXZ1 (Cytocell) reconfirming the coexistence of a Klinefelter cell line and a normal female cell line in the patient's peripheral blood. A STR multiplex system analysis (PowerPlex-Kit, Promega, 21 marker) showed a strong X-chromosome and a weak Y-chromosome signal. In the patient's peripheral blood there were 2 normal alleles and 2 small alleles in 4 markers and 3 alleles in 5 markers indicating chimerism.
Taken together, we assume our female patient to be a natural chimera with XXY/XX in peripheral blood. Few cases about human chimera have been reported and little is known about potential consequences regarding health and reproduction making genetic counseling challenging. Since the presence of Y-chromosome might increase the risk of gonadal tumors, further work-up is recommended to screen for possible gonadoblastoma and ovotestes.

3.P40 Growth hormone therapy in a male patient with growth hormone deficiency short stature isodicentric Y chromosome and three copies of SHOX gene. A clinical case report.

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A 12-year-old male patient was referred to the Genetic Outpatient Clinic due to short stature, microcytosis, anxiety disorder and a familial history of mental disorders (depression, anxiety disorders, adjustment disorders). The patient experienced perinatal complications and scored 2, 4, 7 in APGAR scale after 1, 5 and 10 minutes, respectively. A physical examination revealed non-specific, discrete dysmorphic features. In the array-based comparative genomic hybridization (aCGH) study, a genomic imbalance in the form of a 28.32 Mb duplication of the Y chromosome was found, covering Yp11.32-q11.23 bands and a 269.55 kb deletion of the long arm fragment of the Y chromosome, covering the q12-qter bands (human pseudoautosomal region 2). Additional cytogenetic studies revealed an abnormal male karyotype: 46,X,idic(Y)(q11.23). The multiplex ligation-dependent probe amplification (MLPA) technique confirmed the presence of 3 copies of the SHOX gene and its regulatory regions. On the basis of endocrinological findings (decreased secretion of growth hormone (GH) at night and in stimulation tests and a decreased IGF-1 level in serum), the patient was diagnosed with growth hormone deficiency (GHD). Thyroid gland disorders were excluded. Neither was cortisol deficiency identified. A magnetic resonance imaging (MRI) scan of the pituitary gland did not show any organic changes or disorders in the gland structure. The Whole Exome Sequencing (WES), performed both in the patient and in his parents, did not reveal any additional information about the molecular basis of the presented symptoms. The patient was qualified for a recombinant human GH therapy. This poster presents a detailed genetic evaluation and an initial treatment response.

3.P41 Xp22.31 microduplications involving STS, VCX and PUDP genes

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Introduction:
STS gene deletions are most commonly associated with X-linked ichthyosis. When the deletion involves additional genes, patients may show features of a contiguous gene syndrome including attention deficit hyperactivity disorder (ADHD), communication deficits, and autism spectrum disorder (ASD). The phenotypes associated with Xp22.31 microduplications are not so clear and CNVs in this region have been interpreted either as normal variants, as pathogenic or as variants of unclear clinical significance. Here we present four cases of Xp22.31 microduplications from our Department.
Material and methods:
Array Comparative Genomic Hybridization (aCGH) was performed on DNA samples from four patients (2 female and 2 male) using Agilent 4x180K/8x60K microarrays platforms according to manufacturer's recommendation. Results were analyzed using cytogenomics software (v2.9.2.4/v4.0.3.12; Hg19-GRCh37). HUMARA assay was used for X-chromosome inactivation study.
Results:
Four patients with a Xp22.31 duplication were identified, three with 1,6Mb CNVs overlapping 6467006 to 8131810 genomic positions including STS, VCX, PUDP, MIR4767, PNPLA4, MIR651 genes, and one with a slightly larger duplication of 2,1Mb overlapping 6081799-8164803 genomic positions and including beyond the previously mentioned genes NLGN4X, MIR4770, VCX2 and VCX3A genes. The study in female patients showed random X-chromosome inactivation in one and inconclusive in the other because she was homozygous for the STRs used. Phenotypes included intellectual disability, language delay, development delay, short stature with body disproportion, ASD and ADHD.
Discussion and Conclusions:
Increased gene dosage of X-linked genes may have an impact in the deregulation of normal cognitive development. However, mechanisms such as incomplete penetrance, variable expressivity and, in female patients, skewed X-inactivation may influence the clinical expression. Although we cannot completely exclude that Xp22.31 is a rare population variant, our data, as well as previous reports, suggests an association of duplications at this chromosomal region with neurodevelopmental disorders.

3.P42 22q13 deletion syndrome: a case report

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Introduction: The 22q13 deletion syndrome is a neurodevelopmental disorder characterized by neonatal hypotonia, global developmental delay with intellectual disability of varying degrees, normal to accelerated growth, severely delayed or absent speech and minor dysmorphic features. Some cases have autism and aggressive behavior.
The authors present a de novo 22q13 deletion case.
Clinical Report: 2-year-old boy with polymalformative syndrome, psychomotor development delay, hypotonia, hyperlaxity, deafness, VUR grade 4 with D-edge atrophy, peculiar facies. He was the first child of non-consanguineous couple, with apparent cognitive limitation.
Blood culture and cytogenetic analysis were performed according standards protocols. Cytogenetics analysis revealed a 22q13 deletion in all metaphases analyzed. The karyotypes of the parents were normal. Array Comparative Genomic Hybridization (aCGH) technique revealed a 7,809Mbp deletion in 2q13.2q13.33 region, comprising SHANK3 and 110 more genes.
Discussion: The present case is a de novo 22q13 deletion. The boy has some dysmorphic features and global developmental delay consistent with 22q13 deletion syndrome.
Despite the loss of 111 genes, the haploinsufficiency of SHANK3 gene is the cause of the major neurological features associated with deletion 22q13. This gene is located in 22q13.3 and has a role in synaptogenesis, in synaptic plasticity and in the regulation of dendritic spine morphology.
Every new case of a rare chromosomal alteration should be reported in order to obtain a more precise genotype/ phenotype correlation, improving risk evaluation and genetic counselling.

3.P43 First reports of incomplete penetrance in Xq28 int22h 1 int22h 2 duplication male carriers: the importance of segregation studies

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Introduction:
Xq28 microduplication is the result of NAHR between int22h-1/int22h-2 repeats distant from 0.5 Mb. It is responsible for a syndromic intellectual disability with recurrent infections and atopic disease in males. Non-specific facial dysmorphic features and obesity have also been described. A minority of females with intellectual disability have been reported. To date, all reported evaluated males present mild to moderate intellectual disability so that penetrance is considered complete (El-Hattab et al., 2015 ; Ballout et al., 2020).
After a first puzzling observation of an unaffected male carrier, we decided to study the penetrance of this syndrome in males.
Material and methods:
Through a call for collaboration within the AchroPuce network, we collected 14 families from 7 French university hospitals.

Results:
Among these 14 families, the index case had inherited a Xq28 duplication from an unaffected parent in 12 cases (10 mothers, 2 fathers), inheritance was not known in 1 case and one carrier mother was reported with borderline IQ. Interestingly, segregation studies revealed 3 other unaffected carrier males. Overall, excluding all the index cases referred for cytogenetic microarray, we identified five unaffected carrier males.
Discussion:
This limited number of cases suggest previously undescribed incomplete penetrance, or extremely low expression in Xq28 carrier males. Three disease causing-genes are present in this interval (F8, RAB39B and CLIC2). Haemophilia was never described. Both RAB39B and CLIC2 are related to neurodevelopmental disorders with literature data suggesting an impact on neuronal branching. Hypotheses of a second-hit or modifying (polygenic, environmental) factors are still to be studied but a possible observation bias, with rare CNVs identified in affected patients referred for testing, should be kept in mind in such challenging X-chromosome copy number gains. Segregation studies could help to quantify this newly described incomplete penetrance to adapt genetic counselling.

3.P44 22q11 genetic compensation: 2 new cases

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Calmel et al (2009) reported the case of a father with genetic compensation a 22q11 deletion on one copy of chromosome 22 and a reciprocal 22q11.2 duplication on the other copy of chromosome 22. We report 2 new cases of such a compensation.
Prenatal diagnosis was carried out on amniotic fluid was referred for fetal microcephaly, and hygroma. ARRAY-CGH showed a pathogeneous 1.417Mb 22q11.21 microdeletion between LCR22A and B. FISH using TUPLE1 probe confirmed the deletion on one chromosome 22. At 3 years of age this child showed a bilateral deafness, langage delay, growth delay, and atrial septal defect (ASD).
Analysis of the parents showed a maternal 22q11 deletion between LCR22A and B and surprisingly a paternal 22q11 duplication between LCR22A and D,
A second child was born at 32WG with cleft palate and ASD. Using ARRAY-CGH a duplication between LCR22 B and D was observed. This CNV is the result of the combination of the maternal 22q11 deletion between LCR22A and B and the paternal 22q11 duplication between LCR22A and D, as confirmed by FISH.
A third child was born with the same compensation at 39WG, with birth weight 2.460kg, and OFC 31,5cm. This child at 6 months of age has thymus hypoplasia, microretrognathia, ASD, and cleft palate. ARRAY-CGH showed a 745kb duplication between LCR22B and D.
This report gives a new illustration of the genetic compensation in 2 children with duplication and deletion in 22q11 region. It confirms if needed the use of FISH in genetic counselling.

3.P45 Williams Beuren syndrome: A case report and review of the literature

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Williams-Beuren syndrome is a rare genetic disorder with an estimated prevalence of 1/7500 to 1/20000. It is linked to the 7q11.23 microdeletion, and is characterized by a recognizable clinical phenotype. The objectives of this case report are to describe the clinical, paraclinical and cytogenetic characteristics of this rare disease.
This is a case report of a 10-year-old female patient with a family history of recurrent spontaneous abortion in the mother and a paternal cousin followed for an intellectual disability. The patient presented a retarded psychomotor development, associated with facial dysmorphia. The history revealed the notion of chronic vomiting in the neonatal period, strabismus, precocious puberty, and behavioral disorders, such as hypersociability. A FISH analysis was performed, for suspicion of Williams-Beuren syndrome, and the diagnosis was confirmed. The malformative assessment did not reveal any other abnormalities, in particular no cardiovascular abnormalities.
Williams-Beuren syndrome is a microdeletion syndrome, characterized by a high genotype-phenotype correlation. The testing for the 7q11.23 microdeletion in the parents is necessary to determine whether the anomaly is de novo or inherited and for appropriate genetic counseling..

3.P46 Primary amenorrhea and a reciprocal X autosome translocation: A case report and review of the literature

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Primary amenorrhea is defined as the absence of menarche by the age of 16. It can be isolated or associated with the lack of development of secondary sexual characteristics.
We report the case of a patient referred for primary amenorrhea, who was found to have a reciprocal chromosomal translocation.
The patient was a 19-year-old woman with a family history of recurrent spontaneous abortion in the mother; her maternal cousin had psychomotor retardation. The clinical examination showed the absence of development of secondary sexual characteristics, without any other signs. She was a carrier of a reciprocal translocation: 46,XX,t(X;4)(q21?;q21).
Primary amenorrhea may be isolated or may be associated with other clinical signs. The etiological diagnosis is necessary to allow therapeutic management. If a structural chromosomal abnormality is discovered, the study of the karyotype of the parents is indicated, in order to determine whether the abnormality is de novo or inherited and for appropriate genetic counseling.

3.P47 Designing a Modern Cytogenetic Array and the Benefits of Using a SNP Array Backbone for Cytogenetic Testing

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Cytogenetics Labs want to increase performance and value in their testing pipelines but need guidance on selecting the best platform for their needs. With decreasing costs, whole genome sequencing (WGS) is gaining interest as a platform for copy number variant (CNV) detection but high-density SNP array is currently the most commonly used platform for cytogenetic testing. SNP arrays have an advantage with the ability to robustly detect allelic aberrations, such as regions of homozygosity (ROH), which is coverage dependent for WGS.
We generated a tiered set of genes and regions that are clinically important by surveying prominent cytogenetic laboratories across the globe and cataloging current information for clinical resources, such as ClinGen, OMIM, and DDG2P. For the comparative analysis, we contrasted the probe content of several commonly used arrays for cytogenetic testing to assess the ability to resolve aberrations in these critical areas. The arrays used in the comparison include the popular CytoScan HD offered by Thermo Fisher/Affymetrix, as well as the Global Screening Array (GSA) and the Global Diversity Array (GDA) from Illumina, which offer low-cost options. As each technology uses different types of “probes” we established a uniform measure to compare the different technologies. The statistical

analysis includes a comparison of the inclusion of high minor allele frequency SNPs for the detection of ROH events, probe coverage of the functionally important areas of the genes (canonical exons of genes), and probe distribution genome-wide over these tiered genes and regions. Here we present the results assessing how the arrays compare to each other and to WGS in terms of both coverage and cost. In addition, we looked at the benefit of having SNV and CNV data generated by the same array for the same sample to determine UPD status.

3.P48 Constitutional Ring chromosome 9: Ring formation mechanism revealed by whole genome sequencing and clinic-cytogenomic correlations reviewed from literature

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Constitutional ring chromosome 9 (r(9)) is a rare chromosomal disorder. Cytogenomic analyses and whole genome sequencing (WGS) were performed in a case of r(9). The karyotype showed a mosaic pattern of r(9) and monosomy 9 in 83% and 17% of cells, respectively. Array comparative genomic hybridization detected subtelomeric deletions of 407 Kb at 9p24.3 and an 884 Kb at 9q34.3, and an interstitial duplication of 5.879 Mb at 9q33.2q34.11. WGS revealed that the double strand beaks (DSBs) at ends of 9p24.3 and 9q34.3 looped and anchored by inverted repeats at ends of 9q33.2q34.11 and then microhomology mediated replication and ligation formed this r(9). This is the first r(9) analyzed by WGS to delineate the mechanism of ring chromosome formation. The loss of telomeres by subtelomeric DSBs triggered inverted repeats guided intra-strand annealing, then microhomology mediated replication resulted in the formation of this r(9) with distal deletions and an interstitial duplication. Review of literature found 29 reported cases of r(9) but only seven cases and the present case had clinical and cytogenomic findings. Variable phenotypes of r(9) cases involving critical regions and genes of DMRT, SMARCA2, DOCK8, CER1, FREM1 at 9p24.3 for 9p deletions and the EHMT1 gene for 9q34 deletion syndrome were summarized. Clinico-cytogenomic correlations from these eight cases were documented in the online Human Ring Chromosome Registry (http://web.gxmu.edu.cn/shengwu/HRC/home.asp); this interactive resource could provide information for cytogenomic diagnosis, genetics counseling and clinical management.

3.P49 Copy number variations in a Hungarian pediatric patient cohort and novel potentially pathogenic variants of unknown significance

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Neurodevelopmental disorders are overall frequent and genetically heterogenous pediatric conditions. In the past decade the first tier diagnostic method for uncovering copy number variations (CNVs), possibly the most common genetic etiology in affected individuals, has been array comparative genomic hybridisation (array CGH). However, in Hungary this methodology is not yet a routine cytogenetic test. In a retrospective study we analysed clinical data of the first, relatively large Hungarian cohort of patients whose genetic testing included array CGH.
The 89 children were analysed using various array CGH platforms, results were confirmed using either fluorescence in situ hybridization or polymerase chain reaction. Phenotypes of patients with pathogenic variants were compared to patients with negative results using the chi squared/Fisher exact tests. A total of 36 pathogenic CNVs were identified in 30 patients (33.7%). Macrocephaly ($p=0.045$), abnormal height ($p=0.014$) and/or weight ($p=0.04$), short stature ($p=0.076$) and abnormal perinatal adaptation ($p=0.078$) were more likely to be associated with pathogenic CNVs. Conversely, errors of refraction were slightly more common in the negative group ($p=0.064$).
Variants of unknown significance (VUS; n=37) were found in 22 individuals. We present clinical and genetic data of three boy patients with potentially relevant VUS alterations: 1) a child with seizures, encephalopathy, seizures, syndactyly and coarse facial features carrying a duplication of chromosome 4q24 encompassing disease causing PPP3CA gene and another duplication of 20p11.23 encompassing SLC24A3; 2) a boy with complex neurodevelopmental disorders carrying a paternally (mildly affected) inherited duplication of the chromosome region containing SYNDIG1 gene (20p11.21); and 3) a boy presenting with a phenotype very similar to Leri-Weill osteochondrosis and carrying an Xp22.33 duplication that includes SHOX gene. Our results contribute to unraveling the diagnostic value of rare CNVs.

3.P50 Analysis of reciprocal chromosomal translocations involving chromosome 1 as a genetic factor of limited progeny survival in carriers

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Limited progeny survival is the major concern of couples with pregnancy failures. One of the most important causes of pregnancy loss are fetal chromosomal abnormalities. Parental carriers of reciprocal chromosomal translocation (RCT) are considered to be at high risk for miscarriages, therefore, the examination of familial karyotype is justified in such cases. We report on a couple referred for genetic counseling and cytogenetic evaluations after three miscarriages.
Proband's cytogenetic analysis using G-banding technique revealed a RCT of 46,XX,t(1;8)(q23;p21). The karyotype of proband's partner was 46,XY. The RCT was confirmed by fluorescent in situ hybridization (FISH). The same RCT pattern was identified in the karyotypes of proband's mother and sister who also experienced pregnancy failure. Based on available data, the probability rate for unbalanced karyotype of the offspring was estimated at 0,3% and the risk for miscarriages at 30%.
Additionally, we compared the clinical and cytogenetic data of our patients with those of 77 previously reported couples with RCT involving chromosome 1 (RCT-1). We concluded that RCT-1 carriership has a limiting effect on progeny survival. Furthermore, we observed a correlation between the length of the segment involved in RCT-1 and the type of pregnancy failure. The most common form of pregnancy failure in RCT-1 carrier families were miscarriages. In this report we also discuss the impact of RCT-1 on other types of pregnancy failures.
The RCT carriers need to be identified by classic cytogenetic analyses which are confirmed to be the first tier tool used in recurrent spontaneous miscarriages. Integrated application of classical and molecular cytogenetic methods could provide precise prenatal diagnosis and proper monitoring of all future pregnancies in the family.

3.P51 Cytogenetic evaluation of orofacial clefts

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Background: Orofacial cleft is one of the most common congenital anomalies of the face which includes cleft lip and or cleft palate that causes abnormal appearance of face. It leads to difficulty in speech and feeding, especially in newborns. Genetic and non-genetic factors may lead to orofacial clefts. An association of orofacial clefts to chromosome abnormalities is assessed in the study.
Methods: Conventional karyotyping was done in 133 patients of all the age groups with orofacial clefts to find out the frequency of chromosomal aberrations in orofacial clefts. The patients were referred from the department of Oral and Maxillofacial Surgery, MGM Dental College, Navi Mumbai. The project was started after consent from the patient and institutional ethical approval.
Results: It was found that in orofacial cleft patients, the incidence of chromosomal aberrations is 2.3 % and polymorphic variations is 3.9%. Out of 133 patients 47,XXY was seen in one case and trisomy 21 in two cases, polymorphic variations like pericentric inversion of chromosome 9, 16qh+ and 22 pstk+ were observed in one case each and 9qh+ was seen in 2 cases.
Conclusion: The incidence of chromosomal aberrations and polymorphic variations in patients with orofacial clefts is low. Molecular techniques may be used to further study the genetic components in patients with orofacial clefts.

3.P52 An unexpected karyotype in a newborn with ambiguous genitalia

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Introduction: Disorders of sex development (DSDs) are uncommon congenital anomalies in which there may be an ambiguous or intermediate condition between male and female phenotypes of the anatomical sex. Diagnosis of DSD in a newborn is very disturbing for the parents and even more so when it is associated with an unexpected, abnormal karyotype, resulting in a multisystemic syndrome such as Cri-du-Chat (OMIM #123450).
Material and methods: The authors present the case of a newborn referred for cytogenetic studies due to ambiguous genitalia (bifid scrotum), dysmorphic features (mild retrognathy) and a weak cry. Chromosome analysis with high resolution GTL-banding, obtained from cultured lymphocytes was performed. FISH studies included Cri-du-Chat syndrome specific probes (Cytocell) and 3p and 5p subtelomeric probes (Vysis-Abbot). MLPA technique was performed using panel P036-E1 Salsa® MLPA®

(MRC Holland) for the subtelomeric regions. Parental karyotypes were performed.
Results: The newborn's karyotype revealed a structurally abnormal chromosome 5 with both a deletion and additional material in the terminal band 5p15.2 of the short arm. FISH using Cri-du-Chat probes revealed a deletion and MLPA showed there was both a decrease of amplification in 5p subtelomeric region and an increase of 3p subtelomeric region. The mother's karyotype was an apparently balanced translocation 46,XX,t(3;5)(p26.2;p15.2), thus the proband's karyotype was interpreted as 46,XY,der(5)t(3;5)(p26.2;p15.2)mat.
Discussion: A rare case of a newborn presenting with both Cri-du-Chat Syndrome and partial trisomy 3p, whose main phenotypic features were ambiguous genitalia and minor dysmorphisms is reported. The authors enhance the importance of the combination of high resolution banding with appropriate molecular cytogenetic techniques in the characterization of chromosomal rearrangements, enabling better genetic counselling for patients and their families.

3.P53 An unusual proximal 15q deletion arising from a de novo unbalanced (13;15) translocation in a girl with West Syndrome

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Background: Unbalanced translocations, involving the long arm of chromosome 15 and another chromosomes are uncommon with only few reports in literature. Such rearrangements result in a karyotype with 45 chromosomes with monosomy of the proximal 15q imprinted region.
Clinical report: We report a case of a 6 months female, referred to genetic counselling because of West syndrome. She was born at 40 week's gestation to healthy non consanguineous parents after an uneventful pregnancy. The Apgar scores were 9 at 1 min and 10 at 5 min. Family history regarding early infantile deaths or hypotonia as well as maternal obstetric history was unremarkable. Her birth weight, birth length and head circumference were on the main curves. Physical examination showed facial dysmorphism, short neck, overriding toes and bilateral-club feet. Neurological examination showed axial hypotonia, severe developmental delay, reduced mobility, hyporesponsiveness, and weak primitive reflexes.
Results: A G-banded karyotype obtained from peripheral blood lymphocyte cultures revealed an unbalanced translocation involving chromosomes 13 and 15.
The parental karyotypes were normal indicating that this rearrangement occurred de novo: 45,XX,der(13)t(13;15)(q10;q15),–15dn [ISCN 2016]. FISH analysis performed with SNRPN/PML probe confirmed the deletion of the PWS/AS region on the chromosome 15.
Discussion: We report the first case of a de novo unbalanced translocation involving chromosomes 13 and 15, with an unusual deletion that encompasses the PWS/AS region. In order to characterize the paternal or maternal origin of the lost region, methylation-specific (PCR) will be performed for our patient. The contribution of the second chromosome involved in the rearrangement as well as the size of the deletion on chromosome 15 should also be accurately investigated by array CGH leading to better genotype phenotype correlations.

3.P54 Inherited "pure" 10p15.3p15.2 duplication rearrangement

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Introduction: Microarray-based comparative genomic hybridization (array-CGH) allows the identification of microscopic and submicroscopic chromosomal imbalances, yet it does not clarify the exact chromosomal location of copy number gains/losses. Several 10p partial trisomies have been published but most of these reports are based on patients with a contribution from another chromosome. However, "pure" 10p partial trisomies are rare events, and even rarer without the involvement of a second chromosomal region.
Material and methods: We report on a 7 years-old girl referred for karyotype and fluorescence in situ hybridization (FISH) studies due to a 3,5Mb terminal 10p15.3p15.2 duplication identified by array-CGH. She presents with moderate intellectual and development delay, facial dysmorphisms and a family history of learning disabilities. Chromosome analysis with high resolution GTL-banding obtained from cultured lymphocytes was performed in the proband and her parents. FISH studies included 10p and 2q subtelomeric probes (Vysis-Abbot).
Results: The proband's karyotype revealed a structurally abnormal chromosome 2 with additional material in the terminal band of the long arm. In the

abnormal chromosome, subtelomeric 2q FISH probe showed a normal hybridization, however an extra terminally located subtelomeric 10p15.3 FISH signal was seen, after the normal 2q subtelomeric signal. Proband's father revealed the same abnormal chromosome 2.
Discussion: Four 10p15.3 paternally inherited duplications have been published to date. Our case combines a terminal 10p15.3p15.2 duplication to be found in chromosome 2, which may be considered a "copy number variant", predisposing to a wide range of clinical abnormalities, but only further family investigations, combined with a literature review will allow a more detailed phenotype-genotype correlation for "not-so-pure" 10p partial duplications.

3.P55 Maternal interchromosomal insertion (21;1) leading to 1p22.1p21.3 duplication in three sisters

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Copy number gains involving 1p are rarely described. While a distinctive genotype-phenotype correlation has yet to be established, some clinical features are often present in 1p duplications carriers, such as intellectual disability (ID) and facial dysmorphisms. Here, we describe a familial 1p22.1p21.3 duplication, resulting from malsegregation of a maternal chromosome rearrangement.
A 10-year-old girl was referred to our genetic department due to ID, speech delay and facial dysmorphic features. Two younger sisters also presented with phenotype, one with ID and the other with autism and absence of speech. All three had deep set eyes, broad nose and thick eyebrows. Other three sisters were not available for evaluation. For the index case, a 6.46 Mb duplication was detected by arrayCGH, at 1p22.1p21.3 [arr[GRCh37] 1p22.1 p21.3(92235647_98698096)x3], encompassing 29 OMIM genes, including DPYD gene. Complementary cytogenetics studies, such as karyotype and fluorescence in situ hybridization analysis (FISH) were performed in the parents and evaluated sisters. Duplicated 1p22.1p21.3 region was found to be inserted on the proximal long arm of chromosome 21, as result of a maternally inherited interchromosomal insertion.
Retrospectively, only two clinically significant overlapping 1p22.1p21.3 duplications were reported, both sharing clinical similarities with the presented cases. Overall, global developmental delay, intellectual disability, speech delay, autism spectrum disorder and dysmorphic features were commonly described. Gene content for 1p22.1p21.3 region is poorly characterized with no identified triplosensitivity genes. Despite that, DPYD copy number variations were previously associated with neurological and behavioral phenotype. This familial report reinforces the need of further large screening patient cohorts with the described clinical features in order to provide further evidence of 1p microduplications associated phenotype.
In the new generation sequencing era, arrayCGH and FISH analysis still prove to be valuable tools in clinical genetic diagnosis.

3.P56 Mechanism of formation of a chromosome 18 with a complex rearrangement

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Chromosomal alterations involving a single chromosome, known as intrachromosomal rearrangements, include deletions, duplications, inversions, ring chromosomes, intra-arm rearrangements such as inv dup del, and other complex rearrangements. A number of mechanisms have been described to explain the formation of such rearrangements and they can be identified through the analysis of the DNA sequence at the breakpoints. In this study, we investigated a patient with neuropsychomotor developmental delay, intense hypotonia, epilepsy, microcephaly and dysmorphic features, with a rare intrachromosomal rearrangement in chromosome 18. Karyotyping and CytoScan HD Array (Affymetrix) revealed a 9.7 Mb duplication in 18p and a 25.8 Mb deletion in 18q, as follows: arr 18p11.32p11.22 (136226_9796835)×3,18q21.2q23(52032896_78014123)×1. Fluorescent in situ hybridization (FISH) with bacterial artificial chromosome (BAC) probes and whole genome sequencing (WGS) showed that the extra copy of the short arm could be found attached to the long arm in an inverted position, resulting in the following cytogenomic result: 46,XY,der(18)(pter→q21.2::p11.22→pter)dn. Custom array (Agilent), with enrichment of probes from chromosome 18, was also performed to determine with more precision the putative breakpoint and to facilitate primer design and Long Range PCR execution, with the following result: arr[hg19] 18p11.32p11.22(218740_9797900)×3,18q21.2q23(52036361_77954165)×1. Sanger sequencing of the segments around the breakpoints revealed a more complex rearrangement with the insertion of a 19-nucleotide sequence from a distal region of the long arm, which presented microhomology with both regions involved in the rearrangement. Based on this, we propose that the rearrangement was originated by a single event due to a DNA replication-based mechanism known as MMBIR (Microhomology-Mediated Break-Induced Replication), which can join

different regions of the genome after a replication fork rupture followed by strand switching in the replication process due to similar nucleotides present in these distinct regions. Financial support: São Paulo Research Foundation (FAPESP), Brazil.

3.P57 Clinical and cytogenetic investigation of 10q partial trisomy in a 3 year old male.

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Introduction: Mosaic trisomy 10q is an extremely rare chromosomal abnormality with a heterogeneous phenotype depending on the precise location and size of the duplicated region. It is mainly characterized by developmental delay, craniofacial dysmorphic features, cardiac, renal and skeletal abnormalities. In most cases partial trisomy 10q is a result of a parental balanced translocation or pericentric inversion including 10qter. The most frequent breakpoint is on 10q24 region.
Material and Methods:We report a new case of mosaic 10q trisomy identified in a 3 year-old male patient, the fifth child of two apparently healthy and unrelated parents, who presented with hypoplasia of the distal right upper limb in the forearm and the hand as well as hypospadias. The hypospadias was classified as a mild form where the urethral meatus is located distally on the penile shaft. Detailed examination of the urinary system did not reveal deformities. Abdominal ultrasound and cardio-vascular examination were also normal. The probands psychomotor development was compatible with his age. Conventional karyotyping and Array Comparative Genomic Hybridization(array CGH) analysis were performed.
Results: Cytogenetic analysis revealed a karyotype: 46,XY[20]/46,XY, add(10)(?::q26.1→qter)[10].
Array CGH analysis demonstrated duplication of a 2,494 Mbp on 10q26.13 region in all cells analyzed and a 10,769 Mbp mosaic duplication on 10q26.13 q26.3. Subsequent karyotyping of the parents for further investigation of the case was not possible because the child had been given for adoption.
Conclusion: The patient showed mild phenotypic features including defects of the limbs which is a characteristic manifestation of trisomy 10q25.2-qter. Cytogenetic and molecular cytogenetic analysis revealed one of the smallest aberrations in partial distal trisomy 10q syndrome described to date.

3.P58 Turner syndrome variants identified through karyotyping

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Background: Numerical, structural and combined chromosomal anomalies are known to be the genetic cause for Turner syndrome.The cytogenetic variants associated with this disorder have been described in literature.
Material. Method:Our study includes 38 Turner syndrome patients diagnosed through whole-blood karyotyping (GTG banding, at least 15 cells analyzed and 5 karyotyped for each case).
The average age at testing was 23 years old, the youngest patient being 1 month old and the oldest 42 years and 5 months old.
Results:Out of the 38 cases, 19 (50%) revealed numerical chromosomal anomalies, while in the other structural or both numerical and structural changes were identified. Out of these, 8 were mosaics with a 45,X cell line, 4 of which included a 47,XXX cell line. Approx. 42% of the patients (16 individuals) showed homogenous karyotypes. The other 22 had 2 or 3 cell lines (numerical and/or structural), with or without a normal 46,XX cell line.Among the detected structural anomalies, the most prevalent was the Xq isochromosome, identified in 4 patients (in both a homogenous and mosaic state). Other structural alterations included the X ring chromosome, Xp deletions, X chromosome inversions.The presence of the Y chromosome was observed in 6 patients. A single individual revealed a 46,XY homogenous karyotype. Mosaic 45,X/46,XY and the Y isochromosome were the other detected anomalies involving the Y chromosome.
Conclusions:Our study presents various Turner syndrome cytogenetic variants identified through classical karyotyping. Karyotyping is the genetic test of intent for patients with a clinical suspicion for Turner syndrome or female infertility.

3.P59 A 6179 Kb 13q33q34 deletion in a patient with global developmental delay and speech impairment diagnosed through array Comparative Genomic Hybridisation

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Background:Genetically-induced global developmental delay (GDD) represents a challenge in diagnostics for physicians of all involved specialities.Genetic factors have long been known to cause various types of GDD, including isolated and syndromic forms.
Case presentation: We present the case of a 2 years and 6 months old female patient referred for genetic testing for global developmental delay and speech impairment.
No family history was described.
Method: Genetic testing was performed using the SurePrint G3 CGH v2 Microarray, 8x60K system (Agilent technologies).
Result: The result of the analysis revealed a 6179 Kb deletion on the long arm of chromosome 13 (13q33-q34 chromosomal region).
Interpretation: This region contains 27 OMIM registered genes. The region (and included genes) is not reported in the ClinGen online registry as being associated with pathological phenotypes when haploinsufficient. The Decipher database shows several cases with genomic imbalances overlapping or included in the region identified in the patient (#253197, #317878, #331585, #307816 and #331438) and clinical pictures characterized by moderate GDD, mild facial dysmorphism and speech development impairment.
In conclusion, the identified change may explain the reasons for referral.Parental genetic tessting (constitutional karyotyping / molecular karyotyping) is recommended for determining the de novo/ inherited status of the detected deletion and for possibly identifying the cause of the imbalance.
Conclusion: Array comparative-genomic hybridisation is the test of choice for the genetic diagnosis of GDD of unknown etiology, isolated or associated with other clinical elements.

3.P60 Low grade 45,X/46,XY mosaicism in an infertile male patient

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Background: The genetic causes for male infertility are well documented.
Chromosomal anomalies, both numerical and/or structural, are known to be associated with failure of spermatogenesis and male infertility. Karyotyping is the genetic testing method of choice when diagnosing male and female infertility of unknown causes.
Case presentation: We present the case of a 36 years old male patient with a 3 years history of infertility. The patient`s sperm count revealed a moderate-severe oligospermia.
Method: Whole blood katyoryping was performed (GTG banding, 78 analyzed and katyotyped cells, 2 cultures).
Result:The result of the analysis revealed the following karyotype: mos45,X[3]/47,XY[75].
Interpretation: The result of the analysis revealed an abnormal karyotype with 2 different cell lines (chromosomal mosaicism):- an abnormal cell line with a single X chromosome in the sex chromosomes pair (X monosomy), in 3 analyzed cells (3.8%) - a normal male cell line, with 46 chromosomes (46,XY), in 75 analyzed cells (96.2%). Low grade mosaicism including a 45,X cell line in men has been described in literature. Depending on the number of cells in the abnormal line, the phenotype of the patients range from clinically normal men, to men with infertility or fertility problems, or men with abnormal or ambiguous genitalia.
Conclusion:Karyotyping is the method of choice for male inferility of an unknown cause. The existence of a 45,X cell line in men, depending on the level of mosaicism, does not necessarily need to involve an abnormal sexual development, but in the majority of cases is associated with male infertility.

3.P61 Neurodevelopment and Neuropsychiatric disorders in four new cases of 16p11.2 microdeletion / microduplication syndromes

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500 to 600 Kb microdeletions and microduplications in 16p11.2 are one of the most recurrently detected CNVs in patients with neurodevelopmental and autism spectrum disorders.
In the present study, we report three new unrelated cases of 16p11.2 microdeletion and one new case of 16p11.2 microduplication syndromes detected using 180K CGH array. Patients are aged between 4 years and 28 years and they all present variable developmental and speech delay, hypotonia, motor coordination difficulties, autistic features, seizures and brain congenital anomalies.
All four detected CNVs were between BP4 and BP5 LCRs with a variable size between 533 Kb and 597 Kb. This region involves particularly four genes (QPRT, PRRT2, SEZ6L2 and TBX6) which have been identified as potential candidate genes of the 16p11.2 deletion/duplication phenotype. In our study there are no clear clinical differences between cases of deletions and duplication. In one case a 16p11.2 microdeletion detected in the index case was inherited from his affected mother, supporting the importance of early genetic diagnosis and genetic counseling in these cases

4. Genomics

4.P1 Single cell strand sequencing of a macaque genome reveals multiple nested inversions and breakpoint reuse during primate evolution

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Rhesus macaque is an Old World monkey that shared a common ancestor with humans ~25 Myr ago and is an important animal model for human disease studies. A deep understanding of its genetics is therefore required for both biomedical and evolutionary studies. Among structural variants, inversions represent a driving force in speciation and play an important role in disease predisposition. Here we generated a genome-wide map of inversions between human and macaque, combining single-cell strand sequencing with cytogenetics. We identified 375 total inversions between 859 bp and 92 Mbp, increasing by eightfold the number of previously reported inversions. Among these, 19 inversions flanked by segmental duplications overlap with recurrent copy number variants associated with neurocognitive disorders. Evolutionary analyses show that in 17 out of 19 cases, the Hominidae orientation of these disease-associated regions is always derived. This suggests that duplicated sequences likely played a fundamental role in generating inversions in humans and great apes, creating architectures that nowadays predispose these regions to disease-associated genetic instability. Finally, we identified 861 genes mapping at 156 inversions breakpoints, with some showing evidence of differential expression in human and macaque cell lines, thus highlighting candidates that might have contributed to the evolution of species-specific features. This study depicts the most accurate fine-scale map of inversions between human and macaque using a two-pronged integrative approach, such as single-cell strand sequencing and

cytogenetics, and represents a valuable resource toward understanding of the biology and evolution of primate species.
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4.P2 A high quality bonobo genome assembly and a refined analysis of hominid evolution

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Bonobo and chimpanzee represent the closest living species to humans and diverged only about 1.7 million years ago. Therefore, their genomes are a critical resource for revealing the genetic changes that are unique to human. Using multiple genomic technologies, we constructed a high-quality bonobo genome without guidance from existing reference genomes. In the generated bonobo assembly >98% genes are completely annotated and 99% of the gaps are closed, the majority of full-length mobile element insertions are identified, and we have improved by >6-fold our understanding of bonobo segmental duplication content. We annotated some of the most recently duplicated bonobo genes for the first time. As a result, we present the most complete set of functional changes in the bonobo lineage, including exon and gene losses, structural rearrangements, and gene family expansions. Importantly, the high-quality of the constructed bonobo genome allowed us to revisit incomplete lineage sorting (ILS) at a fine-scale level of resolution because ~75% of the genome could now be systematically compared to the other African ape genomes estimating that ~5.1% of the human genome is closer to chimpanzee/bonobo and >36.5% of the genome shows ILS if we consider a deeper phylogeny including gorilla and orangutan. Notably, we also demonstrate that 26% of the regions of the genome subject to ILS are not randomly distributed. (Manuscript in press on Nature)

4.P3 Non canonical zygotic divisions are characterized by parental genome segregation errors

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The first embryonic division is expected to proceed by duplication and equal segregation of the maternal and paternal haploid genomes. Two resultant biparental diploid daughter blastomeres will form the genetic blue print for further mitotic divisions. Conflicting with this fundamental tenet, genome-wide mosaicism has been detected in severe developmental and pregnancy disorders. The retrieval of genome-wide mosaicism in cleavage-stage embryos of different species prompted the hypothesis that the zygote can cleave directly into three or four cells (multipolar division), simultaneously segregating whole parental genomes into distinct blastomere lines, an event coined “heterogoneic cell division”. To test this, we simultaneously analyzed the genome-wide copy number and haplotype of all blastomeres resulting from 25 bovine zygotes that underwent multipolar division. The large majority of the embryos were polyspermic. Furthermore, all embryos contained whole-genome abnormalities in at least one of their blastomeres. Heterogoneic cell division was confirmed to underly 18 mixoploid and chimeric embryo profiles and occurred via distinct mechanisms resulting in whole-genome segregation errors, including segregation of parental genomes by a tripolar spindle, the pronuclear extrusion of a paternal genome, the operation of an ectopic paternal spindle or the operation of private parental spindles. In nine embryos, blastomeres containing only mitochondrial DNA disclosed cytokinesis without concurrent genome segregation. Heterogoneic cell division may contribute to the high incidence of embryonic arrest and provides an overarching theorem for development of full-grown mixoploid and chimeric individuals, moles and rare forms of twinning in cows and human. To study the

developmental fate of embryos resulting from heterogoneic cell division, a culture system for single blastomeres was optimized. Single blastomeres resulting from both multipolar and bipolar (i.e. normal) divisions developed to blastocyst-stage. Yet, when compared to blastomeres resulting from bipolar divisions, the blastomeres resulting from multipolar divisions arrested at earlier stages more frequently and the synchrony of development between blastomeres originating from the same embryo was lower. Genetic and transcriptomic analysis of these single blastomere outgrowths will allow us to disclose the developmental program of androgenetic, gynogenetic and triploid blastomeres resulting from heterogoneic cell division.

4.P4 A rearrangement involving an inversion and a translocation leading to a complex sSMC: from array to karyotype.

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Complex supernumerary small marker chromosomes (sSMCs) consist of chromosomal material derived from more than one chromosome and constitute one of the smallest subgroups of sSMC. On the other hand, chromosomal inversions are of particular clinical interest due to the possibility of recombination during meiosis that may lead to abnormalities in the carriers' offspring. These structural rearrangements occurring simultaneously are very rare and, if not present in one of the parents or if rearranged during the transmission to the offspring, present a dilemma for interpretation.
Here we present a 11-year-old girl with intellectual disability, severe learning difficulties and some dysmorphic features, with a 23.1Mb terminal gain at 4q32.3q35.2 and a 15.39Mb justacentromeric gain at 21q11.2q21.3, uncovered by Affymetrix Cytoscan HD. To assess a predicted complex rearrangement, namely an sSMCs, and evaluate recurrence risk, karyotype was performed, both in the patient and in her parents. The patient´s karyotype confirmed the presence of a supernumerary der(21) resulting from a paternal balanced t(4;21) and revealed the presence of an unexpected pericentric inversion in one of the chromosomes 4 at p14q21.3. Interestingly, the father's karyotype revealed that the chromosome 4 involved in the balanced t(4;21) presents the same pericentric inversion observed in the patient. Trio SNP-array and QF-PCR confirmed that der(21) is of paternal origin and both normal chromosomes 21 are of maternal origin. Chromosomes 4 showed a biparental origin.
This case is a rare situation in which the presence of crossing-over can be demonstrated morphologically and shows the importance of keeping both cyto-genetics and cytogenomics present in a diagnostic setting as chromosomal microarray analysis may need clarification by karyotype/FISH.

4.P5 Precise detection of balanced chromosomal rearrangements and single nucleotide variations using chromosome conformation capture

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Detection of structural rearrangements, and especially balanced translocations and inversions, is challenging due to limited sensitivity and resolution of next generation sequencing and cytogenetic approaches. Here we propose to combine the chromosome conformation capture (3C) technology with exome enrichment for the simultaneous detection of chromosomal rearrangements and single nucleotide variations.
We performed optimization of the 3C-protocol, achieving uniform exome coverage, improved signal-to-noise ratio, and reduced experiment cost and time. Using an optimized technique, we obtained 3C-libraries for fifteen patients with various congenital pathologies and two model human cell lines, A549 and K562.
Analysis of chromatin contacts in K562 cells showed that chromosomal translocations severely affect the pattern of genomic interactions. Based on these observations, we developed the method for translocations detection, and showed that it captures known chromosomal translocations in K562 and A549 cell genomes. Applying the developed method to data obtained from patients we identified four balanced translocations. The minimal size of translocated loci was less than 100 kb, which could not be captured by routine karyotyping. In addition, we confirmed the presence of large balanced translocation in the genome of a patient with undifferentiated developmental delay and established the boundaries of this translocation with sub-kilobase resolution. Based on the changes of chromatin 3D contacts caused by this translocation, we proposed a

new molecular mechanism underlying the development of the pathology.
In addition to translocations detection, we have shown the utility of the exome-enriched 3C-libraries sequencing for detection of large (megabase-scaled) balanced inversions, various unbalanced rearrangements (deletions, duplications and amplifications) and clinically significant single nucleotide variations. Overall, we have shown that sequencing of exome-enriched 3C-libraries can be used for detection of a broad spectrum of genetic variants.
This study was supported by RFBR grant #18-29-13021

4.P6 AnnotSV and knotAnnotSV: a web server for human structural variations annotations ranking and analysis

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With the dramatic increase of pangenomic analysis, Human geneticists generate large amount of genomic data including millions of small variants (SNV/Indel) but also thousands of structural variations (SV) mainly from next-generation sequencing and array-based techniques. To help identifying human pathogenic SV, we have developed a webserver dedicated to their annotation and ranking (AnnotSV) as well as their visualization and interpretation (knotAnnotSV) at the following address: https://www.lbgi.fr/AnnotSV/.
First, we have tripled the available data sources in our annotation engine (AnnotSV, PMID:29669011), including among others databases such as DGV, gnomAD, DDD, OMIM, intolerance score and known pathogenic SV (dbVar, ClinVar and ClinGen) as well as users own annotations (e.g. patient's SNV/indel...). Second, a phenotype driven analysis based on HPO and Exomiser has been implemented. Third, an automatic SV classification based on the latest ACMG recommendations (PMID:31690835) is available. Testing included users from one research lab and two hospital laboratories. Finally, knotAnnotSV displays the annotated SV in an interactive way including popups, filtering options, advanced colouring to highlight pathogenic SV and hyperlinks to the UCSC genome browser or other public databases. The annotation is available for the SV as a whole (full/compact mode) or divided for each overlapping gene (split/expanded mode). Output can be either visualized in a web browser directly or using a specific link, or downloaded as a tab separated file.
To our knowledge, this makes our webserver the most comprehensive online SV annotation and interpretation tool.
This new version of the AnnotSV web server can be accessed at the following address: https://lbgi.fr/AnnotSV/. The website is available since 2 years and the web traffic is ~12,000 visits in 2020 with a 20% increase per year. Our underlying annotation engine (AnnotSV) has been upgraded to version 3 and is getting more and more citations since its publication in 2018 (40 in total, decomposed in 2 in 2018, 8 in 2019, 17 in 2020 and already 10 in 2021)

4.P7 A case of ring chromosome 21 with hemifacial microsomia, developmental and speech delay analysed with WGS and patient derived iPS

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Introduction: Ring chromosomes are rarely occurring circularized chromatin which can arise during mitosis if the telomeric sequences are lost. Structural variants (e.g. deletions) often accompany the formation of ring chromosomes, leading to neurodevelopmental disorders which severity strongly depends on the affected genes. However, the exact mechanism and biological effect of ring chromosomes is still unknown. Here, we present RD_P26, a 6-year old girl presenting hemifacial microsomia, developmental and speech delay, and a ring chromosome 21, 46,XX,r(21)(p11.2q22.3).
Methods: Characterization with chromosomal micro-array, whole genome sequencing and breakpoint junction (BPJ) PCR unravelled three alternating deletions and two inversions with templated and non-

templated insertions in the BPJs. Remarkably, this loss of genetic material, affecting 29% (70/242) of the protein-coding genes on chromosome 21, is not lethal. Moreover, the ring chromosome is stable through multiple passages of neuroepithelial stem cells (NES), differentiated from patient-derived induced pluripotent stem cells. Furthermore, we analysed the transcriptome of NES from RD-P26 and compared the expression profile to two trisomy 21 patients and three healthy controls.
Results: 11.6% genes (2080/17831) were differentially expressed. of the 171 genes located on chromosome 21, 24% (41/171) were differentially expressed. Within the three deleted segments, 19 genes were significantly downregulated and there were no significant upregulations, consistent with haploinsufficiency. Moreover, the downregulated gene expression was mirrored by an elevated gene dosage in the trisomy 21 patients.
Conclusion: The hemizygous genes located within the deletions are considered as likely causing the patient's phenotype, also other chromosomal rearrangements were found.
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5. Other Cytogenomics Topics

5.P1 Deciphering balanced translocations in infertile males by Next Generation Sequencing to identify candidate genes for spermatogenic disorders

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Male infertility affects about 7% of the general male population. Balanced structural chromosomal re-arrangements are observed in 0.4 to 1.4% of infertile men and are considered as a well-established cause of infertility. However, underlying pathophysiological mechanisms still need to be clarified. We used a strategy combining standard and high throughput cytogenetic and molecular technologies in order to identify candidate genes in three male carriers of balanced translocation and spermatogenesis defect. FISH and whole genome paired-end sequencing were used to characterize translocation breakpoints at the molecular level. Exome sequencing was performed in order to exclude any molecular event independent of the chromosomal rearrangement. Expression study of candidate genes was performed on blood RNA. All translocation breakpoints were characterized in the three patients. We identified four variants: a position effect on LACTB2 gene in patient 1, a heterozygous CTDP1 gene disruption in patient 2, two single nucleotide variations (SNVs) in DNAH5 gene and a heterozygous 17q12 deletion in patient 3. The variants identified in this study will need further validation to assess their role in male infertility. Beside the mechanical effect of structural rearrangement on meiosis, breakpoints could result in additional alterations such as gene disruption or position effect. Moreover, SNVs or copy number variations may be fortuitously associated and could explain the variability of the impact of chromosomal rearrangement on spermatogenesis. This study shows the relevance of combining different cytogenetic and molecular techniques to investigate patients with spermatogenic disorders and structural rearrangements.

5.P2 Simple and complex aneuploidy in premeiotic oocytes detected by aCGH & NGS: evidence for genetic influence and e!ect on fertility

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Aneuploidy is the major cause of embryonic & fetal death. Most errors arise in meiosis I/II in the adult female, strongly correlated with maternal age. Our application of array comparative genomic hybridi-zation (aCGH) and next generation sequencing (NGS) has shown that 10-15% of oocyte aneuploidy is in fact premeiotic (PM) and present in the early embryo, leading to a risk of an aneuploid conception in adult life irrespective of age. Mosaic aneuploidy may be present in the primordial germ cells or may arise during the extensive mitotic divisions of the oogonia. We have seen that there is substantial individual variation in the incidence of PM errors is likely to be related to the genetic background of the oocyte donor. Oocyte DNA was extracted, amplified and analysed using aCGH or NGS [Ion ReproSeq PGS (ThermoFisher) / VeriSeqPGS (Illumina)].NGS or aCGH was performed on 141 immature oocytes [germinal vesicles (GV) & metaphase I (MI) oocytes] and 61 mature oocytes [Metaphase II - 1st PB complexes]. Fifteen (19.2%) of 78 donors had oocytes with PM errors. Oocyte aneuploidy incidence was correlated with the reproductive histories of female partners. In categories 1 & 2 most couples have fertility issues – these two groups have a very

similar incidence of PM errors. Groups 3, 5 & 6 from couples with no known fertility issues all have lower incidences. Two cases in Group 4 stand out, with a much higher incidence which may be related to the genetic factors responsible for the onset of breast cancer in their 30s. Phenotypic & genetic variants have been identified that affect the incidence of complex errors in embryos; we plan to investigate the occurrence of these in our cohort of donors.

Table: Classification of female partners donating oocytes

Classification of female partners based on reproductive histories	Total No. of donors	Total oocytes tested	Oocytes with premeiotic aneuploidy	No. of donors contributing oocytes with premeiotic aneuploidy	No. of oocytes with Simple (SE)/ Complex (CE) Errors
1. Female fertility status unknown or couple infertile	27	76	11 (14.5%)	6 donors	7(SE) ; 4(CE)
2.Primary/secondary female factor infertility	25	48	6 (12.5%)	4 donors	3 (SE) ; 3(CE)
3.Oocyte preservation due to social reasons	3	23	1 (4.3%)	1 donor	1 (SE)
4.Oocyte preservation due to breast Cancer treatment	2	10	5 (50%)	2 donors	1(SE) ; 4(CE)
5.Female carriers of structural rearrangements or monogenic disorders(non-cancer related)	17	30	2 (6.66%)	2 donors	1(SE); 1(CE)
6.Females at increased risk of developing breast/ovarian cancer due to BRCA1/2 gene mutations	4	15	0	0	0
TOTAL	78	202	25 (12.38%)	15 donors	13 (SE) ; 12(CE)

5.P3 Chromosome instability and gene rearrangements in ectopic endometriotic tissue of women with endometriosis

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Endometriosis (EM), a common gynecological disease, is characterized by the presence of endometrial tissue outside the uterus and is associated with pain and infertility. Information regarding EM pathogenesis is limited, mainly due to rare availability of endometriotic tissue and limited number of abnormal cells, but recent molecular cytogenetic studies have identified specific structural chromosomal aberrations.
In the current study, endometrial biopsies were obtained from 18 women during laparoscopic surgery. Endometriosis was histologically confirmed and peripheral blood samples were obtained in order to establish the constitutional karyotype. Written informed consent was provided in all cases.
Endometrial tissue was removed, digested with collagenase and cultured in Dulbecco's Modified Eagle Medium. Karyotyping was done by GTG banding. FISH was performed using commercial probes for 17p13(TP53), 11q22.3(ATM), 8q24(MYC), 11q23(MLL), 14q32(IGH) and 7q22q31 chromosomal regions.
Constitutional karyotypes were normal (46,XX) in 18/20 women, while low X chromosome mosaicism (46,XX/45,X) was observed in 2/20 cases.
Karyotypic analysis of endometrial tissue was successful in 15/18 specimens, revealing no clonal chromosomal abnormalities. However, sporadic chromosomal aberrations, involving mainly chromosomes 9, 11, 19, 20 and X were identified in 9/13 samples and non-clonal supernumerary marker chromosomes in 4/13 (30.8%) cases. Polyploidy was observed in 38.5% cases in 4-11.5% of the analyzed metaphases. Endoreduplication was found in 23.1% of the analyzed samples.
FISH analysis was successful in 13/20 cases. IGH gene rearrangements were observed in 9/13 and MLL rearrangements in 9/11 of the examined cases. Normal hybridization pattern was observed for the p53, ATM, MYC and 7q22q31 genetic loci in all cases. FISH results also confirmed the increased frequency of polyploidy observed by karyotype.
In conclusion, the genomic instability in chromosome level and IGH and MLL rearrangements found in this study, may be implicated in EM pathogenesis.

5.P4 Interstitial deletion of chromosome 20 and isochromosome i(7)(q10) in the bone marrow of patients with Shwachman Diamond syndrome: novel evidence of karyotype instability related to somatic rescue mechanisms

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An interstitial deletion of the long arm of chromosome 20, del(20)(q), and an isochromosome of the long arm of chromosome 7, i(7)(q10), are the most frequent anomalies in the bone marrow (BM) of patients with Shwachman-Diamond syndrome (SDS) and these clonal changes imply milder haematological symptoms and lower risk of myelodysplastic syndromes and acute myeloid leukaemia, thanks to already postulated rescue mechanisms. BM from fourteen patients exhibiting either the del(20)(q) or the i(7)(q10) were subjected to chromosome analyses, FISH and array-Comparative Genomic Hybridization. In eight patients, del(20)(q) was the sole chromosomal abnormality. In 4/5 patients carrying the del(20)(q), further different del(20)(q) clones evolved independently, within a single BM sample, or across sequential samples. An overview of patients with the del(20)(q), also including cases already reported, showed that all the deletions were interstitial with loss of material varying from 1.7 to 26.9 Mb, and all resulted in the loss of the EIF6 gene. The patient with the i(7)(q10) showed a subsequent clonal rearrangement of the normal chromosome 7 across years. This study shows unexpected clonal variations of the chromosome changes and provides further evidence of the striking selection pressure exerted by SBDS deficiency driving karyotype instability and specific clonal abnormalities.

5.P5 Meiotic behavior of sperm chromosomes in carriers of balanced reciprocal chromosome translocations (RCTs): theoretical prediction of quadrivalent vs. experimental data

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Reciprocal chromosomal translocations (RCTs) are one of the most common structural aberrations in human genome (1/700 in new-borns). Balanced RCTs do not affect the phenotype of the carrier but can disrupt spermatogenesis. Approximately, 1% of infertile males reveals RCT. RCTs represent ~16% of all autosomal (lymphocytes) aberrations of oligozoospermic and ~4% of azoospermic males. RCT carriers are at risk for miscarriage and/or developmental disabilities in the offspring, caused by the production of genetically unbalanced gametes' following chromosomal segregation during meiosis. The behavior of the meiotic quadrivalent determines segregation of the chromosomes' resulting in a variety of sperm genotypes. Collation of all genotypes – meiotic segregation pattern is individual for each RCT, similar among the same RCT carriers, and stable during the carrier's lifetime. Theory predicts that meiotic segregation depends on: breakpoints localization (proportion of interstitial (IS), translocated (TS) and centric (SC) segments), type of chromosomes (acrocentric, sex, heterochromatin block), asynapsed regions, number/localization of chiasmata, and functioning of karyokinetic spindle.
The purpose of the study was confrontation of theoretical prediction concerning meiotic behavior of quadrivalents vs. real meiotic segregation pattern results obtained from >250 RCT carriers published so far. The length of the chromosome involved in each RCT, its TS, IS, and CS segments, were counted according to NCBI Genome Data Viewer.
It was found that in most of the RCTs the meiotic segregation pattern followed the theoretical assumptions. However, the theory does not allow to determine the recombination frequency, responsible for production of sperm genotypes other than predicted. Additionally, the deleting mechanisms of genetically unbalanced spermatozoa are not fully effective, leading to disruption in proportion of genotypes observed for given type of segregation. Thus, experimental establishment of meiotic segregation pattern of sperm genotypes is crucial for genetic risk counselling and real probability estimation of miscarriages and/or offspring disabilities.
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5.P6 Molecular cytogenetic analysis of genomic instability of HEK293 cell line derivatives

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Cell lines are a commonly used basic research tools. One of the most commonly used cell lines is the HEK293 line, mainly for its easy transfection. The aim of our study was to analyze the cytogenetic differences between commercially available derivatives of this line: HEK293 T-Rex and HEK293 Flp-In T-REX, as well as the differences between the parent line HEK293 T-Rex and its two derivatives where CRISPR/Cas9 was used to delete genes for DVL1/DVL2/DVL3, and the gene for CK1α. Genome modification by CRISPR/Cas9 is due to its simplicity and efficiency commonly used to delete individual genes or groups of genes (so-called gene knockouts). It typically targets a single target sequence where a double-strand break forms. Consequently, because of an erroneous repair, a shift in the reading frame occurs, which ultimately leads to a functional deletion of the gene. When interpreting the results, it is assumed that the daughter and maternal cells differ genetically only in the edited locus. We believe that this assumption is a significant simplification, as (i) even parental cell lines may have a very complex karyotype, which may require elimination of more or less than 2 alleles, and (ii) during establishment of individual clones cells additional cytogenetic re-arrangements can be selected. In our study we will present the results of mFISH and CGH arrays, that were used to characterize different derivatives of the same line. We propose that cytogenetic analysis is a suitable method to select daughter lines that are most similar to the parent line. The study further showed that in the case of whole genome and proteome level experiments, karyotype analysis of the cell line should be part of the standard validation procedure.

5.P7 Differences in telomere length and inflammation in severe psychiatric disorders

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Individuals with severe psychiatric disorders have a reduced life expectancy compared to the general population. A recent meta-analysis showed a significant overall effect size for telomere shortening across all psychiatric disorders and a recent qualitative synthesis of the literature show increased circulating inflammatory markers.Telomere erosion is caused by several biological insults ,including inflammatory processes.
In this study we measured T-lymphocytes TL with quantitative fluorescent in situ hybridization (Q-FISH) and plasma levels of inflammatory markers in three deep-phenotyped samples of patients with severe psychiatric disorders comprising 40 patients with bipolar disorder (BD), 41 with schizophrenia (SZ), 37 with major depressive disorder(MDD), and 36 non-psychiatric controls (NPC). TL was shorter in SZ and in MDD compared to NPC, while it was longer in BD (modelF6, 137 =20.128, $p = 8.73 \times 10{-}17$, effect of diagnosis, F3 = 31.870; $p = 1.08 \times 10{-}15$). There was no effect of the different classes of psychotropic medications, while duration of treatment with mood stabilizers was associated with longer TL (Partial correlation controlled for age and BMI: correlation coefficient = 0.451; p = 0.001). Levels of high-sensitivity C-Reactive Protein (hsCRP) were higher in SZ compared to NPC (adjusted p = 0.027), and inversely correlated with TL in the whole sample ($r = -0.180$; p = 0.042). Compared to NPC, patients with treatment resistant (TR) SZ had shorter TL (p = 0.001), while patients with TR MDD had higher levels of tumor necrosis factor-α (TNFα) compared to NPC (p = 0.028) and to non-TR (p = 0.039).
Our data show that severe psychiatric disorders present altered TL and peripheral levels of inflammatory markers compared to non-psychiatric controls. Funding: Fondazione di Sardegna F72F16003090002 and FIR2019/20

5.P8 Optical mapping uncovers human specific expansion of 22q11.2 low copy repeats

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Introduction: Low copy repeats (LCRs) are amongst the most complex regions in the human genome. Scrutinizing these structures unraveled their importance during evolution. LCRs play an important role in the disease mechanism of genomic disorders as well. Non-allelic homologous recombination between chromosome 22 LCRs (LCR22s) cause the 22q11.2 deletion syndrome. LCR22-A hyper-variability was uncovered in human. However, it remains unknown whether such variability is human specific or is also a feature of other primates.
Materials and Methods: The LCR22 variability in the Great Apes was charted using an LCR22-specific fiber-FISH. The structures of five chimpanzees, one bonobo, two gorillas, and six orangutans were de novo assembled to assess the level of inter- and intraspecies variation. In addition, we used Bionano optical mapping to chart the overall 22q11.2 structure and to close the gaps in the corresponding non-human primate reference genomes.
Results: The chimpanzee and bonobo harbored the smallest LCR22-A allele observed in humans, containing core duplicons, without intra-species variation. The gorilla haplotype had small expansions compared to the chimpanzee. In contrast, the overall 22q11.2 region is different in the orangutan, including rearrangements in the sequence between the LCR22s, intra-individual structural variation, and LCR22-A haplotypes not resembling the human ones.
Conclusions: For the first time, the LCR22s subunits were evolutionary charted and uncovered lineage-specific differences. The LCR22-A hypervariability is human specific, since no structural variation was observed in chimpanzee or bonobo. This suggests an important role of the locus in human adaptation and evolution.

5.P9 Homologous pairing in spermatogenic cells takes place by preleptotene DSB independent mechanisms in Mus musculus

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In most eukaryotes, the process of homologous pairing ensures meiotic recombination. In Mus musculus, there are controversial data about when pairing takes place since leptotene DSB-dependent and preleptotene DSB-independent mechanisms have been described. To clarify this point, we examined the homologous pairing in premeiotic and meiotic spermatogenic cells of Mus musculus. A methodological approach based on the use of DNA painting probes for the analysis of the entire karyotype by three-dimensional fluorescence in situ hybridization was used. Our results indicate that in the murine model 73.83% of homologous chromosomes are already paired at premeiotic stages (spermatogonia - early preleptotene spermatocytes). Our data evidence a high percentage of homologous pairing before the onset of meiosis and point out that it does not occurs randomly, since it was higher than observed in somatic cells (19.47%) and between non-homologous chromosomes (41%). In our study, we have also observed that premeiotic homologous pairing is asynchronous and independent of chromosome features such as size, GC content, and the presence of NOR regions.
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5.P10 Comprehensive PGT for patients with de novo pathogenic variants following single molecule long read amplicon sequencing based haplotyping.

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Preimplantation genetic testing for monogenic disorders (PGT-M) aims to select embryos devoid of pathogenic inheritable variants from parents to the offspring. Different generic PGT methods, such as Karyomapping, haplarithmisis and OnePGT, cannot be applied for couples where one of the partners is carrying a de novo pathogenic variant. The absence of affected close relatives entangles the process of constructing accurate haplotype using linkage analysis by Single Nucleotide Polymorphisms (SNPs) located upstream and downstream of the pathogenic variant. Current practices for the handling of couples with de novo variants are mostly targeted, require the combination of direct and indirect approaches and often require multiple biopsies. To overcome this limitation, we developed a new method: long-range PCR and subsequent long read sequencing of the

pathogenic variant region enabled haplotype reconstruction and imputation in the genome wide haplotypes.
From February 2017 until December 2019, twenty-two couples with one of the partners carrying a pathogenic de novo variant causing a Mendelian disorder were enrolled for PGT-M in the Centre for Human Genetics, UZ Leuven. Genomic DNA from the partner carrying the variant and his/her parents was used for targeted long-read sequencing on a PacBio RSII/Oxford Nanopore to impute the disease-carrying allele. Subsequently, genotyping of the prospective parents and both grandparents, and DNA amplified from blastomere or trophectoderm biopsies via Illumina HumanCytoSNP-12v2.1 beadchips enabled genome-wide haplotyping using haplarithmisis. Unaffected embryos were transferred. Hence, our developed approach consequently allows comprehensive PGT in a single workflow.

5.P11 Predictors of genomic instability in childhood obesity: a study of multiple variables

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Childhood obesity – a medical condition affecting children and adolescents – is an independent risk factor for cancer and premature mortality in adulthood. Epidemiological evidence suggests that pathological implications from excess adiposity may begin early in life. Obesity in childhood is concurrent with a state of chronic inflammation, a well-known etiological factor for DNA damage. In addition, obesity has been associated with micro-nutritional deficiencies. Vitamin D has attracted particular attention for its anti-inflammatory properties and role in genomic integrity and stability. Here we report the first research to perform a combined, non-invasive investigation of multiple markers of adiposity, systemic inflammation, vitamin D status, DNA damage and genomic instability in children and adolescents. As expected, comparisons between participants with obesity and normal range BMI showed significant differences in anthropometric measures. Significant differences were also observed in some measures of genomic instability. When examining relationships between variables for all participants, significant correlations were found between markers of adiposity, vitamin D status and DNA damage. Through multiple regression analysis we present a model for prediction of genomic instability in children and adolescents. Non-invasive biomonitoring and predictive modelling of genomic instability in young patients with obesity may contribute to prioritisation and severity of clinical intervention measures.

5.P12 A central role of telomere dysfunction in the formation of a unique translocation within the sub telomeric region leading to duplication and partial trisomy

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Telomeres play a major role in maintaining genome stability and integrity. A putative implication of telomere dysfunction in the formation of different kinds of chromosomal aberrations is currently under-investigated. Here, we report a case of a six month old boy with a chromosomal gain encompassing the 11q22.3q25 region, identified by CGH array analysis. The size of duplication is 26.7 Mb and contains 170 genes (OMM1). The partial trisomy results in duplication of the region in question with clinical consequences including bilateral renal dysplasia, delayed development and a heart defect. The karyotype, using specific subtelomeric probes, revealed the presence of an unbalanced t(9;11)(p24;q22.3) translocation with a unique breakpoint involving the subtelomeric region of the short arm of chromosome 9. The karyotypes of the parents were normal. Telomere integrity in circulating lymphocytes from the child and from its parents were assessed using an automatized high-throughput technique based on fluorescence in situ hybridization (FISH) with telomere- and centromere-specific PNA probes followed by M-FISH multicolour karyotype. Short telomeres as well as an increased frequency of telomere loss and formation of telomere doublets were detected in the child's cells. Interestingly,

similar telomere profiles were found in circulating lymphocytes of the father. Moreover, assessment of clonal telomere aberrations involving chromosome 9 and 11 strongly suggest a possible implication of telomere dysfunction in the formation of this rearrangement via chromosome end-to-end fusion and breakage-fusion-bridge cycles.

6. Prenatal Diagnosis

6. P1 Prenatal diagnosis of 5p deletion syndrome: report of 2 cases

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The 5p deletion or Cri-du-chat syndrome (CdCs) results from deletion of a terminal or interstitial segment of the short arm of chromosome 5. Its clinical characteristics are difficult to find prenatally. No specific prenatal ultrasound signs related with CdCS had been proposed since few reports of prenatal diagnosis of CdCS had been published.

Objective: The analysis of 2 cases of prenatal cytogenetic diagnosis of 5p deletion syndrome in fetuses.

Material and methods: Pregnant women were in the high risk group according to ultrasound examination. Cordocentesis was performed under ultrasound guidance after informed consent. Karyotyping via routine chromosome G-banded (320–400 bands) analyses were performed according to standard protocols

Results: Case 1. A 29-year-old woman. Ultrasound examination at 20-21 weeks of gestation detected: congenital heart disease (CHD) in the fetus: ventricular septal defect. Hepatosplenomegaly. Clitoral hypertrophy. Intrauterine growth restriction. Pathologic placental structure.

Chromosome analysis of the umbilical cord blood revealed a karyotype: 46,XX,del(5)(p13).

Case 2. A 19-year-old woman. Ultrasound examination at 29 weeks of gestation obtained: CHD in the fetus: AVC. Dandy-Walker syndrome. Intrauterine growth restriction. Ultrasound markers of chromosomal pathology of the fetus. Chromosome analysis of the umbilical cord blood obtained a karyotype: 46,XX,del(5)(p14).

CHD, Dandy-Walker syndrome, intrauterine growth restriction were previously reported in prenatal cases of CdCS. Though cerebral abnormalities are typical feature, and cardiac anomalies affect 15% to 20% of CdCS patients in postnatal, the prenatal ultrasound features of CdCS remain non-specific.

Conclusion: These cases report highlights the importance of careful examination of basic and extended fetal biometry and structures, as well as soft markers for the detection of chromosomal abnormalities including rare microdeletion/duplication syndromes.

6. P2 Chromosome 18 aneuploidies in prenatal diagnosis

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Introduction: We present six rare cases of aneuploidies involving chromosome 18 identified during second trimester routine prenatal testing. Aneuploidies imply gain or loss of a whole chromosome (full aneuploidy) or part of it (partial aneuploidy). These abnormalities can either involve all cells (nonmosaic state) or just a proportion of them (mosaic state). Our range of aneuploidies covers all of the above: trisomy 18 (mosaic and nonmosaic state), del 18p, i(18q) and i(18p).

Aim: The idea behind this project is to summarize in a single work the different clinical features observed in a fetus with several anomalies, all associated with chromosome 18.

Material & Methods: Sonographies were performed on six pregnant women; Standard Karyotyping, FISH-analysis and CGH-Array were done on amniotic fluid cells.

Discussion: Our six cases had chromosome 18 aneuploidies concerning different areas of the chromosome. We describe three trisomy 18 resulting in three copies of the entire chromosome, of which two are in a nonmosaic state and one in a mosaic state. One is the deletion of the short arm of chromosome 18 where the entire 18p is loss in one of the two copies. An i(18p) present as a supernumerary chromosome means a tetrasomic state of the short arm. The last case is an i(18q) discovered in a fetus with 46-chromosome karyotype in which one homologue is replaced by the isochromosome which implies a trisomy for the “q” arm and a monosomy for the “p” arm. The comparison of the ultrasound features of our cases revealed variable phenotypes but with common traits in fetuses with aneuploidies involving the same chromosomal region.

Conclusion: The investigation of our cases highlighted the presence of choroidal plessis cysts in fetuses with supernumerary copies of the short arm while only the presence of three copies of the long harm lead to cardiac defects.

6. P3 Combination of QF PCR and MCA represents an efficient diagnostic strategy when karyotype is not possible

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Structural aberrations are not detectable by ordinary molecular techniques (QF-PCR and FISH) except when they are not balanced and the regions involved are amplified or deleted
A structural aberration of chromosome 18 was detected in a MCA prenatal diagnostic, but it was wrongly diagnosed by QF-PCR.
This case was referred because of the miscarriage at the 7th week of gestation; maternal age was 37. The result of the QF-PCR on the chorionic villus sample was trisomy 18. The two markers of microsatellits located in the short arm of chromosome 18 used in the QF-PCR analysis were not informative. Karyotype was not available because of the lack of material. The -MCA analysis showed a gain of the long arm of chromosome 18 and a loss of the short arm of chromosome 18, compatible with the presence of an 18q isochromosome.
The 18q isochromosome is a rare structural aberration, it usually has similar characteristics as those of Edwards Syndrome.
In conclusion, the good practises advise the use of all possibly polymorphisms in QF-PCR, and confirm the result with other cytogenetics analysis, karyotype or MCA.

6.P4 Kagami-Ogata Syndrome: prenatal findings in a fetus presenting pulmonary stenosis, polyhydramnios nuchal fold and bell shaped thorax with coat hanger sign.

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Introduction: Kagami-Ogata (KOS) (OMIM #608149) is an imprinting disorder characterised by facial abnormalities, small bell-shaped thorax with coat hanger appearance of the ribs, abdominal wall defects, developmental delay, placentomegaly and polyhydramnios. These clinical features are caused by epimutations (Hypermethylation) and microdeletions affecting the maternally derived MEG3/DLK1:IG-DMR and/or MEG3:TSS-DMR at 14q32.2. We report clinical and molecular features of a prenatally case diagnosed by MS-MLPA showing hypermethylation at MEG3:TSS-DMR.
Methodology: A healthy 42-years-old pregnant woman was referred to our fetal unit at 16 weeks of gestation for echocardiography due to previous daughter affected by pulmonary stenosis. Morphological ultrasound scan showed a pulmonary stenosis, polyhydramnios, nuchal fold of 7mm (p95) and small stomach at 20+3 weeks. During the scan hands remained closed. The 4-D prenatal ultrasound showed a flat face and mild retrognatia.
The following genetic techniques were performed: i) arrayCGH(4x180K, OGT Technologies), ii) NGS gene RASopathy panel, iii) MS-MLPA methylation pattern at 14q32.2, iv) Microsatellite analysis for uniparental disomy (UPD) at 14q32.2.
Results: The prenatal arrayCGH and RASopathy gene panel were normal. After genetic counselling the parents decided a termination of pregnancy at 22 weeks of gestation. Our pregnancy losses protocol includes a radiograph that showed a bell-shaped thorax with coat-hanger appearance of the ribs. The fetal autopsy described a nuchal fold, mild hypertelorism, long filtrum, retrognatia, pulmonary artery stenosis and overgrowth. The hypermethylation level at MEG3:TSS-DMD detected by MS-MLPA confirmed the radiological suspicious of Kagami-Ogata Syndrome.
Conclusion: Kagami-Ogata Syndrome should be considered in fetus with coat-hanger sign with bell-shaped thorax , both features provide a distinctive radiological gestalt that leads to differentiate the Kagami-Ogata Syndrome from other skeletal dysplasias.

6.P5 Investigation of congenital chromosome abnormalities in newborns and infants in Senegal, a low income country

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Pre- and postnatal diagnoses are necessary to detect possible chromosomal and molecular anomalies in the fetus and child, respectively. These types of diagnosis are almost never performed in Senegal. They are done by sending samples to developed countries, which is extremely expensive for the majority of the population. The objective of our work was to set up different chromosomal and molecular diagnostic techniques in order to set up a clinical diagnostic lab in Senegal. The registration of cases is based on all clinical records of patients referred to Pediatric Departments of Dakar Hospitals. Peripheral blood was collected from patients by venous phlebotomy. Cell cultures was set up for karyotyping and Fluorescent In Situ Hybridization (FISH) and Primed In Situ Labelling (PRINS) were used for chromosome analysis. The remaining blood was frozen for DNA extraction and molecular analysis (PCR, Microarrays). In total, 26 patients were sampled including disorders of sexual development, trisomy 21 and trisomy 18. The FISH technique has been developed and used for the diagnosis of all sampled patients. FISH allowed us to diagnose 12 cases of trisomy 21, 14 of disorders of sexual development and 4 of trisomy 18. The karyotype analyses are ongoing for all samples. For the first time Karyotyping, FISH and PRINS analyses of congenital chromosome abnormalities are available in Senegal paving the way for further investigations of chromosome and molecular anomalies for diagnostic purposes.

6.P6 SNP array analysis of spontaneous abortions in 1st and 2nd trimesters

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Miscarriage means a spontaneous loss of pregnancy before 24 weeks of gestation. Studies indicate that spontaneous abortions make up about 15-20% of all pregnancies and about half will occur within the first 12 weeks. Chromosomal abnormalities are the main cause of spontaneous abortions, and are responsible for about 50% of all isolated miscarriages.
The purpose of our study is to evaluate the diagnostic power of SNP arrays to analyse miscarriages in the 1st and 2nd trimesters.
The Genetics Laboratory analysed 183 cases of spontaneous abortion in the 1st and 2nd trimesters, using samples from our Gynaecology Ward. In all miscarriages, foetal malformations had been detected through ultrasound scanning at the Unit of Foetal Medicine and Prenatal Diagnosis. The SNP array analysis was conducted in trios on the DNA extracted from foetal material.
Chromosomal alternations were observed in 51 samples (~28%): 32 with aneuploidies, 6 with triploidy and 14 with microdeletions/micro-duplications. The most frequent aneuploidy involves chromosomes 18, 21, 15 and X, but aneuploids were also observed for chromosomes 5, 7, 11, 13, 14, 16, 20 and 22. Chromosomal microdeletions and microduplications were “de novo” in nine cases and familial in two. Extensive regions of homozygosity (ROHs) were observed in nine cases, plus one case of UPD of chromosome 16 and one of chimerism.
SNP array analysis is shown to be useful and reliable in studying spontaneous abortions because of the extremely low risk of the technique failing and especially for the genetic information gleaned as a result. As this technique can also identify genetic defects, uniparental disomy and extensive ROHs, their aetiology can be established, thus determining the risk of recurrence for the couple in subsequent pregnancies

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6.P7 Prenatal diagnosis of mosaic deletion-duplication of chromosome 20q

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Chromosomal mosaicism (CM) is defined as the presence of two or more populations of cells with distinct genotypes. CM for structural aberrations is a rare event and different mechanisms of origin have been described.
We report a prenatal case referred for fetal cardiac disorder.
Array-CGH performed on DNA from direct amniotic fluid revealed a terminal deletion on the long arm of chromosome 20(q13.33) (~1.22 Mb).
Because this deletion was terminal, although much smaller than the detection limit for karyotyping, chromosomes from the cultured amniocytes were analysed. The cytogenetic study showed no difference between the two chromosomes 20 in most of the metaphases, as was expected, but also revealed the presence of an additional cell line (25% cells) with a derivative chromosome 20 with extra material on the end of 20q. This mosaicism was confirmed because both cell cultures established showed the

same derivative. Therefore, this cell line had to be present in the direct amniotic fluid although in a percentage below the detection limit of array-CGH.
Array-CGH on DNA from the cell culture and FISH with a subtelomeric probe for 20q:
(i) allowed the full characterization of the derivative chromosome 20 as a del dup of chromosome 20 (del20(q13.33) (~1.22 Mb) and dup 20(q13.2q13.33) (~8,8 Mb))
(ii) confirmed the presence of the 1,22Mb terminal deletion in all cells.
Both alterations were found to be de novo.
Considering the variable and unpredictable distribution patterns possible, CM is a challenging issue in prenatal diagnosis. In particular, low-level mosaicism (<15%-20%) can be responsible for the increased risk of erroneous diagnoses.
In this case, only the combined use of aCGH, karyotyping and FISH in both cultured and uncultured amniocytes allowed a precise diagnosis of a mosaic chromosome rearrangement with simultaneous 20p deletion and 20q duplication.

6.P8 Mosaicism involving two different 13q structural alterations

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Mosaicism with structural anomalies involving the same chromosome is uncommon and several mechanisms have been proposed to explain such rearrangements.
We report a prenatal diagnosis referred because of an increased nuchal translucency and other altered ultrasonographic markers. A chorionic villus sample (CVS) was performed and the QF-PCR for common aneuploidies showed a male foetus with a 13q partial trisomy. Karyotype from the cultured CVS, instead of the partial trisomy, revealed a 13q(31) deletion. To clarify these discordant results MLPA (P095 panel) was carried out in the pre-cultured material. The result confirmed the partial trisomy 13(q14.2-q21.33) and revealed a partial monosomy 13(q32.1-qter). DNA extracted from the CVS culture was also analysed using the mentioned molecular techniques: by QF-PCR no 13q partial trisomy was identified and the MLPA test was concordant with the karyotype, with a 13(q32.1-qter) partial monosomy. The sum of all results lead to a conclusion compatible with a mosaicism with, at least, two cell lines: one with a partial 13q trisomy and another with a partial 13q monosomy. Both of them were present in the initial material, as it was shown by the MLPA results (pre-culture), and in the CVS culture only the del(13q) was present. The lack of 13q terminal region STR markers, in the QF-PCR panel, explains why this technique was unable to identify the terminal partial monosomy.
Considering that the study was performed in a CVS and a confined placental mosaicism cannot be excluded, the structural alterations identified are probably responsible for the foetus phenotype.
The variable distribution of the abnormal cell lines may lead to discordant results when analysing different specimens or testing methods, as it happened in our case. Due to the unpredictability of the distribution patterns, mosaicism is challenging especially in prenatal diagnosis and genetic counselling.

6.P9 Whole genome NIPT confirmation of the high performance as a screening test

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During 2020, we implementated expanded NIPT (ExpNIPT).
The proposal was only for singleton pregnancies without any ultrasound sign and only when women asked specifically for it. To limit unnecessary invasive procedures, we chose to screen just a selection of chromosomal anomalies i.e., trisomies 2, 8, 9, 14, 15, 16, 22 and unbalanced structural rearrangement bigger than 7 Mb and compatible with a classical mechanism of chromosomal rearrangement (Kleinfinger et al, 2020).
We performed 1541 ExpNIPT. Indications were maternal serum screening risk (MSS) higher than 1/1000 (75.02 %), low risk population (primary screening or MSS under 1/1000) (21,15 %), previous pregnancy with trisomy (3.57 %) or parental chromosomal anomalies (0.26 %).
Results were normal in 98.25%.
We had one no call.
We reported 0.97% of cases with classical trisomies and 0.71% of cases with ExpNIPT anomalies. Among them, 5 cases presented aneuploidies: one trisomy 16 with normal karyotype on amniotic fluid (AF) (ongoing pregnancy), one trisomy 14 and one trisomy 15 with normal foetal karyotype and no uniparental disomy, one trisomy 14 while the foetal karyotype showed a balanced Robertsonian translocation, one trisomy 22 confirmed on AF. We found

6 unbalanced structural anomalies: all had amniocentesis.Two of these were confirmed (an isochromosome 12p and a terminal deletion of 18p). While excluding the case of trisomy 16, which could be considered as positive even when confined to placenta, the positive predictive value (PPV) was 40% (4/10).
To conclude, ExpNIPT for screening of a limited list of chromosomal anomalies generated only 0.71% of supplementary invasive procedures with a PPV of 40%. Even if more data are necessary, this test appeared already very helpful for the screening of foetal chromosome anomalies.

6.P10 Different genetic analyses in solving a complex prenatal case of 45,X/46,X,r(X)/46XX

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Nearly half of the Turner syndrome (TS) cases are mosaic carrying together normal and abnormal cell lines. Abnormal cells can be monosomic for chromosome X or with possible structural aberrations of X chromosome (isochromosome, ring chromosome etc.).
We report a prenatal case of mosaic Turner syndrome with presence of three cell lines: monosomy X, ring chromosome (X) and normal karyotype.
37-years-old woman underwent amniocentesis at 16th week of pregnancy due to high risk for monosomy X on noninvasive prenatal testing (NIPT). No anomalies were noted at ultrasound of the fetus. Interphase FISH (iFISH) analysis of uncultured amniotic fluid cells showed normal results for chromosomes 13, 18 and 21 and two X chromosome centromeric probe signals.
Chromosomal microarray analysis (CMA) from uncultured amniocytes showed one copy loss of the whole chromosome X approximately in 50% of cells with no other copy number changes.
To interprete the discordant NIPT, iFISH and CMA results, chromosome analysis from cultured amniocytes was peformed. Three different cell lines were found: cell line with X monosomy (24%), cell line with 46 chromosomes missing one X chromosome and having a tiny marker chromosome (24%) and cell line with normal female karyotype (52%).
FISH analysis with X-chromosome centromere probe showed positive signal on the small marker chromosome. Thus it was a very small ring chromosome (X) containing only pericentromeric region which is not covered with SNP markers in CMA.
The final fetal karyotype is: mos 45,X[8]/46,X,r(X)(p11q12)[8]/46,XX[18] which is consistent with all results of different analyses performed. The fetus has mosaic form of TS with X monosomy approximately in 50% of cells, which was also shown at CMA. Due to the cell line with tiny ring (X) the iFISH showed normal result - two copies of X chromosome.

6.P11 Challenges in prenatal diagnosis – a case report

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Mosaic trisomy 12 is a rare chromosomal disorder with few cases diagnosed. Phenotypic variability is wide ranging from normal individuals to severe congenital anomalies. A low-level mosaic trisomy 12 detected in amniocentesis can be related with a favorable outcome, however, it should raise a suspicion of congenital defects. The authors present a trisomy 12 mosaicism detected in a prenatal diagnosis.
A 38-year-old woman underwent amniocentesis at 16 and repeat at 18 weeks of gestation. Cytogenetic and FISH (12 chromosome probe) were performed.
The cytogenetic analysis at 16 weeks of gestation revealed a 47,XY,+12[7]/46,XY[32] in two independent cultures. In the second sample, FISH in uncultured amniocytes revealed an incidence of only 0.42% (2/478) for trisomy 12 and the karyotype was normal. After genetic counseling, the couple decided to interrupt pregnancy. The chorionic villi (CV) showed a 47,XY,+12[10]/46,XY[2] karyotype and FISH revealed 35% cells with trisomy 12. In fetus skin all metaphases analyzed were normal but FISH was positiv in 4% of the nuclei. The anatomo-pathological study of the placenta with fetus revealed the presence of minor dysmorphic features, without structural, cardiac and central nervous system alterations.
The detection of a mosaic in prenatal analysis is always a problem and when the chromosome involved is not frequent the situation worsens. Teamwork is essential, and it is very important to have a good coordination between the obstetrician, laboratory cytogeneticists and the clinical geneticist. Genetic counseling is complicated and the couple must be able to decide with complete knowledge of the situation.

6.P12 Trisomy 22 on NIPT and CVS: an unnecessary "therapeutic" termination of pregnancy avoided by genetic counseling, fetal ultrasound and karyotype examination on AF sample

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A 39-year-old female patient at 18 weeks gestation came to our facility to terminate her pregnancy following a prenatal diagnosis of non-mosaic trisomy 22 on a chorionic villus sample (cytotrophoblast and mesenchyme) performed after a non-invasive prenatal testing (NIPT) positive for trisomy 22.
An ultrasound examination showed a fetus with normal growth parameters and no ultrasound-visible abnormalities. We proposed to repeat fetal cytogenetic testing on an amniotic fluid sample. The examination of 50 metaphases showed a 46,XX karyotype. Interphase FISH showed 300 nuclei with 2 chromosome 22 signals and 3 nuclei with 3 signals. A SNP-Array analysis excluded uniparental disomy 22.
After extensive genetic counseling, the patient decided to proceed with the pregnancy. At the 38th week of gestation, a baby girl was born who weighed 2510 g (-1.66 SD, SGA). The child was in apparent good health and had no dysmorphic features. The placenta was normal in size but had areas of ischemic necrosis and intervillous thrombosis. The umbilical cord had an eccentric insertion and a tortuous course.
Cytogenetic examination of 100 metaphases from peripheral blood lymphocytes showed a 46,XX karyotype. Interphase FISH examination showed 303 nuclei with 2 chromosome 22 signals and 2 nuclei with 3 signals.
We believe that the case we observed is a mosaic trisomy 22 confined exclusively (or almost exclusively) to the placenta, without apparent consequences for the child. It can be classified as CPM III. We believe it may be useful to present it to emphasize the importance of confirming positive NIPT results by karyotype on AF and not on CVS, especially in cases of rare autosomal trisomies (RATs).

6.P13 Haplotyping of preimplantation embryos reveals parent of origin specific mechanisms of aneuploidy formation

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Chromosome instability is inherent to human IVF embryos, but the full spectrum and developmental fate of chromosome anomalies remain uncharacterized. Using haplotyping-based preimplantation genetic testing, we mapped the parental and mechanistic origin of common and rare genomic abnormalities in 2300 cleavage stage and 361 trophectoderm biopsies. We show that while single whole chromosome aneuploidy arises due to chromosome-specific meiotic errors in the oocyte, segmental imbalances predominantly affect paternal chromosomes, implicating sperm DNA damage in segmental aneuploidy formation. We also show that postzygotic aneuploidy affects multiple chromosomes across the genome and does not discriminate between parental homologs. In addition, 6% of cleavage stage embryos demonstrated signatures of tripolar cell division with excessive chromosome loss, however hypodiploid blastomeres can be extruded from further embryo development. Taken together, our data provides further insight to guide embryo selection strategies. Considering that ploidy violations may constitute a significant proportion of non-viable embryos, using haplotyping-based approach to map these events might further improve IVF success rate.

6.P14 Prenatal diagnosis of three cases with Pallister Killian syndrome

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Pallister-Killian syndrome (PKS) is rare chromosomal abnormality characterised by mosaic tissue specific distribution of supernumerary isochromosome 12p. Prenatal sonographic findings suggestive for this aneuploidy are diaphragmatic hernia, ventriculomegalia, polyhidramnios and micromelia of rhizomelic type, although they are not always present. In the first trimester an increased nuchal translucency or cystic hygroma could be found. We

present three prenatally diagnosed cases of Pallister-Killian syndrome.
In the first case, indication for prenatal invasive diagnosis was advanced maternal age and rhizomelic limb shortening. Cytogenetic analysis of cultured amniocytes revealed a male karyotype with additional isochromosome 12p present in 25% of analyzed cells. FISH analysis was used for i(12p) characterization. In the second case amniocentesis was performed due to increased nuchal translucency and rhizomelic limb shortening. A 47,XY+i(12)(p10)/46,XY karyotype with 70% of cells with i(12p) was disclosed. Array CGH and FISH analysis confirmed the diagnosis of Pallister-Killian syndrome. In the first two cases parents opted for termination of pregnancy. In the third case amniocentesis was preformed after first trimester ultrasound finding of fetal cystic hygroma measuring 5 mm. A mosaic karyotype with normal male karyotype in 73% and additional i(12p) in 27% of cells was revealed by classical cytogenetic analysis and FISH, and the characterization of i(12p) was performed using aCGH. Ultrasound examinations at 15 and 19 weeks of pregnancy showed no other structural anomalies. Fetal echocardiography was normal. Parents decided to continue the pregnancy, which is still in progress.
Prenatal diagnosis of Pallister-Killian syndrome is difficult due to tissue limited chromosomal mosaicism and variable phenotype. Optimal prenatal invasive procedure is amniocentesis. FISH and array CGH present a gold standard in diagnostic strategy due to rapid determination of origin of marker chromosome and the level of mosaicsm in a wide variety of tissues.

6.P15 Cell based NIPT and cell-free NIPT in a pregnant woman with mosaicism for Turner Syndrome

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Background: Cell-based Non-invasive Prenatal Testing (cbNIPT) uses extravillous trophoblasts (EVTs) from maternal blood and is comparable with cell-free NIPT (cfNIPT) for the detection of aneuploidy, but the methods differ. This case demonstrates the differences between these methods.
Materials and Methods: A woman with mosaicism for Turner syndrome (27% 45,X in blood) was finally pregnant (GA 10+3) after miscarriages (G4P0). For the cfNIPT analysis (cfNIPT), DNA extraction, genome-wide massive parallel sequencing, and data analysis were carried out as described by the kit manufacturer (Illumina©, San Diego, CA, USA) applying TG TruSeq®_Nano_DNA kit v1.0 + TG_NSQ_500/550 High Output Kit v2.5 and VeriSeq NIPT-Analysis Software v1.
For cbNIPT, three EVTs were enriched from 30 mL blood using a cocktail of antibodies for magnetic activated cell sorting followed by single-cell isolation using FACS. Each isolated cell was subject to whole genome amplification and if non-maternal origin was verified using STR analysis then arrayCGH (Agilent oligoarray,180 kb) was performed.
Results: The cffDNA fraction was 7%, and the cfNIPT analysis showed 2 copies of chromosomes 13, 18 and 21. The result was inconclusive regarding X-chromosomal material, but showed a decreased proportion of X-chromosomal material, compatible with Turner syndrome. No Y-chromosomal material was detected. The results of the arrayCGH of the three EVTs were all compatible with a normal girl, no aneuploidy.
Conclusion: cbNIPT has the potential to screen for aneuploidies and sex chromosome aberrations in a woman with mosaicism for Turner syndrome, where cfNIPT gave an inconclusive result suggesting Turner syndrome in the fetus.

6.P16 Atypical findings from Non Invasive Prenatal Testing: A Case Report

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Background:Non-invasive prenatal testing (NIPT) measures free fetal DNA in the maternal circulation to provide an estimate of risk for the common fetal trisomies. SNP-based NIPT uses common genetic variants to calculate chromosomal number. Atypical findings can increase anxiety and often yield unanticipated results
Case report:A 42yr old woman with an IVF pregnancy, para 0+0, underwent NIPT at 11 weeks gestation. Maternal weight was 57kg with a BMI of 23kg/m2. Panorama™ test reported low fetal fraction of 2.7%. There were no first trimester U/S findings suggestive of aneuploidy. Redraw NIPT was performed and an atypical finding outside the scope of cell free fetal DNA testing was reported. Invasive testing was offered and after considering her options, the patient underwent amniocentesis at 19 weeks gestation despite the absence of fetal anatomical concerns.
Partial trisomy 21 was identified on amniocentesis PCR however fetal karyotyping revealed a normal male fetal karyotype, 46,XY. Array comparative genomic hybridisation revealed a 4.64Mb copy number gain of uncertain clinical significance within the 21q21.1q21.2 region of the long arm of Chromosome 21 (arr[GRCh37] 21q21.1q21.2 (19886439_24526668)x3). The patient began exploring options to proceed to termination of

pregnancy however she was counselled strongly to await parental genotyping array-CGH.
The paternal genotyping revealed the father to be a heterozygous carrier of the same copy number gain, with a normal karyotype 46,XY. The fetus therefore inherited this copy number gain from his phenotypically normal father. Fetal growth was normal throughout the pregnancy and a normal male infant was born by emergency caesarean section at 38 weeks gestation due to breech presentation in labour, weighing 3.24kg.
Discussion:A high risk or inconclusive result on NIPT should always be confirmed by invasive testing. In this case, PCR of fetal cells obtained via amniocentesis also delivered an uncertain result and reassurance could not be provided until karyotyping, array-CGH and parental testing were completed. This case highlights the importance of obtaining a full genetic profile of a fetus before acting on results.

7. Tumour Cytogenomics

7.P1 Cytogenetic complexity in T-cell Prolymphocytic Leukemia

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Introduction: T-cell Prolymphocytic Leukemia (T-PLL) is an extremely rare disease characterized by the out of control growth of mature T-lymphocytes with a very aggressive clinical course. The genetic hallmark of T-PLL is inv(14)(q11q32) but it usually harbors additional changes.
Aim: In this study, we report 6 cases of de novo T-PLL and discuss the distinctive clinical, morphological, immunophenotypic, cytogenetic features and outcome of this entity.
Patients-Methods: Our cohort included 4 men and 2 women with a median age at diagnosis of 73.5 years (range, 45-83). Cytogenetic analysis was performed at diagnosis in 5/6 and in relapse 1/6. Flow cytometry confirmed T-PLL with a T-helper (CD4+/CD8-) phenotype. Five patients had splenomegaly, 2 hepatomegaly, and 3 had developed lymphadenopathy.
Results: All patients had complex karyotype (CK) with 5-13 abnormalities (median 10.5). Five patients had inv(14)(q11q32) and one translocation involving the band 14q11. The most common aberration found in all pts was an abnormal chromosome 8, with loss of 8p in 4 and gains 8q in 2. Deletions of chromosomes 17p, 11q, 6q, 12p were observed in 2, 4, 4, and 2 patients respectively. Five patients received 1-3 lines of treatment (median: 2). Three out of 5 patients received Alemtuzumab, with 1 achieving complete response (CR), 1 partial response, while 3 had no response. Two patients underwent allo-HSCT: one is alive in CR 60 months from diagnosis and the other patient died 28 months from diagnosis and 18 months after allo-HSCT in CR due to sepsis. At the end of the study, 2 patients were alive, with follow-up from diagnosis of 1-60 months (median 15.4) in the whole cohort. Median overall survival (OS) after cytogenetic analysis was 3,47 months.
Conclusion: T-PLL is aggressive mainly in those with complex karyotype and often resistant to conventional treatment. Allo-HSCT is associated with more durable outcomes.

7.P2 Cytogenetic abnormalities in secondary Acute Myeloid Leukemia (sAML): after antecedent hematologic disorders (sAML - AHD) versus therapy-related (tAML)

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sAML-AHD has been recently linked with better outcomes compared to tAML. We studied the prevalence, the variety, and the patterns of chromosomal abnormalities as well as their impact on disease outcomes in sAML-AHD and tAML patients. We studied 149 patients with sAML with Median age 58 years old, M/F: 85/64. Cytogenetic analysis was performed in 145/149 patients (44 tAML and 101 sAML). Karyotype was normal in 58. Abnormal karyotype was found in 87 (29/44 cases in tAML, and 58/101 in sAML-AHD). Favorable karyotype was observed in 4 patients with tAML: t(8;21) in 3 and inv(16) in one. Only one patient in sAML-AHD had inv(16)(p=0.013). Poor cytogenetics were detected in 55 patients (tAML: 20/44, 45.4% and sAML-AHD: 35/101, 34.6%. Standard risk karyotype was detected in 85 patients (tAML: 20/44, sAML-AHD: 65/101 (p=0.033) and complex karyotype in 37 patients (tAML:13/44, sAML-AHD:24/101). Thirteen out of 29 tAML patients had a median number of 8 abnormalities while 24/58 in sAML-AHD with median number of 6 abnormalities. Chromosome 7 abnormalities were the most prevalent ones (29/87). Abnormalities of chromosome 11 were found in 19 patients. Abnormalities of 11q were more prevalent in tAML (p=0.00027). No correlations with any other chromosomal aberrations were observed. Complete remission was achieved in 63/149 patients (20/45 tAML and sAML-AHD 43/104). Refractory disease and relapse rates were high (78/149 and 37/63) in both groups. The only significant difference between sAML-AHD and

tAML was age (p=0.003). Survival outcomes were dismal (125/145 died), with no significant difference between the two groups. In conclusion, abnormal and complex karyotype was detected in high frequency 60% and 42.5% respectively in both groups without any statistically significant difference. Novel targeted treatments or referring patients to alloHCT are warranted.

7.P3 Increased diagnostic yield for FISH analysis in Multiple Myeloma using bone marrow samples with ≥ 0.5% myeloma cell fraction by immunophenotyping

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Introduction: Multiple myeloma (MM) is a genetically complex plasma cell neoplasm that evolves from pre-malignant stages following genomic evolution, leading to the proliferation of malignant plasma cells and production of monoclonal immunoglobulins. Diagnosis and classification of MM rely on morphology, immunophenotyping, chromosomal abnormalities, and molecular genetics analyses. Specifically, FISH analysis is important in determining the prognosis of MM. Our study illustrates higher diagnostic yield of cytogenetic testing if an inclusion criterion of MM cell fraction ≥0.5% is applied.
Materials and Methods:A hundred and ninety bone marrow samples of patients with suspected newly diagnosed or relapse MM were immunophenotyped for MM cell fraction between February 2019 to February 2020.
A subgroup of samples was selected based on MM cell fraction ≥0.5% for inclusion in the cytogenetics analysis. Enrichment for CD138+ cells from bone marrow samples was performed using MACSprep MM CD138 Microbeads autoMACS Pro Separator Kit. FISH was performed using P53/DLEU, CDKN2C/CKS1B, IGH, IGH/CCND1, IGH/FGFR3, IGH/MAF and IGH/MAFB, centromeres 7, 9, 11 and 15 probes.
Results: Initially, 50 patient samples underwent FISH testing without applying a cell fraction criterion. In this group, 12 samples were < 0.5% MM cells and had normal FISH; samples ≥ 0.5% MM cells had abnormal FISH. Following this observation, we performed FISH on 62 additional samples passing the criterion of ≥0.5% myeloma cells; only one had normal FISH. Altogether, 99% of samples ≥ 0.5% MM cells had an abnormal result.
Conclusions: Our study highlights the limitations of cytogenetic evaluation for myeloma bone marrow samples with myeloma cell burden below 0.5% by immunophenotyping. To get reliable cytogenetic results with specific clinical implications, we suggest performing FISH analysis on samples with ≥ 0.5% MM cells.

7.P4 Comparative study of MDS between the Western and Asian people from cytogenetics to clinical characteristics

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The world population is genetically and environmentally diverse. In particular, genetic differences related to an ethnic factor may underlie differences in cancer phenotypic expression. Therefore, we systematically compared the genetic characteristics including the cytogenetic and gene mutation of myelodysplastic syndrome (MDS) between Asian and Western countries over the last 20 years. We found that 5q deletion (del(5q)) aberrations and mutations in TET2, SF3B1, SRSF2, and IDH1/2 are more frequently reported in Western MDS patients, while trisomy 8, del(20q), U2AF1 and ETV6 mutations are more frequent in Asian MDS patients. Next, we investigated whether the cytogenetic differences are related to the epidemiology and the clinical characteristics of MDS. We found that Asian MDS cases show a 2- to 4-fold lower incidence and a 10-year younger age of onset compared to the Western cases. A higher proportion of Western MDS patients fall into the very low- and low-risk categories while the intermediate, high and very high-risk groups are more represented in Asian MDS patients, according to the Revised International Prognostic Scoring System. Treatment approaches differ between Western and Asian countries owing to the above discrepancies, but the overall survival rate within each prognostic group is similar for Western and Asian MDS patients. Altogether, our study highlights the cytogenetic variation of MDS in a different area, greater risk MDS in Asians supported by their genetic profile.

7.P5 Laboratory hunting for Ph-like acute lymphoblastic leukemia

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Introduction. Philadelphia chromosome (Ph)-like acute lymphoblastic leukemia (ALL) is characterized by an expression profile similar to Ph+ ALL, but lacking the BCR/ABL1 fusion gene. As it can only be reliably detected by expression profiling, there is still no standardized diagnostic test enabling its prompt recognition. Here we introduce an approach how to detect it by a combination of widely available cytogenetic and molecular genetic techniques.
Methods. Data on immunophenotype and cyto-genetics of 179 ALL patients diagnosed during the last 8 years were analysed to select patients with potentially Ph-like ALL. CRLF2 gene rearrangement was tested by FISH (59/65 patients) and next generation sequencing was done by Archer FusionPlex ALL kit in 34 patients.
Results. (Cyto)genetic aberrations detected in ALL patients using standard diagnostic work up served to select cases with fusions mutually exclusive with Ph-like ALL. In 6 (10%) of further tested samples CRLF2 rearrangement was confirmed. While CRLF2/P2RY8 fusion gene was later confirmed also by NGS, IGH/CRLF2 was detected by FISH only. Due to a lack of material, NGS was performed only in a half of potentially Ph-like cases, confirming other Ph-like fusions in 10% of tested samples again. Three fusions involved in tyrosin kynase signaling were found, two from the JAK-STAT (EPOR/IGH and PCM1/JAK2), and one from the ABL signaling class (TRF2/PDGFRb). Using FISH analysis, rearrangements of genes IGH, JAK2, and PDGFRb were later confirmed. The patients with the confirmed Ph-like ALL (N=9) shared the main characteristics of this genetic subgroup; they were predominantly males (7/9), young adults (4/9) with normal karyotype (7/9), and poor disease course (8/9).
Conclusions. The obtained frequencies, genetic, and patients' characteristics were in accordance with the literature data, which confirms that the proposed approach allows reliable detection of Ph-like ALL at reasonable costs and acceptable workload applying widely available techniques.

7.P6 Tendencies of cytogenetic analysis of patients with acute myeloid leukemia – an 11 year single centre experience report

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Introduction: Conventional cytogenetic analysis (CCA) - a relatively dated method, still holds a valued position in the diagnostic and evaluation algorithm of hematological diseases, despite the constantly evolving genomic era. Hereby, we focus on the heterogeneous Acute myeloid leukemia (AML) with a diagnostic process that requires routine CCA on bone marrow samples.
Materials and methods: We have conducted a retrospective analysis on patients with current diagnosis AML who underwent CCA in the Laboratory of Medical genetics, UMHAT "St. Marina", Varna, for eleven years – 01.2010 through 12.2020. Karyotyping was performed with GTG-banding technique in accordance with the International System for Human Cytogenomic Nomenclature.
Results: We have assessed 723 samples of 422 patients – 225 (53.3%) males, 197 (46.7%) females, and 11 (2.6%) of all - children. 125 (29.6%) patients were tested more than once in order to monitor their treatment response and overall disease evolution. Out of 606 successful CCA with a resolution mode 150-200 bands, karyotype was normal in 369 (60.9%) and abnormal in 237 (39.1%). The latter was mainly presented by complex karyotype in 83 (13.7%), trisomy 8 in 14 (2.3%), inv(16) or t(16;16) in 13 (2.1%) and t(8;21) in 10 (1.7%). According to the European Leukemia Net (2017) risk stratification, 23 (3.8%) belonged to the favourable, 469 (77.4%) - to the intermediate, normal karyotype included, and 105 (17.3%) - to the adverse risk group. AML comprised 29.6% of all bone marrow samples for the period – a share steadily growing through the years for both new and re-assessed patients.
Conclusion: CCA is an essential part of the AML evaluation process since cytogenetic findings carry certain prognostic and diagnostic values. Noteworthy limitations in terms of resolution, turnaround time and possible lack of success lead to a marked necessity of subsidiary molecular-genetic method.

7.P7 Conventional Cytogenetics in a developing country; achieving highest quality testing with CAP standards. A rewarding challenge

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Introduction and Objective: Cytogenetics, a complex, yet the gold standard, genetic testing in hematopoietic neoplasms, some solid tumors, prenatal diagnoses, newborn dysmorphology, history of spontaneous abortions and endocrinology

disorders. Cytogenetics is a bird's eye view of the whole genome. Reliability and accuracy of the results require the highest level of competency. The current study highlights the challenges of initiating and implementing this testing, especially in developing countries, lacking the presence of a well-structured training program.
Methods: 40 hematological malignant bone marrows were included in an optimization and validation program, from July 2019 to July 2020 at Indus Health Network, Karachi, Cytogenetics department. A pre-planhned model was prepared to overcome challenges such as Hematopathologist training, lab infrastructure, establishing standard operating procedures, technique optimization, technologists training and competency assessment as well as standardizing a concise and clear reporting system. An accuracy study was conducted to measure our ability to report such a complex testing using the international nomenclature in parallel with a national reference laboratory and an international CAP accredited laboratory. Evaluation for chromosome abnormalities recognition and proper 2016 ISCN karyotype nomenclature was performed between the three laboratories.
Results: 40 patient samples, age range of 1-60 years. 77.5%(1/40) B-ALL, 2.5%(1/40) T-NHL, 5%(2/40) AML, 5%(2/40) CML, 5%(2/40) ET/Myelofibrosis and 5%(2/40) MDS. Compared to the International laboratory, our results showed 92.5% (37/40) of concordant cases. Minor discordances, related to subtle changes or nomenclature differences, were found in 3 cases. The discordances had no impact on patient's outcome, since the cases showed multiple abnormalities and were originally stratified in the unfavorable prognosis group. The national reference laboratory had 60% concordance with 40% discordance compared to international laboratory.
Conclusion: These results highlighted the signifi-cance of a well-structured strategy for the initiation and implementation of a highly complex testing following international standards to produce accurate and reliable results. However, with experience, the technique will be further enhanced. The international partnership and the continuous collaboration and review of cases is a great help in achieving highest competency.

7.P8 Comparing the genomic profiles of Glioblastoma with the peritumoral zone and their primary isolated cells reveals specific signatures

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Glioblastoma (GBM) is the most common and malignant brain tumour. Treatment failure and tumour recurrence are due to the extreme heterogeneity of GBM, which primarily reflects the coexistence in the tumour core (TC) of different cell populations, including the Glioma Stem Cells (GSCs), and secondly the contribution, still to be defined, of the peritumoral brain zone (PBZ).
In this work, using array-CGH platform, we investigated the genomic profiles of 15 TCs and 7 PBZs of GBM; in addition, for 9 cases and 4 cases, respectively, we compared the genomic profiles with those of the related primary cell cultures (GSCs and PBZ cells).
The preliminary analysis on 15 TCs confirmed the intertumor heterogeneity and a high percentage of copy number alterations (CNAs) in GBM canonical pathways. In particular, PI3K pathway was affected in 73%, Rb pathway in 67% and p53 pathway in 20% of TCs. In addition, the CNA load correlates with a worst prognosis. Comparing the genomic profiles of 9 TCs with the matched GSCs we evidenced a robust similarity, albeit with an evident clonal selection. Furthermore, a correlation is observed between the percentage of shared CNAs and worst prognosis. The comparison between 7 TCs with their matched PBZs did not show a good sharing of the genomic profiles, highlighting PBZ exclusive alterations and a possible correlation between PBZ CNA load and worst prognosis. Similarly, the genomic profiles of 4 PBZs and the relative PBZ cells, did not show a good correlation. The same emerged from the comparison between the genomic profiles of the two types of cell cultures, matching GSCs with PBZ cells.
Our data confirmed the preservation of the genomic profile from TCs to GSCs, strengthened by bioinformatics analysis using DAVID, which identified an impairment of pathways related to the development and progression of cancer and focal adhesion. Finally, we improved the knowledge of specific PBZ genomic profiles.

7.P9 A genetically engineered in vitro model to understand infant AML with t(7;12)(q36;p13)

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The mechanisms behind infant Acute Myeloid Leukaemia (0-2-year olds) are poorly understood. A particularly intriguing and aggressive subtype carries the chromosomal translocation t(7;12)(q36;p13) involving the MNX1 and ETV6 genes. Although previous studies have shown that t(7;12) is likely to result from the ectopic overexpression of the MNX1

gene rather than from the formation of a fusion oncogene, the lack of a research model and the limitations imposed by available genetic tools has hindered understanding of disease biology. Thanks to the advances in gene editing technologies, we have recreated the leukaemia-associated t(7;12) in vitro using a vector-free CRISPR/Cas9 approach, to serve as a research model. By recapitulating breakpoints seen in t(7;12) patients, we designed guide (sg)RNAs to be delivered as RNP complexes via electroporation. We successfully recreated the translocation in the K562 leukaemia cell line and in human CD34+ haematopoietic progenitors, demonstrating that the rearrangement is achievable in vitro. The genomic and cytogenetic features of the engineered translocation were confirmed by FISH and PCR amplification of genomic fusion junctions. Nonetheless, the translocation was progressively lost in extended liquid cultures and serial replating colony-forming assays, suggesting that it may not confer sustained proliferative or survival advantage. As suggested by earlier studies, the presence of t(7;12) alone might not be sufficient for the establishment and/or maintenance of the malignant clone. This genetically-engineered model is a promising tool to better understand the genetic and phenotypic implications of this specific rearrangement in the initiation and progression of its associated infant leukaemia.

7.P10 Impact of cytogenetic aberrations on outcomes of allo-HSCT patients with myelodysplastic syndromes

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Introduction. Cytogenetics is an important prognostic factor in patients (pts) with myelodysplastic syndrome (MDS). However, the current scheme for assessing cytogenetic risk is based on patient groups treated with chemotherapy only, but not allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Aim. To evaluate the impact of cytogenetic aberrations upon outcomes of allo-HSCT in a mixed cohort of patients with MDS.
Material. The study included 70 patients with de novo and secondary MDS (62 pts and 8 pts, respectively), who had undergone allo-HSCT in our Institute from 2008 to 2020. The age of the patients ranged from 1 to 67 years (median - 36 years).
Results. Cytogenetic abnormalities were detected in 45 (64%) pts. All patients were divided into 5 prognostic groups according to IPSS-R. The "very good" and "good" prognostic groups included 2 (3%) and 23 (33%) pts, respectively. The "intermediate" prognosis group consisted of 11 (16%) pts, the "poor" and "very poor" prognosis groups were presented with 27 (38%) and 7 (10%) pts, respectively. A complex karyotype (CK) was presented in 8 (11%) pts. One abnormal cytogenetic clone was identified in 41 (58%) pts, two or more abnormal clones were in 11 (16%) pts. In univariate analysis, the probability of 3-year overall survival (OS) in the common "very good, good, intermediate" group, "poor" and "very poor" cytogenetic groups was 63%, 45% and 25%, respectively (p=0.015). Patients without CK had significantly higher OS than those with CK (56% vs. 25%, p=0.015). OS and EFS were significantly higher for pts with one abnormal cytogenetic clone compared with patients with ≥2 abnormal clones (55% vs. 11%, p=0.0008 for OS; 43% vs. 9%, p=0.03 for EFS).
Conclusion. The study showed the prognostic value of chromosomal aberrations remains important in the transplant cohort of patients with MDS.

7.P11 Copy number intratumor heterogeneity predicts relapse in chemotherapy-naive stage II colon cancer

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Intratumor heterogeneity (ITH) driven by chromosomal instability (CIN) fuels genome evolution prompting metastatic progress and therapy failure. In stage II colon cancer (CC), around 10-15% of patients experience a recurrence after curative intended surgery. Here, we investigate the prognostic

and predictive value of tumor aneuploidy and ITH for disease relapse in stage II CC. We combined SNP array, targeted next-generation sequencing, FISH and immunohistochemistry analyses on a retrospective cohort of 84 untreated stage II CC patients. Levels of ITH were assessed by measuring the proportion of subclonal copy-number alterations (CNAs) and mutations, defined as present in <85% of the cancer cell population. Genomic and histopathological markers were tested for their association with time-to-recurrence (TTR), and downstreamed to machine-learning analysis for prediction of individual relapse risk. Tumors from recurrent patients (N = 38) exhibited greater proportion of CNAs compared to non-recurrent (N = 46) (mean 31.3% vs. 23%; P = 0.014). Candidate chromosome-specific aberrations displaying higher prevalence in relapsed cancers included the gain of chromosome arm 13q (P = 0.02) and loss of heterozygosity at 17q22-q24.3 (P = 0.03), both associated with shorter TTR. CNA load positively correlated with ITH levels (R = 0.52; P < 0.0001), indicating ongoing CIN. Multi-probe FISH evinced increased copy-number diversity of the chromosome 13q in relapsed lesions (P < 0.0001). Consistently, subclonal copy-number heterogeneity was associated with elevated risk of relapse (P = 0.028; hazard ratio, 2.20), which we did not observe for subclonal mutations. The clinico-genomic model rated an AUC of 0.83, achieving a 10% incremental gain compared to clinicopathological markers alone. In conclusion, tumor aneuploidy and copy-number ITH were predictive biomarkers of disease recurrence in early-stage CC, and improved the discriminative performance in comparison to clinicopathological information.

7.P12 Analysis of cytogenomic and clinical features of pediatric T-acute lymphoblastic leukemia (T-ALL)

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T-ALL is a clinically and genetically heterogeneous disease that comprises 10-15% of newly diagnosed pediatric ALL. Despite the use of intensive chemotherapy, relapse occurs in approximately 20% of patients whose outcome remains dismal. Although several recurrent genetic aberrations were identified, their concurrent occurrence and the prognostic implication remain unclear.
We performed retrospective molecular cytogenomic analysis of bone marrow cells of children with T-ALL with the aim to determine recurrent chromosomal aberrations and their combinations and to identify those with significant impact on survival. Diagnostic samples were analyzed by conventional and molecular cytogenomic methods, i.e. FISH (Abbott Molecular, Dako), mFISH/mBAND (MetaSystems), MLPA (MRC Holland) and CGH/SNP array (Agilent). For overall/event-free survival Kaplan-Meier analysis with Mantel-Cox test was done.
We examined archived material of 66 children with T-ALL (20 girls and 46 boys, median age 7.9 years). Chromosomal aberrations were detected in 91% of patients. The most frequent were the deletion of CDKN2A gene in 46/66 patients (29x homozygous, 17x heterozygous), rearrangements of TCR loci in 18/66 children (11x TRA/TRD, 7x TRB), and TLX3 gene rearrangement in 15/66 cases. All chromosomal changes occurred in various combinations, however, TLX3 aberrations never appeared along with TCR loci rearrangements. 48 patients are living in the first (45x)/second (3x) complete remission, 18 children died. The best outcome was associated with TRA/TRD locus translocations. Conversely, patients with TLX3 rearrangement had a worse prognosis.
We detected chromosomal aberrations in the vast majority of children and showed that they are more likely found in various combinations than individually. Nevertheless, TRA/TRD locus translocations correlated with favorable and TLX3 rearrangements with poor outcome independently of additional aberrations. Thus, they could facilitate patients´ risk stratification, especially identify those at an increased risk of relapse.
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7.P13 Chromosomal instability in HAP1 cell lines revealed by multiplex fluorescence in situ hybridisation (M-FISH)

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HAP1, a near-haploid human cancer cell line, is widely used in gene editing by CRISPR/Cas9 technology for genetic screens, since its haploid nature means only one allele requires modification. It carries the Philadelphia chromosome and an

additional 30Mb fragment of chromosome 15 inserted into chromosome 19.
Being a cancer cell line, HAP1 is prone to genetic instability, which is further compounded by its tendency for 'diploidisation'. CRISPR/Cas9 gene editing coupled with prolonged in vitro cell culturing can cause unintended 'off-target' cytogenetically visible mutations.
To gain an insight into chromosomal instability (CIN) and karyotype heterogeneity we characterised 19 HAP1 cell lines, including two double haploids, using M-FISH, a single cell-based assay. We focussed on novel numerical (N) and structural (S) CIN and explored the potential causal factors that triggered this. For each cell line we examined its ploidy, gene editing status and its length of in vitro cell culturing.
Sixteen of the 19 cell lines had been edited with passage numbers ranging from 10 to 35. Diploidisation in the 17 near-haploid cell lines ranged from 4% to 33% and percentage of CIN in [1n & 2n] metaphases ranged from 7% to 50%, with two cell lines showing no CIN. The most common S-CIN observed were translocations, both non-reciprocal and Robertsonian along with gain of deleted chromosomes, in [1n] metaphases. Interestingly, 11 cell lines showed a prevalence of S-CIN associated with chromosome 13, with Robertsonian translocation being the most common. Furthermore, we showed for the first time that the additional chromosome 15 fragment is inserted into 19p rather than 19q.
In summary, our study has revealed karyotype instability in most HAP1 cell lines and emphasizes the importance of maintaining the cell lines at low passage and the need for regular monitoring to prevent karyotype heterogeneity which may have implications for downstream applications.

7.P14 Duplication of the long arm of chromosome 1 in primary myelofibrosis is a malignity factor

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Primary myelofibrosis (PMF) is one of the Myeloproliferative neoplasms (MPN), which presents a preferential proliferation of megakaryocytes and granulocytes in the bone marrow (BM). One of the causes of morbidity and mortality in PMF is the progression to Acute Myeloid Leukemia (AML).
We present a clinical case, of a female individual, 68 years old at the time of the initial diagnosis, who presented moderate anemia and thrombocytosis, and diagnosed as myeloid metaplasia with myelofibrosis. The karyotype performed in the BM, resulted in a duplication of the long arm of chromosome 1, del(1)(q21q32). The patient remained stable and without therapy for 5 years having performed a myelogram at this time, and a bone biopsy that showed an advanced myelofibrosis. In parallel, cytogenetic studies and search for V617F mutation in the Jak2 gene, indicated the absence of the mutation V617F, and confirmed the presence of the dup(1)(q21q32). the patient started therapy with an erythropoietin substitute. Currently, with 8 years of evolution of the disease, she has no clinical complaints, without transfusions and maintaining therapy.
The dup(1)(q21q32) associated with MPN is a rare anomaly and is associated with AML evolution. Since the patient understudy did not evolve to AML, FISH, and high-resolution microarray studies were performed. The studies confirmed the observed breacpoints and did not show other changes Based on the patient's clinical history and results, we suggest that the dup(1)(q21q32) alone does not induce an evolution to AML and that the duplication of genes correlated with this pathology (ex: ARNT, among others) is not a sufficient factor for the development of a more aggressive progression. However, more studies should be carried out in order to clarify the role of this alteration in NM.

7.P15 Cryptic translocation t(5;11)(q35;p15) resulting in NUP98 / NSD1 gene fusion in adults with de novo acute myeloid leukemia (AML)

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The NUP98/NSD1 fusion, a product of the cryptic translocation t(5;11)(q35;p15.5), is a recurrent genetic change in cytogenetically normal patients with AML. It occurs most frequently in children (16%) and young (2%) AML patients, very rarely in adult patients.

The coexistence of internal tandem duplication of FLT3 gene (FLT3/ITD) found in more than 70% of NUP98/NSD1 positive patients is always associated with a poor prognosis and results in high frequency of induction failure.
The aim of this study was to determine the incidence of the NUP98/NSD1 fusion gene in adults with AML and NUP98 rearrangement.
Since 2017, we examined the bone marrow cells of 268 newly diagnosed AML patients using conventional karyotyping in combination with molecular cytogenomic techniques: FISH (Abbott, MetaSystems), mFISH/mBAND (MetaSystems). We used RT-PCR followed by direct sequencing to detect fusion genes.
In nine out of 268 cases we identified rearrangement of the 11p13-15 region. We confirmed t(5;11)(q35;p15.5) with NUP98/NSD1 gene fusion in four of them (4/268; 1.5%). Sequence analyses proved the NUP98/NSD1 transcript, arising from fusion of NUP98 exon 12 with exon 6 in NSD1 gene, in all four patients (2M/2F; FAB M4/M5b; range, age 42, 54, 64 and 64 years). FLT3/ITD mutation was detected in all of them. Specific primers and a probe have been designed to monitor minimal residual disease during intensive therapeutic treatment of the patients.
Our study demonstrated the occurrence of t(5;11)(q35;p15.5) with NUP98/NSD1 gene fusion also in adults over 60 years of age. With respect to the poor prognosis of the patients with NUP98/NSD1 fusion gene, we suggest pre-screening of NUP98 gene rearrangement using FISH in cytogenetically normal AML patients with confirmed FLT3/ITD mutation.
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7.P16 Copy number alterations in HNSCC: clinical implications for therapeutic optimisation

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Over 90% of head and neck cancers are classified as squamous cell carcinoma (HNSCC) and more than 60% of HNSCC patients are diagnosed with locally advanced disease.
Induction chemotherapy prior to chemoradiation with cisplatin or cetuximab has been associated with survival benefits, though there are differences between patients. Hence, we queried whether genomic profiling could define tumour subtypes and clinical outcome in primary HNSCC.
We studied 144 primary HNSCC samples of patients within a multicentre clinical trial (TTCC-2007-01). DNA samples were processed for identification of somatic copy number alterations (SCNAs) using the OncoScan FFPE Assay while mutational status was determined by targeted massive sequencing on prevalent cancer-associated genes.
of 144 patients who received induction chemo-therapy, 123 showed complete response (CR) or partial response (PR), while 21 got stable disease (SD). In the whole series, gains on 3q and 8q and losses on 3p and 21p were the most frequent broad changes though no statistically significant differences were found between SD and CR+PR. Regarding focal changes (FCS), losses on 11q25 were more frequent in SD (80,95%) than in CR+PR (56,91%) ($p<0.05$). All cases with SD showed losses on 11q25 or FCS<90. NCAPD3, located in 11q25 locus, could be implicated in response to induction chemotherapy. Also, 81% of the SD group showed pathogenic mutations in TP53.
After induction treatment, 141 cases were randomized to receive radical treatment: cisplatin+RT or cetuximab+RT. 57 out of 97 analysed patients showed complete response (CR). We identified statistically significant genomic SCNAs associated with CR in both arms of treatment, such as the loss of the 1q22 region.
Altogether, this study identified several genomic regions of interest and a candidate gene, NCAPD3, associated with induction chemotherapy in HNSCC. Our results suggest that genomic alterations could be used as biomarkers for therapeutic optimisation.

7.P17 Balanced and unbalanced translocations in a multicentric series of 2,843 patients with chronic lymphocytic leukemia

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Chromosomal translocations in chronic lymphocytic leukemia (CLL) are very rare, and therefore systematic analysis of large series of cases are needed to allow the identification of recurrent rearrangements, breakpoints involved, as well as target genes affected. The aims of the present study were to identify new translocations and their clinical impact and to establish their frequency in a large cohort of 2,843 CLL patients. By conventional cytogenetics, 250 translocations were identified in 215 (7.5%) patients, 186 (74%) were apparently balanced and 64 (26%) were unbalanced. All chromosomes were involved in translocations, except Y chromosome. The chromosomes more frequently translocated were in decreasing frequency chromosomes 14, 18, 13, 17, 1, 6, 2, 3, 8, and 11. Translocations were found in the karyotypes either as the unique chromosomal abnormality (27%), associated with another alteration (24%) or as a part of a complex karyotype (48%). A large proportion of rearranged breakpoints involved genes related to CLL such as IGH (14q32), RB1, MIR15A, MIR16-1 (13q14), BCL2 (18q21), IGL (22q11.2), TP53 (17p13), IRF4, (6p25-p23), ATM (11q22) and CDK6 (7q21). Overall, 76 novel CLL translocations were identified, including a recurrent t(8;11)(p21;q21-23). Whole-genome sequencing and copy-number microarray data of 24 cases with translocations confirmed all rearrangements, enabled refinement of 3 karyotypes and all breakpoints at gene level. The projected survival and time to first treatment significantly decreased linearly with the number of translocations. In summary, this study allowed to establish the frequency of translocations (7.5%) and to identify new translocations in a cohort of 2,843 CLL patients.

7.P18 Importance of cytogenetic study in Non-Hodgkin Lymphoma: a case report

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Non-Hodgkin Lymphoma (NHL) is a type of cancer that affects the lymphatic system. It includes a number of clinicopathologic subsets of lymphoid neoplasms with heterogeneous features, that differ in molecular features, growth patterns and how they respond to different types of treatment. Waldenstrom Macroglobulinemia (WM) is an uncommon lymphoma characterized by the accumulation of lymphoplasmacytic cells that produce monoclonal immunoglobulin M (IgM). Mean age at diagnosis is 65 years and this disease may have an asymptomatic phase or the patient refers anaemia and asthenia in primary symptoms. 30% of WM patients will have a second neoplasm and complications resulting from marrow or other tissue infiltration of monoclonal IgM. The most common genetic testing is somatic mutation of MYD88 (L265P).
The authors report a case of a 79-year-old man with suspected LNH/ WM, with asthenia and nodular tissue in lung for study. Laboratory test showed monoclonal IgM (3.51 g/dL), anemia (9.80 g/dL) and blood imunophenotype was monoclonal small lymphocytes.
Cytogenetic analysis was performed using stimulator cultures (TPA and DSP/IL2) and FISH technique for the chromosome 13, 17, t(4;14) and t(11;14).
The karyotype was 46,XY,t(11;18)(q21;q21)[32]/ 46,XY[3] and normal FISH results. The present case has a balanced translocation between the long arms of chromosomes 11 and 18, which involves the API2 and MALT1 genes and lead to an API2-MALT1 gene fusion. The chimeric fusion transcript (API2-MALT1) induces a strong enhancement of NF-κB activity and several cell apoptosis inhibitors. This t(11;18) is described in marginal zone B-cell lymphomas of the mucosa-associated lymphoid tissue (MALT) type.
Conventional cytogenetics study was important for the diagnosis of this patient, since it enabled the identification of the NHL specific for this translocation and it offers critical information regarding to the prognosis and therapeutic strategy.

7.P19 Atypical deletions of 5q with retained commonly deleted regions (CDRs) in myelodysplastic syndromes (MDS)

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Interstitial del(5q) is a recurrent cytogenetic aberration in myelodysplastic syndromes (MDS). The

extent of del(5q) varies in individual cases, but region 5q31 is deleted in most of them. Two different commonly deleted regions (CDRs) have been identified: the proximal 5q31.2 CDR associated with a high-risk MDS, and the distal CDR 5q32–5q33 involved in the pathogenesis of MDS with isolated del(5q). However, rare cases of atypical del(5q) with retained known CDRs have also been reported. The aim of this study was to determine the frequency and clinical significance of atypical del(5q) in a large cohort of MDS patients.

During 1993-2019 we examined bone marrow cells of 3714 MDS patients by conventional G-banding and I-FISH (Abbott, MetaSystems). Extent of del(5q) was analyzed using mBAND (MetaSystems) and/or array CGH/SNP (Illumina, Agilent). del(5q) was detected in 920/3714 patients (24,8%). Most of them had large deletions spanning whole region 5q31 and both defined CDRs. Atypical deletions with retained CDRs were identified in 9/920 cases (1%; 4M/5F; median age, 73 years). In five patients del(5q) was a sole abnormality, in four cases it was detected in combination with additional chromosomal aberrations. In all patients, deletion was localized proximally to the 5q31 region. The size of the deleted segment ranged from 25.46 to 53.19 Mb (median 38.51 Mb) and the region 5q14.3 - q21.3 (26.79 Mb) was deleted in all nine cases. Many candidate genes, whose haploinsufficiency could lead to malignant transformation, have been identified in this region (for example CCNH, CHD, MAN2A1, ARRDC3, ELL2, etc.). of the nine patients, only one lives 5 months after diagnosis, eight patients died (median OS 13.5 months).

Our results suggest that del(5q) may occur outside the defined CDRs. Although these findings are extremely rare, they show that also genes located outside known CDRs may contribute to the malignant progression of MDS. The identification of these genes will lead to better understanding of the MDS pathogenesis and may contribute to identification of new therapeutic targets.

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7.P20 Effect of ovarian steroid hormones on heteroploid uterine leiomyoma cells in vitro

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Our previous study of uterine leiomyomas (ULs) with an apparently normal karyotype revealed cryptic tetraploid and monosomic cell subpopulations that were differently represented in cultured and uncultured tumors. This implies that their growth is regulated by the tumor microenvironment. Considering that ULs are ovarian steroid hormone-dependent tumors, in the present study we checked whether estrogen and progesterone have an effect on the frequency of heteroploid cells (tetraploid and aneusomic for chromosomes 7, 16, X) in culture conditions. Using interphase FISH with centromeric DNA probes (Vysis CEP7 (D7Z1), Vysis CEP16 (D16Z3), and Vysis CEPX (DXZ1)), we simultaneously analyzed the copy number of chromosomes 7, 16, and X in twelve ULs. In each UL, chromosome copy number was screened in 1000 cells cultured until passage 1 in the presence of 10-8 M estrogen and 10-6 M progesterone and in 1000 cells cultured without hormones (control). All ULs included both normal disomic cells representing a predominant subpopulation and heteroploid cells. The pattern of heteroploid cells mostly consisted of monosomic and tetrasomic cells. The frequency of heteroploid cells varied from 0.8% to 9.1% (mean 4.2%) in the control samples and from 1.1% to 4.7% (mean 2.8%) in samples treated with estrogen and progesterone. The samples treated with estrogen and progesterone had lower frequency of heteroploid cells compared to the controls (Wilcoxon signed-rank test, $p=0.0488$) and demonstrated the tendency to decrease the number of tetraploid cells and increase the number of cells with monosomy X. Our results suggest a specific effect of ovarian steroid hormones on in vitro growth of UL cells with numerical chromosome abnormalities, thus providing new directions for the studies on the hormonal treatment of ULs with abnormal karyotype.

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7.P21 Molecular cytogenetic characterization of two new head and neck squamous cell carcinoma cell lines

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Head and neck squamous cell carcinoma (HNSCC) includes epithelial malignancies of the oral cavity,

pharynx and larynx. Most of the HNSCC cell lines reported do not come from the primary tumour site and its molecular characterization is not available. The aim of this work was to characterize new HNSCC cell lines and to study their response to autophagy-modulating drugs, an emergent therapeutic strategy for cancer treatment.
Two cell lines were newly established from oropharyngeal (32816) and laryngeal (32860) squamous cell carcinomas. Their characterization was done by karyotyping, array-CGH and microarray expression profiling. Sensitivity to chloroquine, metformin, paclitaxel and panobinostat was tested by MTT cell viability assay and cell cycle analysis by flow cytometry.
We found little genomic differences between 32816 and 32860 in karyotyping and array-CGH. Both cell lines share common alterations associated with HNSCC, but we also found small regions specifically altered for each line. Loss on 3p11.1-3p26.3 was only identified in the 32860 cell line. We found that there are 1313 differentially expressed genes between both lines ($p<0.05$). of these genes, 767 are overexpressed and 546 are underexpressed. The Autophagy Related 7 gene, ATG7, located in 3p25.3 locus, is differentially expressed between both lines, with a statistically significant less expression in the 32860 cell line (p=0,0052; FDR=4,64E-05). The loss of expression in this region can be associated with the 3p11.1-3p26.3 genomic deletion. We compared the sensitivity to different autophagy modulators of our two cell lines, derived from tumours at different locations. The 32860 cell line was more resistant to metformin, paclitaxel and panobinostat than the 32816. Both cell lines showed a similar sensitivity to chloroquine. This difference in treatment response can be due to the molecular alteration of 3p region in 32860.
In conclusion, we established two new cell lines derived from different HNSCC locations that are good models to study this type of cancer. Our results suggest that genomic alterations in HNSCC depend on tumour localization and could determine therapeutic response.
This project was funded by FIS-FEDER: PI18/01476.

7.P22 High deletion 13q rate negatively affects the time to first treatment in CLL

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The most common anomaly in chronic lymphocytic leukemia (CLL) is deletion of 13q14 [del(13q)] that is associated with good prognosis when it is a sole anomaly. However, the clinics of CLL cases with isolated del(13q) are quite heterogeneous. Recently, it has been put forward that recurrent gene mutations in NOTCH1 and SF3B1 gene are associated with advanced disease stage and poor prognosis. In our study, we aimed to research the reason for the clinical heterogeneity of the CLL cases with isolated del(13q).
Sixty-eight cases with isolated del(13q), detected by FISH, were included in the study. Del(13q) data of cases were examined and NOTCH1 and SF3B1 gene mutations were investigated in 43 of 68 by sanger sequencing.
In NOTCH1 gene, 7541_7542delCT frameshift mutation was found in 1/43 cases. When the cases were examined in terms of del(13q), it was observed that 6 cases had biallelic deletion. It was determined that 77% (52/68) of the cases had deletion rate below 80%. As a result of the statistical analysis, it was determined that the cases with deletion rate over 80% were statistically associated with shorter time to first treatment (TTFT).
The results support the data that NOTCH1 and SF3B1 gene mutations are observed more frequently in the advanced stages of CLL and isolated del(13q) is observed more frequently in the early stages. As a result of our study, it was concluded that high del(13q) ratio has a significant effect on clinical heterogeneity in isolated del(13q) cases and deletion rates should be considered in clinical applications.

7.P23 Two distinct mature B-Cell neoplasms in a patient with independent cytogenetic clones

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Introduction: The identification and characterization of recurrent chromosomal changes contributes to the classification of mature lymphoid neoplasms, namely chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). CLL is characterized by progressive accumulation of phenotypically mature malignant B lymphocytes and displays clinical and biological heterogeneity. Chromosome aberrations such as trisomy 12, del(13q), del(11q), and del(17p) can be detected in more than 80% of the cases. MCL morphology is variable, has a progressive clinical course and the t(11;14)(q13;q32) is present in virtually all cases of MCL independent of their morphologic or clinical presentation.
Case: A 72-year-old man with a history of prostate cancer and dyslipidemia was admitted at our Institute in January 2019 presenting lymphocytosis and the suspicion of MCL and CLL.
Material and Methods: A peripheral blood sample from the patient was cultured with synthetic CpG-oligodeoxynucleotide (DSP30) and Interleukin 2 (IL-

2). CBA was performed with GTL banding. Fluorescence in situ hybridization (FISH) was performed on uncultured blood cells with the probes ATM (11q22)/SE 11; SE 12 (D12Z3) Green; DLEU1 (13q14)/13qter; CCND1/IGH t(11;14) Fusion and TP53 (17p13)/SE 17 (Kreatech).
Karyotype and FISH results were described according to ISCN.
Combined cytogenetic results:
47,XY,+12[19]/46,XY,t(11;14)(q13;q32),inc[1].nuc ish(CCND1,IGH)x3(CCND con IGHx2)[50/100],(D11Z1,ATM)x2[100],(D12Z3x3)[7/100],(DLEU1,13qter)x2[100],(TP53,D17Z1)x2[100]
Conclusions: The karyotype and FISH analysis revealed the presence of two cytogenetically independent clones, one harboring trisomy 12 and another presenting a translocation t(11;14)(q13;q32), involving the IGH locus and the CCND1 gene. Trisomy 12 is the most common cytogenetic aberration in CLL and t(11;14)(q13;q32)/IGH-CCND1 is present in > 95% of MCL cases.
Combined cytogenetic results allowed the identification of two independent clones, reflecting the coexistence of two mature B-Cell neoplasms in the same patient.

7.P24 Case report: the use of optical genome mapping (Bionano) to understand a complex mechanism of KMT2A rearrangement in an AML case.

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Diagnostic of AML M5 (FAB classification) is proposed after cytology and cytometry analysis in an eighty-six year old woman with 46% myeloblasts in the bone marrow. Standard karyotype shows a translocation t(10;11)(p12;q23) in seventeen mitoses. KMT2A rearrangement was confirmed by FISH analysis using a gene specific break apart probe. However, informative metaphases observation show that the KMT2A rearrangement is situated on the short arm of a single chromosome 10. KMT2A-MLLT10 fusion is known to result from an inversion which can occur either before or after the t(10;11) translocation. More FISH or molecular biology experiments could be used to determine the exact mechanism in this particular case. An RT-PCR Hemavision test would have been informative, but was not possible due to the lack of RNA. Optical genome mapping (OGM) is a new, all in one, technique allowing the detailed characterization of all structural rearrangements (balanced or not) genome-wide . In our work, we used the OGM technique to understand the mechanisms leading to this particular KMT2A rearrangement. As expected, we observed a KMT2A-MLLT10 fusion resulting from several anomalies involving chromosome 10 and 11. We conclude that the analysis of this AML case nicely confirms the utility of OGM to resolve complex aberrations using just one assay.

7.P25 Cytogenetic findings and survival analysis of patients with Philadelphia-negative myeloproliferative neoplasms; A report from the main referral center in the south of Iran

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Background: Philadelphia-negative myeloproliferative neoplasms (Ph- MPN) are clonal myeloid disorders for which cytogenetic examination is carried out to assess clonality and to stratify patients into prognostic groups. Herein, we report for the first time the cytogenetic findings of a series of Iranian Ph- MPN patients.
Methods: This retrospective cross-sectional study was conducted on all patients with a confirmed diagnosis of Ph- PMN whose cytogenetic findings were available. Telephone contacts were done to find the overall survival (OS) time and primary myelofibrosis (PMF) patients were categorized according to the proposed cytogenetic risk stratification models. The data were compared to the available evidence.
Results:Thirty-six patients (male/female: 28/8, mean±SD age =57.8±13.72 years) consisting 30 (83.3%) PMF, 5 (13.9%) essential thrombocythemia (ET), and one (2.9%) MPN, unclassifiable patients have been entered the study. Sixteen (53.3%) PMF patients showed a variety of numerical and structural abnormalities including three novel findings but all the other patients had normal karyotypes. The mean OS time was significantly better in early/prefibrotic PMF patients than their fibrotic phase counterparts. The frequencies of different risk categories and their OS times were as expected.
Conclusion: The low frequency of MPN in this study maybe due to the fact that most MPNs, especially Polycythemia Vera cases, were diagnosed without cytogenetic assessment in recent years. Overall, it looks that the normal karyotype rate and different types of karyotype findings in our population is comparable to other studies and also the proposed cytogenetic risk stratifications fits well to our data.

7.P26 Case Report: A rare atypical BCR-ABL1 transcript in a T cell acute lymphoblastic leukemia

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The detection of a Philadelphia chromosome (Ph) in T-acute lymphoblastic leukemia (T-ALL) is a relatively rare event1. Here, we report a case of a 37-year-old woman with early-T-cell precursor ALL immunophenotype. Cytogenetic analysis reveals a complex pseudodiploid karyotype: 46,XX,del(6)(q2?3),del(7)(p15p11),t(9;22)(q34;q11),-22,+mar.
Array-CGH confirms a heterozygous partial deletion of a part of the short arm on chromosome 7 including the IKZF1 gene, and demonstrates a homozygous deletion of the CDKN2A gene. FISH analysis shows a profile of major breakpoint cluster region (M-BCR) with extra-signal dual color translocation probe (1F1R1G1ES) while the multiplex RT-PCR detects a BCR-ABL P190 transcript. A targeted Next-Generation Sequencing (NGS) form RNA demonstrates a rare e6a2 P195 BCR-ABL1 fusion transcript never reported in Ph-positive T-cell ALL and detects also an expression of a TLX3 (HOX11L2) transcript. In addition, NGS from DNA identifies two mutations classified as likely pathogenic involving the BCORL1 and WT1 genes2,3. Although these mutations are usually linked to acute myeloid leukemia, it's interesting to find these in a patient with T-ALL. This patient was included in the GRAALL-2014 (ATRIALL) protocol. Three months after the beginning of the treatment, the patient was in clinical remission with a negativ minimal residual disease (MRD) based on NGS analysis of her TCR specific gene rearrangement. To date, six months after her inclusion in the protocol, she is still in clinical remission.

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7.P27 Analysis of chromosomal instability in Chronic Lymphocytic Leukaemia

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Chromosomal Instability (CIN) is a dynamic state characterised by a heightened rate of chromosomal losses or gains. While relatively uncommon in human cells, CIN is pervasive in cancer. Through genome destabilization, CIN leads to genomic heterogeneity, which under selective pressure can drive clonal evolution and profoundly impact cancer progression and therapeutic response. CIN has been well explored in solid tumours, but has been less extensively studied in in haematological malignancies. Chronic Lymphocytic Leukaemia (CLL) is a malignancy of mature B cells, which can exhibit a range of genomic and chromosomal aberrations, many of which can be related to the disease course and are thus utilised as biomarkers for prognostic stratification. So far, studies of chromosomal instability in CLL have been limited in number and scope. There have been a few studies reporting a higher baseline frequency of micronuclei (MN) in lymphocytes of untreated CLL patients when compared to age-matched controls. While reports of increased CIN in association with telomere shortening and TP53 deletion would suggest a link with poor outcome, CIN has also been found in a CLL patient with a 13q14 mono-allelic deletion encompassing the DLEU and RB1 genes, the most favourable prognostic biomarker in CLL. Here we report our analysis of CIN in CLL patients with different cytogenetic (TP53 deletion, DLEU/RB1 deletion, ATM deletion, trisomy 12, chromosome Y loss, telomeres shortening, complex karyotypes) and immunophenotypic profiles (Zap70, CD38, CD40, CD180 and the B-cell receptor isoforms IgM and IgD). Our observations suggest CIN as a biological occurrence to be considerably more frequent than previously reported and to be present across the CLL prognostic spectrum, with no specific association to any particular subgrouping. Our results warrant further investigations into CIN as an independent prognostic biomarker and influencer of genomic complexity in CLL.

Read by Title Abstracts

R1 Study of chromosomal heteromorphisms in 800 women

Mariya Levkova [1], Mariya Tsvetkova [1], Tsanka Ruseva [2], Mari Hachmeriyan [1], Milena Stoyanova [1], Valentina Miteva [1], Dinnar Yahya [1], Lyudmila Angelova [1]
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R2 Inversion of chromosome 10 in two infertile males

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R3 Cytogenetic evaluation of children with short or tall stature – a single center study

Milena Stoyanova [1], Mari Hachmeriyan [1], Dinnar Yahya [1], Mariya Levkova [1], Valentina Miteva [1], Mariya Tsvetkova [1], Tsanka Ruseva [2], Violeta Iotova [3], Lyudmila Angelova [1]
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R4 Cytogenetic diagnosis of prenatal samples results of 4 years of clinical experience

Ariadna Escalona [1], Maria Angels Rigola [1], Susana Caballero [1], Mireia Sero [1], Jordi Duran [2], *Sandra Andreu* [1]
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R5 Pre and perinatal aspects of sex chromosome abnormalities and other gonadal dysgeneses

Eva Pinti [1], Anna Lengyel [1], Gyorgy Fekete [1], Iren Haltrich [1]
Semmelweis University, IInd Department of Pediatrics, Budapest-Hungary [1]

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E.C.A. is now also present on Social Media. Here are announcements of interesting articles that we have posted on Facebook. The articles and news items are related to cytogenomics or to biology in general. If you have relevant articles that you would like to share, please contact mariano.rocchi@uniba.it.

LINE-1 ACTIVITY TO EXPLAIN EARLY SPONTANEOUS MISCARRIAGE?

A well-known genetic cause of recurrent pregnancy loss is the presence of a balanced chromosomal rearrangement, as identified by karyotyping in about 4% of couples tested. More recently, exome sequencing has enabled the identification of causal embryonic lethal genes. A novel hypothesis to explain early spontaneous miscarriage has been proposed by Lou, Goodier and Qiang, Dept. of Genetics, Northwest Women's and Children's Hospital, Xi'an, China (BMC-Reproductive Health). LINE-1 retroelements are the most active autonomous transposable elements in mammals, including humans. Novel copies are generated when a LINE-1 RNA integrates into the genome by target-primed reverse transcription. About 5% of newborns have such a novel insertion, which can lead to inactivation of genes associated with hereditary disease. In addition, recombination between LINE-1 elements can generate deletions, duplications and rearrangements, and any novel insertion introduces splice sites, promoters, poly-adenylation signals and transcription factor binding sites.
The hypothesis of Lou et al. is based on the observation that, in both mouse and humans, the cellular environment of early embryonic cells is less restrictive to LINE-1 transposition. This may be associated with the successive waves of demethylation in the early embryo, as methylation of the LINE-1 promoter is inversely correlated to LINE-1 expression.
One way this hypothesis can be tested is by measuring LINE-1 methylation status and levels of LINE-1 derived nucleic acids and proteins in miscarriage samples. Possibly, an elevated LINE-1 insertion rate causes embryonic lethality by inactivation of essential genes. In addition, an accumulation of unintegrated LINE-1 DNAs that try to integrate into the genome may cause multiple genomic lesions and distract regulatory factors necessary for normal cellular processes.

DIFFERENCES BETWEEN MONOZYGOTIC TWINS

Each cell of our body is subject to a huge number of mutations. We have, however, a very complex and efficient repair systems which correct the vast majority of them. Some mutations, however, escape repairing. As a consequence, differences among different cells accumulate as these differences start at the formation of the embryo. The Stefansson's group (Nature Genetics) took advantage of the very early embryo mutations to analyze the formation of monozygotic twins. In order to be sure that the mutations originated from the early embryo, the study analyzed the genomes of identical twins, their parents, their children, and their spouses.
They measured the shared/specific point mutations (average difference 5.2 mutations), inferring how the twins originated. The more uneven the split, the greater was the difference between the twins. The authors observed "instances where a twin was formed from a single cell lineage in the pre-twinning cell mass and instances where a twin was formed from several cell lineages".

GENES AND COGNITIVE ABILITIES

Mendel's laws of inheritance published in 1866 were rediscovered in 1900, independently by three botanists Hugo de Vries, Carl Correns and Erich von Tschermak. This paved the way for genetic research, including that in man. Some of it, however, did not end well. The book "The Kallikak Family: A Study in the Heredity of Feeble-Mindedness" (1912), by Henry H. Goddard, used a simplistic interpretation of Mendel's laws to trace the hereditability of intelligence, moral ability, and criminality in a large family. This book has been very influential in the infancy of the eugenics movement, first in America and then in the world (in Germany in particular). In the last 100 years since then our understanding of human heredity has come a long way.
A recent paper in Molecular Psychiatry reports a genome-wide analysis study which estimates that ~10,000 genes modulate cognitive ability. The authors, Huguet et al., conclude: "these results suggest that a large proportion (56%) of the coding genome covering all molecular functions influences cognitive abilities. One may therefore view the genetic contribution to cognitive difference as an emergent property of the entire genome not restricted to a limited number of biological pathways".

RECENT HISTORY OF HOMO SAPIENS

This month two articles have taken stock of the recent evolution of *Homo sapiens*. The one in Nature (Origins of modern human ancestry), is a strictly "scientific" paper. The second (An evolutionary timeline of *Homo sapiens*), which appeared in Smithsonian Magazine, is very well illustrated, also reconstructing what the face of our ancestors, or close relatives like Neanderthals, must have looked like.

PHENOTYPIC IMPACT OF ROBERTSONIAN TRANSLOCATIONS ON THEIR CARRIERS

Robertsonian translocations (RTs) are detected in roughly 1 per 800 referrals for prenatal diagnostics. While carriers of RTs are thought to harbor themselves no phenotypes, they are at increased risk for miscarriages, infertility, uniparental disomy, and aneuploid offspring because of production of unbalanced gametes. A recent cohort study with a median follow up time of 24 years in the United Kingdom revealed a significantly increased risk for breast cancer for cariers of a rob(13:14). Also the risk for non-Hodgkin lymphoma and childhood leukemia was elevated, albeit that this risk was not related to a specific type of RT. Poot and Hochstenbach reviewed the literature regarding potential mechansisms by which RTs may arise, and the effect of RTs on nucleolar structure in view of their pathological effects in somatic cells (Molecular Syndromology). The authors also discuss the abilities and limitations of current molecular and cytogenetic methods to detect RTs in a clinical diagnostic setting.

ONGOING RETROVIRAL INVASION OF THE KOALA GERMLINE AND ITS CONTRIBUTION TO CANCER

Retrovirus-like elements occupy a considerable part of the genome of placental mammals (about 8% in humans). They are also present in marsupials such as the koala. Now, Gayle McEwen et al. from the Leibniz Institute for Zoo and Wildlife Research in Berlin have discovered that insertions of elements of the KoRV retroviruses family contribute to the high rate of leukemia, lymphoma and other cancers in koala populations in New South Wales and Queensland in Australia. In contrast to humans, where retroviruses in the germline have been mutated into inactive genome elements during million years of evolution, the koala germline is currently being invaded by exogenous retroviruses, a process starting about 50,000 years ago. There are on average 100 retroviruses in the koala genome and about two-thirds of these are full-length, intact elements that are capable of re-integration at novel positions in somatic cells. By comparing the DNA from cancer cells to that of the germline, McEwen et al. demonstrate that tumors contain newly integrated KoRV copies at positions not present in the germline. These affect gene expression, leading to increased cell division rates, and, ultimately, to cancer. Hotspots for insertion are in regions with known cancer-related genes. Such knowledge of KoRV biology is not only essential for guiding koala species conservation efforts, but may also give us insight in how (repeated) invasions of the germline by retrovirus-like elements have contributed to the structure of our genomes.

De novo STRUCTURAL MUTATIONS

Next generation sequencing (NGS) technologies have made it possible to define the rate and patterns of point mutations in the human germline. Defining the rate of structural mutations is more challenging. This is because they are relatively rare and therefore much larger samples would be required. The other reason is that NGS technologies based on short reads (150-300 bp) are not suitable for structural variations. On the other hand, ultra long sequencing technologies (reads >100kb) are prohibitively expensive for large samples. With this limitation in mind, Belyeu et al. (Am J Hum Genet-2021 in press) have used NGS for a family-based study of germline mutations among 9,599 human genomes from 33 families and 2,384 families from an autism dataset. They found a rate of 0.160 events per genome in unaffected individuals. The rate in autism families was significantly higher (0.206 per genome). In both groups 73% of de novo structural mutations arose in paternal gametes. At variance with point mutations, they did not find an increase of structural mutations with parental age; this suggests that different mechanisms are involved in point mutations and structural changes.

HYPERTROPHIC CARDIOMYOPATHY EXPRESSION: ROLE OF EPIGENETICS AND ENVIRONMENTAL FACTORS.

Repetti et al. (PNAS, 2021) report nine identical twin pairs with sarcomere protein gene variants and two with unknown disease aetiologies who were discordant for morphological expression of a Hypertrophic CardioMyopathy (HCM). The authors conclude that epigenetics and environmental factors contribute to the progression of the disease. These factors might include microbial infection, diet or exercise.

Chen et al. (Nature Biotechnology, 2016) reported “A comprehensive screen of 874 genes in 589,306 genomes led to the identification of 13 adults harbouring mutations for 8 severe Mendelian conditions, with no reported clinical manifestation of the indicated disease”. The interpretation was that there was something in their genomes that was buffering the effects of these deleterious mutations. A paradigmatic example in this respect is the mutation that causes the persistence of foetal haemoglobin (HbF) which substantially attenuates beta-hemoglobinopathies.

The report by Repetti et al. suggests that genome identity is not enough! The complexity of our genome is complex.

THE DEMOGRAPHY OF DOWN SYNDROME IN 39 EUROPEAN COUNTRIES

Trisomy-21/Down syndrome is a major cause of mental retardation. For the first time, a systematic survey has been made of the demography of Down syndrome (DS) in Europe. The authors Gert de Graaf, Frank Buckley and Brian Skotko are involved in Down syndrome education programs in the Netherlands, UK and USA, respectively. In the March 2021 issue of the European Journal of Human Genetics they present estimates of the nonselective and actual live birth prevalence of DS for the years 2011-2015 in 39 European countries. An estimated number of 417,000 people with DS were living in Europe in 2015, which would have been about 572,000 without selective pregnancy termination. The average reduction of the 2011-2015 live birth prevalence was 54% among these countries, varying from 0% in Malta and 8% in Ireland (countries with restrictive termination regulations) to an average of 38% in Eastern Europe and 62% for the rest of Europe (with the highest in Spain, 83%). There was a significant correlation between this percentage and the Gross Domestic Product per capita ($r = 0.73$; $p<0.000$). Such differences may reflect the wealth of a country and participation in prenatal screening/testing programs. These numbers provide insights into the effects of prenatal screening and testing and may serve to improve education and health policies for people with DS.

ETHICS OF "OPPORTUNISTIC" WHOLE EXOME/GENOME SCREENING

As more and more patients with an indication for genetic testing are having their whole exome or whole genome sequenced, the possibility emerges to search for pathogenic variants that are not associated to the initial reason for the genetic test. Should such variants (so-called secondary findings) be actively looked for, because they could be of direct benefit for the health or reproductive choices of the patient and the patient's family? Organizations such as the American College of Medical Genetics and Genomics, the French Society of Predictive and Personalized Medicine, and Genomics England state that "actionable" pathogenic variants should be routinely and systematically looked for and should be reported. In the March 2021 issue of the European Journal of Human Genetics, a committee on behalf of the ESHG, headed by Guido de Wert and Wybo Dondorp from the Department of Health, Ethics and Society of Maastricht University, the Netherlands, argues for a more cautious approach. In their publication, the authors discuss the wider ethical context of what they call "opportunistic genome screening" (OGS), with an emphasis on a balanced application of normative principles of proportionality, autonomy, economy and justice. They conclude that it is too early for OGS as a professional clinical standard, and point to cascade testing as an alternative to OGS. Please have a look at the paper for the details of this ongoing and important medical-ethical discussion.

EVOLUTION OF SWEATING IN HUMANS

Homo sapiens and chimpanzees have very similar genomes. It is commonly believed that their differences mainly depend on the expression of their genes.
A recent article in PNAS provides a paradigmatic example. Endurance running is a peculiarity of Homo sapiens compared to all other primates and has played an important role in its evolution. This characteristic mainly depends on a very efficient human thermoregulatory sweating, because of a very high density of water-secreting eccrine sweat glands (about 10 times more than in chimpanzees). Studies in mice have indicated the Engrailed 1 gene (EN1) as responsible for seeding the sweat glands. The PNAS paper now reveals that enhanced expression of this gene depends on the regulatory element hECE18, which has accumulated mutations during the evolution of Homo sapiens

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SEX DETERMINATION IN CREEPING VOLE

Chromosomal sex determination varies among the different classes of chordata. For birds, see this Trends in Genetics review. In mammals, the XY system is almost omnipresent; the Y chromosome contains the sex determining gene (SRY) and a few other functional sequences. Indeed, in its evolutionary trajectory, the Y has lost the vast majority of its genes compared to its X partner.
Jennifer Graves, in her seminars, often joked that the Y chromosome is small, almost useless, and has no evolutionary prospects; it will be lost. This is exactly what has been hypothesized for the Y chromosome of the rodent creeping vole (Microtus oregoni). Couger et al. in their recent article in Science, by sequencing the sex chromosomes of Microtus oregoni, were able to reconstruct the evolutionary history of the system. The Y chromosome has actually been lost, and the sex-determining chromosome is highly homologous to the X. Consequently, females are X0 and males XX (the second X carrying SRY). They were also able to show that the male X chromosome has already begun the well-known degeneration that occurred in the mammalian Y chromosome. The X0 / XX system also has implications for X inactivation in order to ensure a functional balance.

PLACENTAL MOSAICISM

Chromosomal mosaicism present in the placenta but absent in the fetus is a well-known phenomenon. In a recent article in Nature, Coorens et al. sequenced 86

bulk placental samples and 106 microdissected placental samples in order to reconstruct the phylogeny of placental cells. The task was achieved by monitoring the somatic mutations that cells accumulate during their replication. The authors point out that the number of these mutations, including copy number variations, in the placenta is unmatched in any other normal tissue. One of the most interesting findings is the discovery of bottlenecks that allow normalization of zygotic aneuploidy and segregation of abnormal cells into the placenta. This was very evident in a case of chromosome 10 trisomy, where the extra chromosome was contributed by the mother: trisomy 10 was detected in a bulk sample; the embryo, the umbilical cord and a portion of the placenta contained only two chromosomes 10 but both of maternal origin.

PARENTAL GENOME UNIFICATION AT THE STARTING POINT OF THE EMBRYO IS HIGHLY ERROR PRONE

Cytogenetic analysis of spontaneous abortions revealed, in the 1970s, that half of them were due to aneuploidy, and this was thought to be the tip of the iceberg. For example, autosomal trisomies lacked the expected counterpart, the monosomies. Since then, the many stages from *gametes* to the early development of the embryo have been investigated. In this regard, an important seminal work was the one published in 2009 by the group of J. Vermeesch (Nature Medicine). They showed that chromosomal instability is common in human embryos at the cleavage stage and, more importantly, that normal development of the embryo can occur by selection against abnormal blastomeres. This conclusion has been supported and clarified by the recent work of Coorens et al. (Nature, 2021) who found that abnormal cells can be segregated into the placenta.
An article by Cavazza et al. (Cell, 2021) now reveals that another important stage, underlying the development of the embryo, is involved in the generation of aneuploidy and chromosomal abnormalities in general.
After fertilization, the parental genomes polarize towards each other and the chromosomes of the two pronuclei cluster at the pronuclear interface, ready for unification. This process, the paper points out, is highly error-prone.

CENTROMERE ORGANIZATION AND EVOLUTION OF CHROMOSOME 8

As pointed out in an earlier post (The X-chromosome telomere-to-telomere, no gap), the third generation of DNA sequencing technology allows the assembly of an entire chromosome end-to-end, including the centromeric region, which had not been possible with previous sequencing approaches. Logsdon et al. (Nature) report, for the first time, the telomere-to-telomere sequence of an autosome, chromosome 8. The most interesting part of this work concerns the centromere, of which the sequence has been investigated for its organization as well as for its evolution. The latter task was accomplished by comparing the human sequence to the one of the homologous centromeres of chimpanzees, orangutans and macaques. The comparison showed that the alpha higher-order repeats are located peripherally and are older than the centrally located monomeric alpha satellites. Comparative molecular cytogenetic analysis had already shown that alpha satellite DNA evolves rapidly (Archidiacono et al. 1995). Logsdon et al. now report that the alpha satellite mutation rate is 2.2 times that of the rest of the genome.

DNA DAMAGE AS A UNIFYING CAUSE OF AGEING

The dream of defeating the ageing process is as old as human civilization. Given the global ageing of the population, efforts in developing interventions against ageing has also attracted many drug companies. But the -sine qua non- condition for achieving this goal requires the understanding of the causes of ageing. In this regard, the number of studies in humans and model animals is enormous, and different causes of ageing have been proposed: mutations, chromosomal changes, activity of transposable elements, deletions in mitochondrial DNA, telomere shortening, protein abnormalities (e.g. crosslinking, oxidation…), accumulation of insoluble protein aggregates, abnormalities in gene function leading to cancer, epigenetic defects, progressive decline in heart function, irreversible changes in major arteries etc. In conclusion, many details have been revealed, but we still lack an overview of the phenomenon. Recently, epigenetics has come to the fore (Nature, 2020). Now, an article in Nature (2021) points to the "Central role of DNA damage as a unifying cause of ageing". The thesis is that all the above-mentioned hypothesized causes of ageing can be traced back to DNA damage.

EXPRESSED PSEUDOGENES

Wikipedia defines pseudogenes as "non-functional segments of DNA that resemble functional genes. Most arise as superfluous copies of functional genes, either directly by DNA duplication or indirectly by reverse transcription of an mRNA transcript".
Decartes suggested that we must doubt everything. Troskie et al. (Genome Biology) did just that – doubted pseudogenes. Using long-read DNA sequencing, they identified hundreds of new transcribed pseudogenes expressed in tissue-specific patterns.

MUTATIONAL LOAD IN EUROPEANS

Next-generation sequencing of human genomes has revealed millions of genetic variants and from sequencing parent-child trios we know how many de novo variants arise in the offspring (Nature Genetics). Some of these variants have been annotated as deleterious, i.e. causing disease. This latter group of variants constitutes the mutational load. Several studies have attempted to define this load in specific populations. The results may differ based on the different parameters used in the study, i.e. algorithms, statistics, sets of investigated genes and sequencing approaches (exon sequencing or direct genomic sequencing). Furthermore, differences in allele frequency between populations may have been caused by a genetic drift (e.g. population bottleneck, founder effect).
Fridman et al., in their recent paper in Am. J. Hum. Genet., investigated the mutational burden in Dutch and Estonian populations (4,120 and 2,327 unrelated individuals, respectively) by testing the exons of 1,929 OMIM genes that are associated with an autosomal recessive monogenic disorder. They found that, on average, each individual carries 2.3 (range 0-11) (Dutch) or 2.0 (range 0-9) (Estonian) pathogenic or likely pathogenic (PLP) variants. of these 1,929 genes, 1,119 are associated with severe phenotypes and frequency of PLPs was 1.5 (range 0–8) and 1.1 (range 0–6) in the Dutch and Estonian cohorts, respectively. They comment that these figures represent a lower bound-estimate due to their strict selection criteria.
The main objective of the study was to provide figures on genetic risk for the two populations and for the European population in general. They estimated that 0.8% –1% of unrelated European couples are at risk for a child with a severe AR condition (~ 225 per 100,000 births). 90% of this risk is due to the ~ 100 most frequent genes. The risk increases by ~ 16 times if the partners are first cousins (~ 3,400 per 100,000 births). Many other details on risks and selection against deleterious variants are reported in the study.

THE COMPLETE SEQUENCE OF A HUMAN GENOME

In two earlier posts we have reported the sequencing of entire chromosomes (X and 8) from telomere-to-telomere (T2T). The T2T consortium has now finished the end-to-end sequencing of an entire haplogenome. There are no gaps; the centromeres as well as the short arms of the acrocentric chromosomes are included. The results have been deposited on bioRxiv and the paper will, most likely, appear soon in a high-level journal. This version was called T2T-CHM13. The CHM13 cell line is haploid, and was chosen because the absence of a second haploid set could greatly facilitate the task.
This version will surely be the starting point of a new human reference genome. Some of the authors belong to the UCSC genome centre. And, in fact, this version is already on UCSC (t2t-chm13-v1.0).

CHROMOSOME INSTABILITY: A PARALLEL BETWEEN SPECIES AND CANCER EVOLUTION

A few years ago the "omics" of single cells made it possible to analyse a population of cancer cells using bioinformatics tools typically used to study the phylogeny of species, that is, the evolution of species. Since then, several articles have been published on this topic. An article in press in Trends in Genetics focuses on the parallels between the chromosomal changes that have occured over millions of years of evolution and the very rapid chromosomal changes that occur in the evolution of cancer. This publication is not a classical paper; it is classified as a "Forum" and is very easy to read.
The authors illustrate the “major principles connecting species and somatic karyotype evolution” and suggest that “karyotype plasticity, despite being necessary for speciation, comes with a cost: a predisposition to somatic genomic instability and cancer”.

TRANSGENERATIONAL EFFECTS TO EXPLAIN THE RISE IN PREVALENCE OF MENTAL HEALTH DISORDERS

The prevalence of mental health disorders (MHD), such as ADHD, autism and mood disorders, has increased 2-3 fold during the last two decades in the USA, and probably in other high-income countries as well. Also rates of suicide and self-harming behavior in teenagers and young adults increased. In a paper in the March 2021 issue of the Eur J Hum Genet, 29, 387–395 (2021) Anthony Monaco, president of Tufts University in Medford, USA, provides a model to explain this important problem for society.
The model goes beyond traditional explanations based on DNA variants, because these only account for a fraction of the heritability and family clustering of MHD. The model includes transgenerational effects by three possible mechanisms, each supported by evidence from studies in rodents.
1) Epigenetic changes of the genome: in utero exposure to maternal stress or toxic agents leads to epigenitic changes of the fetal genome that affect long-term gene expression of the progeny.
2) Changes of the microbiome: an unhealthy vaginal microbiome has effects on health and behavior of male offspring, and affects gene expression in the hypothalamus.
3) Changes in the sperm content of non-coding RNAs, such as miRNAs and tRNAs by stress or poor diet have an effect on the behavior of the progeny, as brain micro-RNAs can be transmitted to the germ line and to the progeny by sperm.
There are indications that such mechanisms exist in humans as well. Environmental conditions that may

play a role are substance use (drugs and alcohol), poor diet and obesity, as the rise in MHD coincides with increases in marijuana, cocaine, heroin, high sugar caffeinated soft drinks and processed foods rich in sugar and additives. This occurred 15-20 years before the rise of MHD, sufficient for a transgenerational effect to have taken place. Cooperatively, these environmental factors may have dysregulated reward pathways in basal ganglia, thereby sensitizing the brain to further rounds of exposure in the next generation.
Empirical support for this model comes from 11 neuropsychiatric and 3 metabolic disorders as they occur in 128,989 families in the USA (based on Insurance claims), for which it is shown that epigenetic effects explain much of the heritability. The good news is that epigenetic effects are reversible by reducing parental exposures to adverse environmental factors - a herculean effort for society but at the benefit of all.

HETEROSIS

The yield and quality of maize has been significantly increased over the past century, by common application of hybrid seeds (heterosis) and advanced management technologies. Now production has reached a plateau in many countries.
Heterosis (hybrid vigor) in plants is highly exploited to increase the production of many crops. Among them corn, sugar beet, cotton etc. etc. But not much is known about the genes involved and their molecular mechanisms of action. Xiao et al. (Genome Biology 22, 148, 2021) exploited a new experimental approach, in maize, to identify the quantitative trait loci involved and their mode of action. This paves the way for an optimized design of hybrids by using appropriate gene modifications in the parental lines.

SEPARASE DEREGULATION AND CHROMOSOME NONDISJUNCTION IN TUMORS

The separase protease severs the cohesion between sister chromatids and ensures progression towards anaphase. Deregulation of separase has been shown to lead to incomplete removal of cohesin, which results in prolongation of the metaphase, which in turn leads to the formation of anaphase bridges and to cytogenetically abnormal daughter cells.
Chindo et al., in their article in Cell Reports (Cell Reports 34, 108652, 2021) used a probe to very accurately detect the activation/suppression of sepasase activity during the metaphase-anaphase transition. Their data indicates that prolonged metaphase in cancer cells can lead to early and insufficient activation of separase, leading to nondisjunction of chromosomes in the anaphase.

REWRITING THE GENETIC CODE

CRISPR-Cas9 has allowed the manipulation of genomic DNA fragments. The 2020 Nobel Prize to Doudna and Charpentier underscored its importance. This technology is usually used to modify some bases of the genomic DNA, and much of the debate has focused on applications in humans.
There are other fields of application of CRISPR and related technologies where the manipulation of the genetic material is incredibly more profound. A striking example is reported by Ostrov et al. in Science 372, 1057-1062, 2021 in *Escherichia coli* (commented on by Jewel and Chatterjee, same issue).
Earlier, in 2016, authors of an article in Science (353, 819-822, 2016) designed, and partially generated, an *E. coli* in which a few appropriate triplet codons were replaced, using CRISPR-Cas9, by synonymous codons. The corresponding tRNAs were deleted. There were no substantial consequences for the bacterium, but the infecting phages were not able to synthesize their proteins due to the absence of some tRNAs. The *E. coli* had become immune to phages!
The authors of the paper by Ostrov et al. have gone further. They have created an *E. coli* in which some codons were forged to encode three non-canonical amino acids. In this way they ensured phage immunity and the potential to produce a range of novel proteins.

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COMMITTEE

ECC SCIENTIFIC PROGRAMME COMMITTEE

E.C.A. News

- The 13th European Cytogenomics Conference will take place on-line this year on Saturday 3 Sunday 4, and Monday 5 July 2021.
- The 2021 General Assembly of the E.C.A. with Borad elections will take place in Milan on 1 September 2021.
- Renewal of the Board in 2021. The following members are due for replacement or re-election in 2021: J-M. Dupont (France), J. Garcia-Sagredo (Spain), M. Rocchi (Italy), E. Syk Lundberg (Sweden), and R. Vanni (Italy).
- According to the statutes, lists for the board election may be sent to the President until 1 July 2021.

E.C.A. Fellowships

- The **E.C.A.** offers two **Fellowships** for the following courses:

  European Advanced Postgraduate Course in Classical and Molecular Cytogenetics
  to be held in Nîmes (France) March 2022.

  Goldrain Course in Clinical Cytogenetics
  to be held in Goldrain Castle (South Tyrol, Italy) 20-28 August 2022.

- The fellowships **include the course fees and the accommodation** during the lectures in Nîmes or in Goldrain but **do not include travel expenses**.
- Applications with CV, list of publications and a letter of recommendation should be addressed to the course organizer (see pages 93 and 94).

EUROPEAN CYTOGENETICISTS ASSOCIATION (E.C.A.)

European Advanced Postgraduate Course in Classical and Molecular Cytogenetics

Director: Professor Jean-Michel Dupont, Paris – France

The course is scheduled to be held in Nîmes, France in March 2022. However, an online version will be organized, if the restrictions due to the pandemic are still in place.

2022 Course provisional programme

This approximately 55-hour theoretical part of the course attempts to cover the field of cytogenetics in the broadest sense. The topics can be divided into the following categories:

Technical aspects:

Classical Cytogenetics: Cell culture techniques; Chromosome staining methods (Q-, G-, C-, R-banding and high-resolution banding);

Molecular Cytogenetics: Methods and principles of Fluorescence In Situ Hybridization (FISH) and MFISH; Array CGH; Application of Massively Parallel Sequencing to Cytogenetics; Production and use of molecular probes; Database use in Cytogenetics;

Laboratory quality assessment.

Clinical cytogenetics:

Basics: Frequency of chromosome disorders; Cell cycle, mitosis and meiosis, gametogenesis; Heterochromatic and euchromatic variants; Numerical chromosome abnormalities; Structural abnormalities: translocations, inversions, insertions, deletions, rings, markers; Risk assessment for balanced abnormalities; X inactivation; numerical and structural abnormalities of the X and the Y; Mosaicism; Chimaeras; ISCN 2020.

Clinical: Phenotype of common autosomal and gonosomal aneuploidies; Chromosome abnormalities in recurrent abortions; Cytogenetics and infertility; Microdeletion syndromes; Uniparental disomy and its consequences; Genomic imprinting; Genetic counselling and ethical issues in cytogenetics.

Prenatal diagnosis: Indications, methods and interpretation; Risk assessment for chromosomal abnormalities; Non-invasive methods using foetal nucleic acids and foetal cells in maternal blood; Pre-implantation diagnosis.

Cancer Cytogenetics: Molecular approach to cancer cytogenetics; Predisposition to cancer, Chromosome instability syndromes; Chromosome mutagenesis; Solid tumors; Clinical application in onco-haematology.

Other:

Genome architecture; Structure of chromatin; Structure of metaphase chromosomes, Mechanisms of chromosome abberations; Origin of aneuploidy; Evolution and plasticity of the human genome; Animal cytogenetics; Plant cytogenetics.

15th Goldrain Course in
Clinical Cytogenetics
August 20 to 28, 2022

LOCATION

Goldrain Castle, Goldrain, South Tyrol, Italy
Website of the venue: **www.schloss-goldrain.it**

COURSE DESCRIPTION

The course is focused on phenotypic findings, mechanisms of origin and transmission, correlations of clinical patterns with chromosomal imbalance and modern ways of diagnosis of the latter. Special attention is paid to an understanding how deletions and/or duplications of chromosomal segments cause developmental defects. The course also addresses the optimal application of the diagnostic possibilities, both pre- and postnatally and including molecular cytogenetic methods for a precise determination of segmental aneuploidy.

TOPICS

Dysmorphic findings in chromosome aberrations: formation and interpretation – The adult and elderly patient with a chromosome aberration – Follow-up studies in patients with chromosome aberrations – Clinical findings associated with chromosome aberrations – Microdeletion syndromes: clinical pictures – prenatal cytogenetic diagnosis – Mosaics and chimeras – imprinting and uniparental disomy - Epidemiology of chromosome aberrations – Chromosome aberrations in spontaneous abortions and stillborns – Harmless chromosome aberrations – Risk assessment in structural chromosome aberrations Extra small supernumerary chromosomes – Genomic variation: a continuum from SNPs to chromosome aneuploidy – Pre-implantation cytogenetic diagnosis – Ultrasound findings indicative of chromosome aberrations – Ethical issues in the context of cytogenetic diagnosis – Non-invasive prenatal cytogenetic diagnosis.
ISCN - Practical exercises in cytogenetic nomenclature – Accreditation of cytogenetic laboratories - Accreditation of cytogenetic laboratories – Optimal use of available techniques in clinical cytogenetics – NGS – SNP arrays and Array-CGH: principles, technical aspects; evaluation of the results – MLPA - QF-PCR - FISH techniques and their interpretation – Introduction and practical exercises with database for phenotypical and variant interpretation - Students presentation of cases with difficult-to-interpret chromosome aberrations. Introduction to modern genetic editing techniques. - Practical exercises will be offered with the ISCN system for chromosome aberrations and with cytogenetic, genomic, and phenotypical databases.
- Students will have the opportunity to present their own observations and cytogenetic findings which are difficult to interpret.
- The students will have the opportunity to perform a test at the end of the course.

DIRECTOR

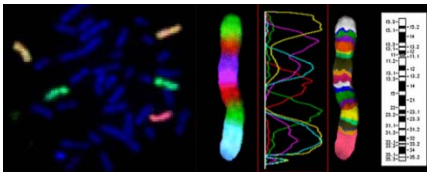
A. Schinzel (Zurich, Switzerland)

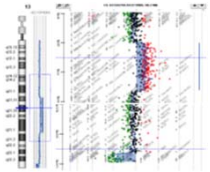
FACULTY

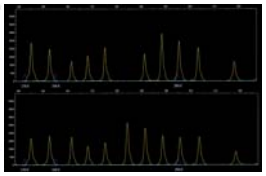
D. Bartholdi (Berne, Switzerland), A. Baumer (Zurich, Switzerland), P. Benn (Farmington CT, U.S.A.), J.M. Dupont (Paris, France), N. Kurtas (Florence, Italy), E. Klopocki (Würzburg, Germany), K. Madan (Leiden, The Netherlands), K. Miller (Hannover, Germany), R. Pfundt (Nijmegen, The Netherlands), G. van Buggenhout (Leuven, Belgium), M. Vismara (Zurich, Switzerland), J. Wisser (Zurich, Switzerland), O. Zuffardi (Pavia, Italy) and others

For further questions please write directly to Albert Schinzel at **schinzel@medgen.uzh.ch**

Full fee is Euro 1600 for a single room or Euro 1450 (VAT included) in a 2-bed-room. It includes tuition, course material, free access to internet during the course, accommodation for 7 nights, all meals, beverages during the breaks and a ½ day excursion.







E.C.A. PERMANENT WORKING GROUPS (PWG)

PWG: MARKER CHROMOSOMES.

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Please remember that the sSMC homepage can now be reached at
http://cs-tl.de/DB/CA/sSMC/0-Start.html
and UPD/ heteromorphisms and M-FISH pages at
http://cs-tl.de/ and http://cs-tl.de/DB.html

PWG: CYTOGENOMIC TOXICOLOGY AND MUTAGENESIS.

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PWG: ANIMAL, PLANT, AND COMPARATIVE CYTOGENOMICS.

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PWG: CYTOGENOMICS OF HAEMATOLOGICAL MALIGNANCIES.

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PWG: CANCER CYTOGENOMICS, SOLID TUMOR STUDIES.

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PWG: CYTOGENOMICS AND SOCIETY

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PWG: CYTOGENOMICS.

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PWG: PRENATAL DIAGNOSIS.

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PWG: CLINICAL AND MOLECULAR APPROACHES TO CYTOGENETIC SYNDROMES.

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Please see the programme of the PWGs on pages 2 and 3 of this newsletter.

Abstract added in proof

Predicting CNV effects on brain structure, function and risk for psychiatric conditions

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Background: Copy number variants (CNVs) are routinely identified in patients with neuro-developmental and psychiatric disorders. Still, their quantitative effects on essential traits such as cognitive ability and the risk they confer to conditions such as autism or schizophrenia are poorly understood. We have estimated that close to 50% of coding genes show measurable effects on cognition when deleted, and new data shows that this is also the case for duplications. However, the link between the function of genes and their effect size on cognition and risk for psychiatric disease remains obscure.

Hypothesis: Genes -and corresponding CNVs- with the same functional characteristics will have similar effect sizes on cognitive ability and risk for psychiatric conditions.

Methods: CNVs were identified in over 300000 individuals from unselected and autism cohorts with general intelligence assessments. All genes encompassed in CNVs were scored based on intolerance to haploinsufficiency, their developmental expression trajectories, cellular expression signatures, and spatial patterns of expression in the brain provided by different bulk tissue and single-cell transcription data resources (i.e., Allen Human brain Atlas, PsychENCODE).

Results: We mapped the estimated effect size of over half of the coding genes on cognitive abilities and risk for autism when deleted or duplicated.
Models could predict the effect size of any CNV on cognition with an 80% accuracy. The effect sizes of genes on cognition were associated with their expression along the primary neuroanatomical hierarchy of the human brain, situating early sensory cortical areas toward the bottom and higher-order association areas toward the top. We developed models, and a web-based tool is available to assist genetic counseling in interpreting CNVs.

Perspectives: These models are being adapted to include the additional variance explained by common and rare genomic variants across the genome and pave the way for precision diagnosis and counseling.

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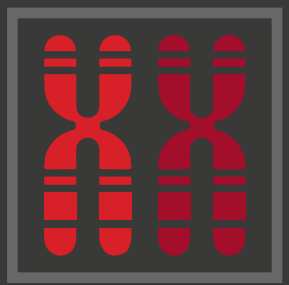

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