to be a relatively common mechanism for GIN associated with brain diseases. Additionally, chromothripsis is demonstrated for the first time to affect the diseased brain without cancerization. Accordingly, one may suggest that molecular alterations to genome stability maintenance machinery resulting in tissue-specific GIN (or chromosome instability) could be an underappreciated cause of a wide spectrum of complex diseases as it is the case for a number of cases of common brain disorders.
Supported by the Russian Science Foundation (Grant #14-35-00060); studying the adult brain is supported by ERA.Net RUS Plus Programme.

O3

Whole genome characterization of array defined clustered CNVs reveals two distinct complex rearrangement subclasses generated through either non homologous repair or template switching

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Molecular Cytogenetics 2017, **10(Suppl 1):**O3

Introduction
Clustered copy number variants (CNVs) as detected by chromosomal microarray are often reported as germline chromothripsis. However, such cases might need further investigations by massive parallel whole genome sequencing (WGS) in order to properly define the underlying complex rearrangement.
Methods
22 carriers of clustered CNVs, previously referred to the Departments of Clinical Genetics at the Karolinska University Hospital (Stockholm, Sweden) or Kennedy Center, Rigshospitalet (Copenhagen, Denmark) for a clinical chromosome microarray due to congenital developmental disorders, were sequenced using either Paired-End or Mate Pair libraries. To utilize the WGS data for structural variant analysis, a WGS caller (TIDDIT), a pipeline (FindSV) and a visualization program to picture the rearrangement end-product were developed.
Results
By combining read depth and discordant read pair analysis 154 junctions were characterized (range 4–26; median = 5) and an overall connectivity picture is given in 21 cases. These rearrangements were sub-classified depending on the patterns observed:
(1) Cases with clustered deletions only (e.g. del-nml-del-nml-del) often had additional hidden structural rearrangements, such as insertions and inversions, that may be the result of multiple simultaneous double-strand DNA breaks followed by non-homologous repair typical to chromothripsis.
(2) Cases with only duplications (e.g. dup-nml-dup) or combinations of deletions and duplications (e.g. del-nml-dup-del-nml-dup-nml-del), demonstrated a pattern of inversions, deletions and duplications more consistent with serial template switching during DNA replication (chromoanasynthesis).
Conclusion
Multiple copy number changes clustered on a single chromosome may arise through both chromothripsis and chromoanasynthesis.

O4

Defining haplotypes of complex structural variation using multicolour fibre FISH amylase CNV study

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Molecular Cytogenetics 2017, **10(Suppl 1):**O4

In spite of the rapid development in genome technology, the characterization and validation of complex structural variants (SVs) involving tandem low copy repeats and multi-allelic gene families remains a challenge. Recently we have been exploring the potential of combed DNA-fibres in the characterisation of complex SVs in human populations by multicolour fibre-FISH. The human amylase gene cluster harbours the salivary (AMY1) and pancreatic amylase genes (AMY2A and AMY2B). Early comparative genomic studies revealed copy number variations (CNV) in the amylase genes, which were shown to be linked with human adaptation to dietary starch intake, and implicated in predisposition to obesity. We have employed an array of high-resolution measurement methods, such as, whole-genome sequencing, paralogue ratio test (PRT), and optical mapping, together with fibre-FISH to characterise the amylase SV haplotypes in different populations. The results validate haplotype structures for AMY1 and show that pancreatic amylase genes underwent at least five independent rearrangements to create new haplotypes, one of which contains five copies each of the AMY2A and AMY2B genes in sub-Saharan African population. Fibre-FISH not only enabled a direct visualization of the SV haplotype structure by delineating the order, orientation, and absolute copy number of amylase genes, but also provided direct evidence for an inversion event that accompanies higher order expansion of AMY2A and AMY2B and a non-homologous junction where the upstream sequence of AMY2A interrupts one copy of AMY1. Such rearrangements were not detected by other methodologies initially, thus vindicating the use of fibre-FISH as the method of choice for validating complex SVs.

O5

Balanced X autosome translocation suggests association of AMMECR1 disruption with hearing loss short stature bone and heart alterations

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Molecular Cytogenetics 2017, **10(Suppl 1):**O5

Female balanced X-autosome translocations have been associated with absence of functional copies of the gene mapping at the breakpoint through disruption of the derivative chromosome and inactivation of the normal X-chromosome. We report on a nine year-old girl with karyotype 46,X,t(X;9)(q23;q12)dn, disproportionate short stature, septal

atrial defect, scoliosis, bone dysplasia, hearing loss, and normal cognition. Array-CGH and breakpoint sequencing confirmed the full complement of genetic material, whereas replication banding and HUMARA assay showed preferential inactivation of the normal X-chromosome. Both junction points were determined at the nucleotide level. The autosomal breakpoint affects a heterochromatic region, while the X-chromosome breakpoint was mapped between the AMMECR1 and RGAG1 genes. Whereas expression of the latter was unmodified, RT-qPCR showed absence of AMMECR1 expression in blood and lymphoblastoid cells of the proband. Concordantly, we identified an individual with nonsense mutation in AMMECR1 and short stature, septal atrial defect, radioulnar synostosis, hearing loss, and psychomotor and speech delay. We also describe three individuals with missense mutation and one with AMMECR1 duplication and overlapping phenotypes. The encoded protein contains evolutionary conserved nucleic acid-binding RAGNYA folds and localizes to the nucleus. AMMECR1 is co-expressed with genes implicated in cell cycle and translation regulation, five of which were previously associated with growth and bone alteration syndromes. Our knockdown of the zebrafish orthologous gene resulted in animals with features reminiscent of the patients' phenotype such as shorter tails, thinner bodies, kinked tail-ends, poorly defined somites, pericardial edema, tachycardia and hydrocephaly. Part of the knockdown phenotypes were rescued by co-injection of mRNA from the human ortholog. Our results suggest that AMMECR1 is potentially involved in cell cycle control and linked to a new syndrome(s) characterized by hearing loss, short stature, bone and heart alterations.

Consent to publish: The authors confirm that written informed consent was received by the patient for publication.

O6

Monitoring Guide RNA Synthesis for CRISPR Cas9 Genome Editing Workflow

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Molecular Cytogenetics 2017, **10(Suppl 1):**O6

Bacterial clustered regularly interspaced short palindromic repeats (CRISPR) - associated protein 9 (Cas9) system has increased in popularity as a genome editing tool for targeted mutations, insertions, deletions and gene knock-out studies. CRISPR genome editing has also proved superior to Zinc-finger nucleases (ZFN) and transcription activator- like effector nucleases (TALENs) due to its simplicity and easy programmability. In CRISPR, a guide RNA (gRNA) is used to recognize and introduce a double standard break (DSB) in a target DNA. The DNA repair mechanism triggered after the break is then exploited to introduce an insertion/deletion (indel) in the case of non-homologous end joining (NHEJ), or precise genetic modification if a homology-directed repair (HDR) pathway is triggered.

A critical part of the CRISPR/Cas9 tool is the design and synthesis of the gRNA that comprises T7 promoter sequence, target sequence, and protospacer adjacent motifs (PAM). Monitoring the transcription of the gRNA is critical to the workflow to ensure successful gene editing. Here we present an automated electrophoresis approach for monitoring the synthesis, integrity, and functional activity of gRNAs created for a CRISPR-Cas workflow.

O7

Chromosome territories in mice spermatogenesis: a new three-dimensional methodology

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Molecular Cytogenetics 2017, **10(Suppl 1):**O7

Chromosomes occupy specific nuclear regions called chromosome territories (CT) which are arranged in cell-type specific non-random patterns and are involved in genome regulation. In spermatogenic cells, a non-random distribution of chromosomes has been demonstrated in pachytene and metaphase I spermatocytes and in spermatozoa. However, most studies have been carried out using two-dimensional strategies, in particular spermatogenic stages and evaluating few chromosomes.

To overcome these limitations, we have developed an in situ fluorescent hybridization (FISH)-based protocol to approach the three-dimensional study of CTs along spermatogenesis. Testicular tissue from fertile mice C57BL6/6 J was enzymatically disaggregated. To preserve the nuclear structure, cell suspensions were spread out on polylysine-coated slides and fixed with paraformaldehyde. Subsequently, slides were frozen in liquid nitrogen and treated with pepsin. Three successive FISH rounds were carried out using the customized kit Chromoprobe Multiprobe® OctoChrome™ Murine System (Cytocell Ltd, Cambridge, UK) designed to identify the entire mouse karyotype. This kit uses seven different combinations of three whole chromosome painting probes directly labeled in three different fluorochromes. Afterwards, SYCP3 and H1T were identified by immunofluorescence staining to categorize among pre-meiotic cells, meiotic figures (discriminating all stages from leptotene to metaphase I and metaphase II), post-meiotic cells and spermatozoa. Serial optical sections of all cell types were obtained with a TCS-SP5 confocal microscope coupled to an imaging analysis system (LAS AF-1.8.1). After processing images by ImageJ, Matlab developed scripts were used to align, normalize and process nuclei in order to determine chromosome volume and proportion, chromosome radial position and chromosome relative position.

The application of the methodology developed allows the establishment of CTs throughout all spermatogenic stages providing a new basis to study the relationship between chromosome positioning and genome regulation.

Financial support: CF-180034 (UAB, Spain), DPI2015-65286-R/SAF2016-77165-P (MINECO, Spain) and CERCA Programme (Generalitat de Catalunya, Spain). M Solé is recipient of a grant from UAB (PIF/2015).

O8

A geography of clones mapping the tumour genome over anatomic space in children with cancer

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Molecular Cytogenetics 2017, **10(Suppl 1):**O8

Objectives

To chart genetic intratumour diversity in childhood cancer over multiple anatomic locations and time points during treatment in order to (1) delineate common routes of cancer cell evolution, (2) gain information on intrapatient variability of clinical biomarkers, and (3) assess clinical correlations to evolutionary patterns.

Methods

Patients were included based on the availability of two or more informative samples from the primary tumour, taken with a minimum intersample distance of 10 mm. A total of 55 patients with neuroblastoma (n = 24), Wilms tumour (n = 23) or rhabdomyosarcoma (n = 8) were subjected to multiregional analysis of tumour tissue with high resolution whole genome genotyping arrays (all patients) complemented by whole exome sequencing and targeted deep DNA sequencing (n = 20). Between two and 20 tumour samples were analyzed per patient resulting in total of 240 informative tumour samples.

Results

The majority of cases exhibited intratumour genetic diversity with branching evolution, including variability of several suggested clinical