

A case of ring chromosome 21 with hemifacial microsomia, developmental and speech delay analysed with WGS and patient-derived NES cells

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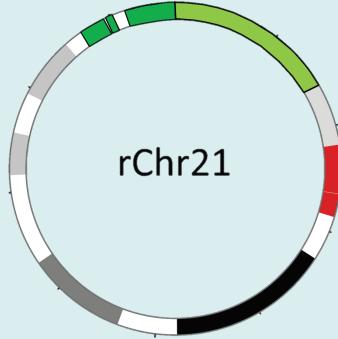


Figure 1: Ring chromosome showing G-banding (gray, black), centromere (red), confirmed (dark green) and hypothesized (light green) deletions

Ring chromosome

Karyotyping of blood, iPS and NES cells confirmed the presence of the ring chromosome in all three cell types. This highlights the fact that the ring is stable over passages and during differentiation, also suggesting a ring reformation process taking place every cell cycle.

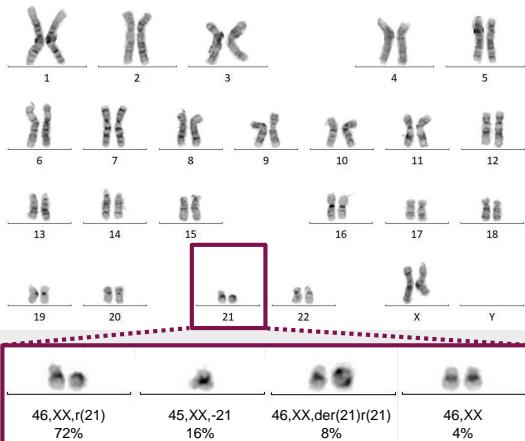


Figure 2: Karyogram from NES cells, showing the prevalence [%] of the chromosome 21 with four coexisting haplotypes.

Methods

We used chromosomal microarray, mate-pair and PCR free whole genome sequencing (WGS) for the genomic characterization. Breakpoint junction PCR (BPJ) and Sanger sequencing were used for confirmation. The gene expression analysis was performed by RNA Seq on NES cells from the patient compared to 3 healthy individuals.

Conclusion: Ring chromosomes are rarely occurring circularized chromatin which can arise during mitosis if the telomeric sequences are lost. Here, we present a 6-year old girl, carrying a ring chromosome 21, 46,XX,r(21)(p11.2q22.3). Genomic analysis unraveled three alternating deletions and two inversions with templated and non-templated insertions. Gene expression showed that 19 genes located in the deletions were downregulated suggesting haploinsufficiency. Moreover, the ring chromosome is stable through multiple passages of neuroepithelial stem cells (NES), differentiated from patient-derived induced pluripotent stem cells. The hemizygous genes located within the deletions are considered as likely causing the patient's phenotype.

Structural variants

WGS found three deletions flanking two inversion on the terminal region of chr21p. BPJ analysis confirmed results and further revealed templated insertions in each of the junctions.

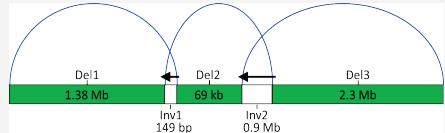


Figure 3: Length, orientation and position of three confirmed deletions and two inversions located on chr21p:term

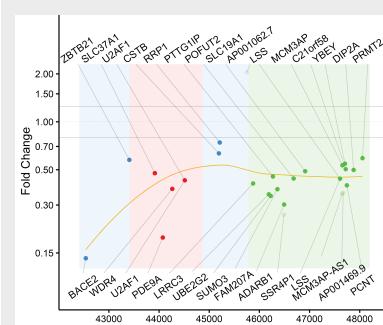


Figure 4: RNA Seq analysis shows downregulated protein coding genes (solid dots) and non-protein coding genes (pale dots) located in deletions (red, green) and 1 MB surrounding (blue).

RNA Seq analysis

RNA Seq revealed 10.7% and 18.6% differentially expressed protein coding genes genome wide and on chr21, respectively, of which 19 genes are located in deleted regions (red, green). It suggests that these 19 genes are haploinsufficient. Allele specific expression confirmed equal allele usage among the ring and linear homologue chromosome (not shown).

In vitro model with stem cells

A long term invitro system has been established using NES cells derived from patient-derived iPS cells. These cells showed neuronal differentiation and neurite growth forming axon-like structures within 45 days. This system can be exploited for physiological assays like axonal activity or cell migration to be linked to the patient's phenotype.

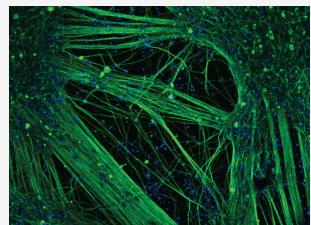


Figure 5: Differentiated neuroepithelial stem cells (NES), d45, labelled for TUBB3 (green) and DAPI (blue).