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Reduced cajal body number in a patient haploinsufficient for *COIL*

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Introduction

In this study we present a child and his mother who are both carriers of an apparently balanced translocation t(11;17) and with similar clinical symptoms including developmental delay, long face and bulbous nose, proximal symphalangism, bilateral cutaneous syndactyly of 2nd and 3rd toes and mild scoliosis. At the chromosome 17 breakpoint we revealed presence of a microdeletion, which includes among others *COIL*, *NOG* and *MSI2* genes. *COIL* encodes for p80 coilin, the molecular marker of the Cajal Bodies (CBs), which are subnuclear domains that contain a wide variety of components, including factors involved in splicing, pre-rRNA processing, histone pre mRNA 3´ maturation as well as transcription factors (1). CBs have recently been implicated in the assembly and/or modification of the RNA-processing machinery (2). *NOG* encodes for noggin and is essential for cartilage morphogenesis and joint formation (3). *MSI2* (*musashi 2*) encodes an RNA binding protein that regulates the expression of target mRNAs at the translation level and is thought to play a role in the proliferation and maintenance of stem cells in the central nervous system (4).

Results

Cytogenetic analysis of the mother and the child revealed an apparently balanced translocation t(11;17)(p15.5;q23.2). We mapped both chromosome breakpoints with FISH using BAC clones and revealed a ~2Mb deletion at 17q23.2 (Fig.1). This microdeletion spans 16 known genes, including *COIL*, *NOG*, and *MSI2* (Fig.2).

We investigated the Cajal body number of the transformed lymphoblastoid cells, and the patients CBs were present in only ~2.5% of the cells, as opposed to ~34% in the control cell-line (Fig.3). These results suggest that happloinsufficiency of *COIL* gene leads to reduced number of Cajal bodies.

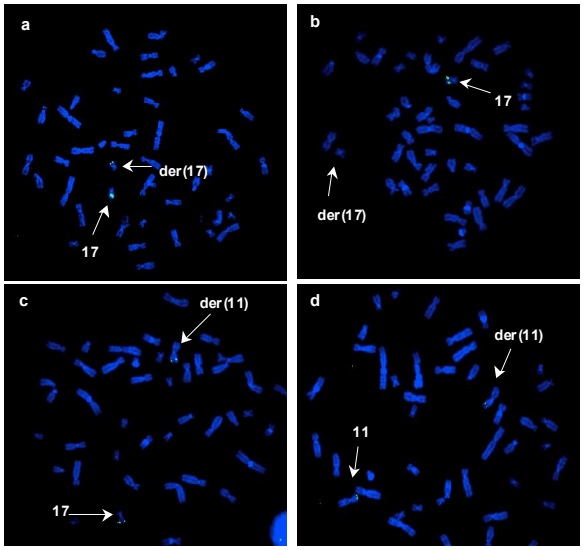

Fig.1 - FISH results for the 2 chromosomes: (a) BAC RP11-515e23, proximal to the breakpoint, showing signals on normal 17 and der(17), (b) BAC RP11-45j24, deleted, showing signals on normal 17q, (c) BAC RP11-159d12, distal to the breakpoint, showing signals on normal 17q and der(11p) and (d) BAC RP11-304m2, proximal to the breakpoint, showing signals on normal 11p and der (11p).

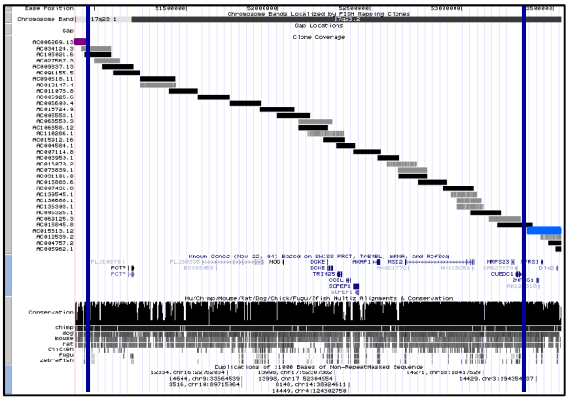

Fig.2 - Human genome browser image showing positions of proximal (purple), distal (light blue) and deleted clones (between dark blue lines), known genes in the region, conservation and duplications of >1000 bases of non-repeatmasked sequence.

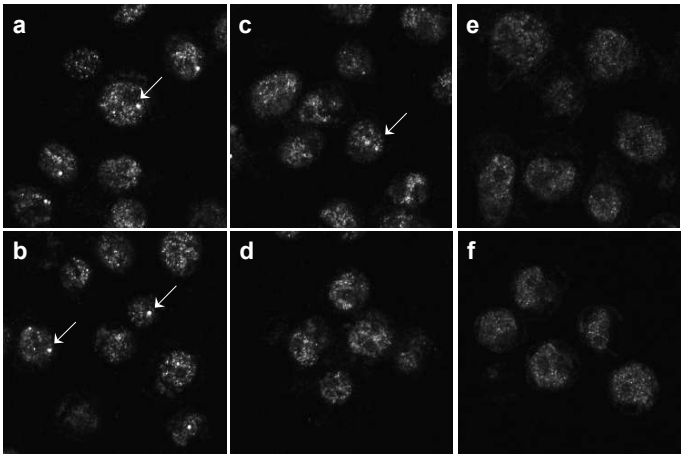

Fig.3 - Cajal Bodies of LBV transformed cell lines (a,b) control cell line, (c,d) mother cell line and (e,f) child cell line. The cells were incubated with anti-coilin antibodies (a,c, e, polyclonal anti-coilin; b, d and f, monoclonal anti-coilin). Arrows indicate cajal bodies.

Discussion and Conclusion

The phenotypes of the patients may be explained by haploinsufficiency of the genes deleted at 17q23.2. One of the genes deleted from this region is *COIL*. Deletion of exon 2-7 of *COIL* led to reduced viability in homozygous mice (5). The mouse knockout model has shown that full-length coilin is essential for proper formation and/or maintenance of CBs and recruitment of snRNP and SMN complex proteins to CBs (5). If Cajal Bodies, key organelles involved in RNA processing in general, are in significantly lower numbers, main molecules necessary for RNA processing may be absent or reduced, resulting in alterations in RNA-splicing and –transcription. The reduction of CBs in the patients suggests that haploinsufficiency of *COIL* could have phenotypic consequences. The limb defects (symphalangism, syndactyly) are consistent with the deletion of *NOG*, mutations of which are associated with symphalangism (3). Similarly, mental retardation may be caused by any of the unknown genes including *MSI2,* which is expressed during CNS development of in mice (4).

Materials and Methods

Chromosomes were prepared from peripheral blood lymphocytes cultures and by analysis of GTG-banded chromosomes (International System for Human Cytogenetic Nomenclature, ISCN 1995), a t(11;17)(p15.5;q23)mat was identified (Fig. 1). The translocation breakpoints were mapped by fluorescence in situ hybridisation (FISH) using bacterial artificial chromosomes (BACs) from the RP11 library, provided by the MCN Reference Centre in Berlin (http://www.molgen.mpg.de/~cytogen/). FISH was carried out using 250ng BAC-DNA labelled indirectly with biotin-14-dATP by nick translation and hybridised to metaphase chromosomes prepared from peripheral blood lymphocytes of the patient. Signals were visualised using avidin-FITC detection system. Chromosomes were counterstained with DAPI and the signals were analysed using a Leica DMRB epifluorescence microscope equipped with a Sensys 1400 CCD camera (Photometrics, USA) and an IPLab Spectrum imaging software (Vysis, USA) on a Macintosh computer. The Cajal Bodies of LBV transformed cell lines from the 2 translocation carriers and a control were immunolabeled with anti-coilin antibodies and antibodies that recognise a CB U2 snurp specific protein.

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