High-resolution karyotyping by oligonucleotide microarrays : the next revolution in cytogenetics

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Chapter 4.2

A subtle familial translocation t(3;21)(p26.3;q22.3): apparently healthy boy with a 3p deletion and 21q duplication

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Abstract
Here we report on the clinical and cytogenetic results in a family carrying a cryptic translocation involving chromosome 3pter and 21qter detected by Single Nucleotide Polymorphism (SNP) array and subtelomeric Fluorescent *In Situ* Hybridisation (FISH) analysis. The index patient, with mild mental retardation (MR) in combination with minor dysmorphic features, inherited the derivative chromosome 21 resulting in a partial trisomy of the short arm of chromosome 3 and a partial monosomy of the long arm of chromosome 21. Her apparently healthy brother inherited the derivative chromosome 3 resulting in a terminal deletion of the short arm of chromosome 3 and a terminal duplication of the long arm of chromosome 21. We discuss the different phenotypes for the two genotypes and argue for the importance of reporting these imbalances to achieve accurate genetic counseling in prenatal and postnatal diagnosis.

Introduction
Several patients with chromosome aberrations involving chromosomes 3pter and 21qter have been reported. The first case with the 3p deletion syndrome was described in 1978 (Verjaal and De Nef, 1978), and is characterized by low birth weight, developmental delay, growth retardation and dysmorphic facial features (Malmgren et al., 2007; Barber et al., 2008; Fernandez et al., 2008). The smallest region of overlap for all reported 3p deletion patients is 1.5 Mb on chromosome band 3p26, including the candidate genes *CHL1* (MIM# 607416), *CNTN4* (MIM# 607280) and *CRBN* (MIM# 609262) (Cargile et al., 2002; Dijkhuizen et al., 2006). The clinical manifestations of the trisomy 3p syndrome are quite variable depending on the size of the duplication (for review see Schinzel, 2001). The syndrome includes psychomotor retardation, mental retardation (MR) and minor dysmorphic features.

Genotype-to-phenotype correlations for partial monosomy and partial trisomy of chromosome 21 have recently been reviewed (Lyle et al., 2009). Deletions of the terminal 21q22.2q22.3 region produce a mild phenotype including MR and holoprosencephaly (Lyle et al., 2009). Cases with terminal duplications of 21q22 are rare. Most patients have a Down syndrome phenotype including cognitive impairment, congenital heart disease and characteristic facial and physical appearance (Lyle et al., 2009).

Conventional karyotyping can detect chromosomal abnormalities larger than approximately 5 Mb. For detection of smaller abnormalities Fluorescent *In Situ* Hybridisation (FISH)-, Multiplex Ligation-dependent Probe Amplification (MLPA)-,and whole genome high resolution array - analyses are necessary (Knight and Flint, 2000; de Vries et al., 2005; Rauch et al., 2006; Hoyer et al., 2007; Kriek et al., 2007; Gijsbers et al., 2009). In 5% of patients with MR with or without multiple congenital abnormalities subtelomeric abnormalities are identified (Flint et al., 1995; Knight et al., 1999; de Vries et al., 2003). Nevertheless, there are subtelomeric rearrangements reported in unaffected normal individuals and therefore considered to be not pathogenic (Hengstschläger et al., 2005; Balikova et al., 2007). Hence it is of great importance to report these polymorphisms for genetic counseling in prenatal and postnatal screening.

Here we report on the segregation of a submicroscopic familial reciprocal translocation between the subtelomeric regions of the short arm of chromosome 3 and the long arm of chromosome 21, and describe the different phenotypes in two siblings who inherited the different unbalanced products.

Materials and methods

Index patient

The index patient in this family was a 5-year old girl (Fig. 4.2.1a and b), the first child of healthy, unrelated parents. She was born after an uneventful pregnancy after 42 weeks of gestation with a birth weight of 2500 g and birth length of 43 cm. She spoke her first words before her first year and walked unsupported at the age of 2 years. At 2 years and 8 months delayed motor and speech development was noted. She had mild MR (IQ 65) and clinical examination showed frontal bossing, hypotelorism, thin and long face, hoarse voice and laxity of all joints.

Williams syndrome was excluded by FISH analysis with the DNA probe LSI ELN/ LSI D7S486, D7S522 (Vysis, Il, USA).

Brother of index patient

The 2.5-year old, younger brother (Fig. 4.2.1c) of the index patient was born after an uneventful pregnancy after 40 weeks of gestation. His birth weight was 3350 g. He walked unsupported at 14 months. His motor and speech development was normal. Clinical examination showed no dysmorphic features. At 14 months mild strabismus was noted. Before the age of 2 years he was able to count and to recognize figures. He will be kept under review in case later onset health problems emerge.

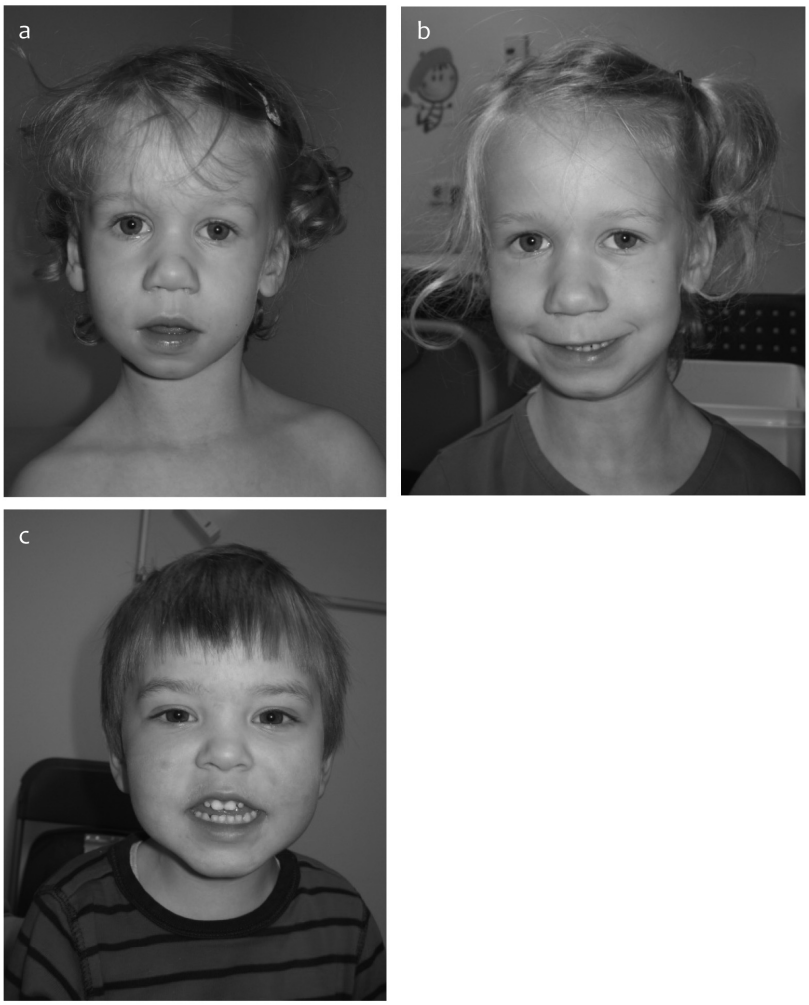

Figure 4.2.1 Pictures of the index patient and her apparently healthy brother. (a) (b) Facial picture of the index patient at the age of 3 (a) and 4 (b) years. Note frontal bossing, hypotelorism and thin and long face. (c) Facial picture of her brother at the age of 2 years.

Conventional karyotyping and FISH analysis
Conventional cytogenetic analysis on GTG-banded chromosomes from cultured lymphocytes and FISH analysis were performed according to standard techniques (Dauwerse et al., 1990).

SNP arrays
DNA was extracted from whole blood by a Gentra Puregene DNA purification Kit (Gentra Systems, Minneapolis, USA), following the manufacturer's instructions. The Affymetrix GeneChip Human Mapping 262K *NspI* array contains 262.262 25-mer oligonucleotides, with an average spacing of approximately 12 kb (Affymetrix, Santa Clara, CA, USA). An amount of 250 ng DNA was processed according to the manufacturer's instruction. SNP copy number was assessed using the software program CNAG Version 2.0 (Nannya et al., 2005).

The result of the index patient was added to the DECIPHER database (Wellcome Trust Genome Campus, Hinxton, Cambridge, UK) when consent was obtained (ID LEI248616).

Results
Conventional karyotyping showed no abnormalities in the index patient (Fig 4.2.2a). SNP array screening, however, revealed a terminal duplication on the short arm of chromosome 3 and a terminal deletion on the long arm of chromosome 21 (Fig 4.2.2b). The size of the aberrations was 3 Mb (from probe SNP_A-1971271 to SNP_A-2081957, respectively at 73.603 bp and 3.085.004 bp according to http//:www.ensembl.org, Ensembl release 56 – Sept 2009) and 5 Mb (from probe SNP_A-2019989 to SNP_A-2020813, respectively at 43.020.221 bp and 48.069.930 bp). FISH analysis confirmed the presence of a derivative chromosome 21, originating from a translocation between the short arm of chromosome 3 and the long arm of chromosome 21 (Fig 4.2.2c).

FISH analysis revealed a normal karyotype for the father, while the mother was carrier of a *de novo* balanced t(3;21)(p26.3;q22.3) (Fig 4.2.2d). The brother of the index patient inherited the derivative chromosome 3 resulting in a 3 Mb deletion of the terminal part of the short arm of chromosome 3 and a 5 Mb terminal duplication of the long arm of chromosome 21 (Fig. 4.2.2e).

Discussion
The presence of a double chromosome imbalance complicates genotype-phenotype correlations in patients. Our index patient carrying the derivative chromosome 21, resulting in a partial trisomy of 3p and a partial monosomy of 21q, presented mild MR, frontal bossing, hypotelorism, thin and long face, hoarse voice and laxity of all joints. The phenotype of trisomy 3p patients is quite variable and depends on the size of the duplication (Schinzel, 2001). The duplication of 3p in the index patient is 3 Mb and contains three genes: *CHL1*, *CNTN6* and *CNTN4*. In the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation/), all these genes are reported as gains in normal individuals. Therefore we suggest that the 3p duplication in our patient is probably not contributing to the phenotype. The 5 Mb deletion on chromosome 21, however, contains approximately 88 genes and is not described in normal individuals according to the DGV. Despite the phenotypic variability in partial monosomy 21, there are some common features among the reported cases including craniofacial, skeletal and cardiac effects, genital malformations and severe MR (Lyle et al., 2009). Yet, most

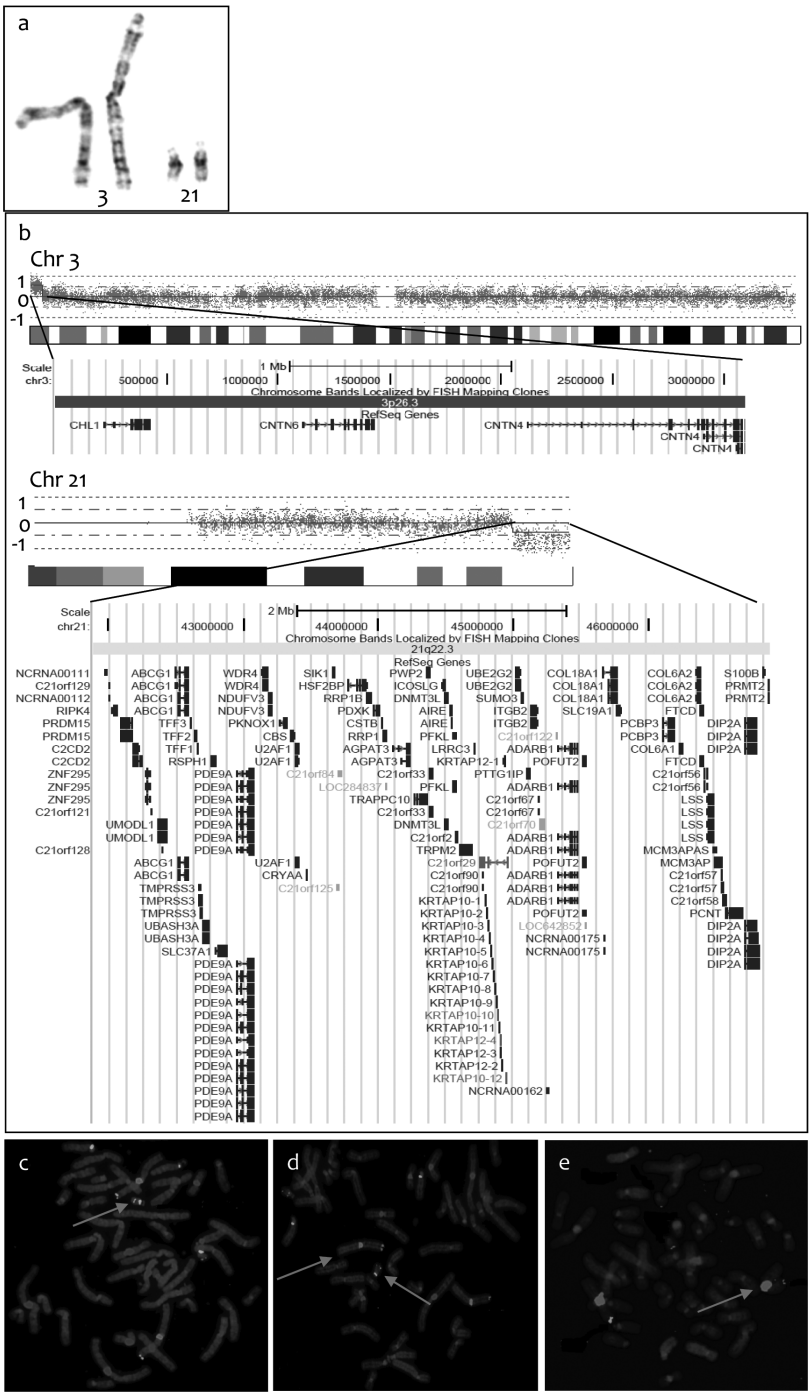

Figure 4.2.2 Cytogenetic and molecular analysis of the familial t(3;21)(p26.3;q22.3). (a) Conventional karyotyping revealed no abnormalities for chromosomes 3 and 21 in the index patient. The arrow indicates the aberrant chromosome 21. (b) SNP array analysis (Affymetrix GeneChip Human Mapping 262K NspI) results for the index patient demonstrating a 3 Mb duplication on the short arm of chromosome 3 and a 5 Mb deletion on the long arm of chromosome 21. Genes involved on both regions, UCSC Human Genome Browser (http://genome.ucsc.edu), Mar 2006 build (hg18). (c) FISH analysis confirmed the presence of the derivative chromosome 21 (black arrow), originating from a translocation between 3p (green; GS-1186B18; Flint) and 21q (red; GS-63H24; Flint). Control probes used for centromere chromosome 3 (red; CEP3; Vysis) and satellites of chromosome 13 and 21 (green; a-sat 13/21; Cytocell). (d) FISH analysis for the mother of the index patient demonstrated that she is carrier of the balanced t(3;21)(p26.3;q22.3) (black arrows) (probes: GS-1186B18, GS-63H24, CEP3 and a-sat 13/21). (e) FISH analysis for the brother of the index patient showing the derivative chromosome 3 (black arrow) (probes: GS-1186B18, GS-63H24, CEP3).

of these patients have a larger deletion than our patient. The most terminal 10 Mb of chromosome 21 contains approximately 130 genes and monosomy of this region contributes to a milder phenotype (Lyle et al., 2009). Accordingly the relative mild phenotype of our index patient is most likely explained by the even smaller distal 21q deletion. The phenotype might be the result of a gene-dosage effect of a combination of genes. Moreover, five of the 88 genes have been associated with MR; *CBS* (MIM# 236200), *COL6A1* (MIM# 120220), *CSTB* (MIM# 601145), *S100B* (MIM# 176990) and *SLC19A1* (MIM# 600424) (http://www.ncbi.nlm.nih.gov/sites/entrez).

The brother of the index patient, carrying the derivative chromosome 3, resulting in a terminal deletion of the short arm of chromosome 3 and a terminal duplication of the long arm of chromosome 21, shows an apparently healthy phenotype. The distal 3p deletion syndrome is reported with a recognizable phenotype, including developmental delay, low birth weight, growth retardation and several dysmorphic features (Malmgren et al., 2007; Barber et al., 2008; Fernandez et al., 2008). The critical region was defined as a 1.5 Mb region on chromosome 3p26, containing the candidate genes *CHL1*, *CNTN4* and *CRBN* (Cargile et al., 2002; Dijkhuizen et al., 2006). The genes *CHL1* and *CNTN4* map within the brother's deletion excluding haploinsufficiency of these genes as a cause for MR. Nevertheless, cases have been reported with a deletion of the distal 3p region without any phenotypic effects (Knight et al., 1995; Shrimpton et al., 2006; Takagishi et al., 2006; Hoo et al., 2008). Shrimpton et al (2006) even postulated that the distal 3p26 deletion is probably associated with normal intelligence and normal physical features. However, it is possible that mutations in one of the candidate genes on the normal chromosome 3 are responsible for an abnormal phenotype. This could also be suggested for the candidate gene *CRBN*, which was not deleted in our patient, but in both phenotypically abnormal and normal previously reported cases (Knight et al., 1995; Dijkhuizen et al., 2006). Another explanation might be that the distal 3p region is a susceptibility locus for MR, and therefore the deletion is not presenting MR in all 3p- cases. A further possible explanation might be the involvement of epigenetic and environmental factors.

Since terminal deletions of the 3p26 may be associated with a normal phenotype we suggest that prenatally detected de novo distal 3p deletions may warrant further molecular analysis to accurately size the deletion.

Patients with partial trisomy 21 are rare and the Down syndrome phenotype in most of these cases has been associated with duplication of 21q22 (Horn et al., 2003). A 250 kb trisomy of the distal subtelomeric region of 21q22.3 was reported as a benign variant (Bonaglia et al., 2007). Here we show that a terminal duplication of at least 5 Mb on distal 21q22.3q22.3 is not related with an abnormal phenotype. While a deletion of this region is associated with an abnormal phenotype in our index patient.

In conclusion, a genotype-phenotype correlation is difficult to make in regions with large number of genes and in regions in which deletions as well as duplications have been reported in healthy individuals. Yet, reporting these pathogenic and polymorphic rearrangements is of great importance for genetic counseling and prenatal screening. The detailed clinical description of our cases, along with a precise cytogenetic designation of chromosome breakpoints, allows further refinement of genotype-phenotype correlation for terminal imbalances in 3p and 21q.

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