Short communication

Combined deletion 18q22.2 and duplication/triplication 18q22.1 causes microcephaly, mental retardation and leukencephalopathy

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ABSTRACT

Chromosome 18 abnormalities rank among the most common autosomal anomalies with 18q being the most frequently affected. A deletion of 18q has been attributed to microcephaly, mental retardation, short stature, facial dysmorphism, myelination disorders, limb and genitourinary malformations and congenital aural atresia. On the other hand, duplications of 18q have been associated with the phenotype of Edwards syndrome. Critical chromosomal regions for both phenotypes are contentious. In this report, we describe the first case of an 11-year-old male with a combined interstitial duplication 18q22.1, triplication 18q22.1q22.2 and terminal deletion 18q22.2q23 with phenotypic features of isolated 18q deletion syndrome and absence of phenotypic features characteristic of Edwards syndrome despite duplication of the suggested critical region. This report allows for reevaluation of proposed critical intervals for the phenotypes in deletion 18q syndrome and Edwards syndrome.

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1. Introduction

Anomalies of chromosome 18 are among the most common autosomal abnormalities with a duplication of chromosome 18 occurring in approximately 1/3600 to 1/10,000 live births (Cereda and Carey, 2012) and a deletion of 18q reported in approximately 1/40,000 live births (Cody et al., 1999). Distal deletions of 18q are particularly frequent and appear to cause a variable phenotypic spectrum including growth deficiency, microcephaly, midface hypoplasia, congenital aural atresia (CAA), genital-tourinary malformations, myelination disorders, hypotonia and mental retardation (MR) (Cody et al., 1999; De Grouchy et al., 1964; Strathdee et al., 1995; Wertelecţă and Gerald, 1971). Proximal interstitial deletions involving bands q12 to q21 have been less frequently described (Harris et al., 1975; Krasikov et al., 1992; Schinzel et al., 1991). Furthermore, partial duplications of chromosome 18 have been intensively analyzed by various authors in order to identify critical regions for the core phenotype of Edwards syndrome. The classic Edwards syndrome with mental and developmental delay, growth deficiency, craniofacial dysmorphism, clenched hands with overlapping digits and internal organ malformation is associated with a duplication of the entire chromosome 18 (Binkert et al., 1990). However, partial trisomy 18 with mild to severe phenotypes of Edwards syndrome have also been reported (Boghosian-Sell et al., 1994; Frys et al., 1978; Matsuoka et al., 1981; Neu et al., 1976). Attempts to identify critical regions remain inconclusive. Hence, aneuploidies of chromosome 18 and the determination of genotype/phenotype correlations are of great clinical relevance. The advent of oligonucleotide array comparative genomic hybridization (array CGH) has enhanced the field of chromosomal aberration detection. Using array CGH coupled with conventional genetic techniques, we identified and described for the first time a case of combined interstitial duplication 18q22.1, triplication 18q22.1q22.2 and terminal deletion 18q22.2q23 presenting with leukencephalopathy, aural atresia, microcephaly, short stature and mental retardation.

2. Material and methods

2.1. Karyotype

For chromosome analysis peripheral blood lymphocytes from the index patient and his parents were karyotyped using standard protocols for cultivation and GTG banding.

Abbreviations: 18q deletion syndrome, 18q — syndrome; Array CGH, array comparative genomic hybridization; CNS, central nervous system; CAA, congenital aural atresia; FISH, fluorescence in situ hybridization; UCSC, genome browser hosted by the University of California, Santa Cruz; GH, growth hormone; Kaufman Assessment Battery for children, K-ABC test; MRI, magnetic resonance imaging; MR, mental retardation; MBP, myelin basic protein gene; NCBI, National Center for Biotechnology Information; OMM, Online Mendelian Inheritance in Man.

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2.2. Array CGH analysis

We obtained blood samples from the index patient and his parents after written informed consent. Genomic DNA was isolated from peripheral blood lymphocytes according to standard procedures. Patient and female reference DNA (Promega, Mannheim, Germany) were labeled with Cy3 and Cy5 using the Genomic DNA Enzymatic Labeling Kit (Agilent, Santa Clara, USA) and mixed according to the manufacturer’s protocol. The mixture was hybridized on a Microarray Kit 244A (Agilent) and incubated for 48 h in a hybridization oven. The chip contains 236,000 60-mer oligonucleotides plus controls resulting in an average resolution of 8.9 kb. After scanning the chip on the Agilent Microarray Scanner, the data was processed with the Feature Extraction software and analyzed with the Agilent Genomic Workbench version 6.5. DNA of the parents was tested with the father’s DNA as patient and the mother’s DNA as reference DNA.

For the validation of the array-CGH results, the Cytochip ISCA 180K (BlueGnome, Cambridge, UK) was used as a second array platform. This chip contains 180,000 probes with an average spacing of 25 kb in disease regions and up to 100 kb in the backbone region. Patient and reference DNA were labeled with the Cytochip Oligo dUTP labeling kit (BlueGnome) and hybridized according to the manufacturer’s protocol. Data was analyzed with the BlueFuse Multi software version 2.6 (BlueGnome). Interpretation of the results was based on the Ensembl genome browser (human genome build GRCh37/hg19).

2.3. Fluorescence in situ hybridization (FISH)

Cultured blood lymphocytes from the patient and his parents were harvested according to standard protocol to produce metaphase chromosomes. FISH analysis of the parents was performed on metaphase plates with whole chromosome painting probes for chromosome 18 (MetaSystems, Altlusheim, Germany) as well as subtelomeric probes for 18p and 18q (Abbott, Wiesbaden, Germany). Signals were analyzed using the ISIS software tool (MetaSystems, Altlusheim, Germany) (online Supplementary Fig. 1).

2.4. Database search

Literature search was performed to identify and review critical intervals for Edwards and deletion 18q syndrome using the databases of the National Center for Biotechnology Information (NCBI), the Online Mendelian Inheritance in Man (OMIM), the genome browser hosted by the University of California, Santa Cruz (UCSC) and the Charité University hospital library (search terms: Edwards syndrome, duplication chromosome 18, deletion chromosome 18, 18q, 18q22, microcephaly, mental retardation and leukencephalopathy).

3. Results

3.1. Phenotype

The index patient is the first of two children of non-consanguineous, healthy German parents. His younger brother is healthy and family history was unremarkable with respect to neurologic disorders. The patient was born at term without complications after an uneventful pregnancy: birth weight 2580 g (15th centile), length 48 cm (3rd centile) and head circumference 33 cm (10th centile). Dysmorphic features including hypertelorism, epicantus, broad nasal bridge, secondary microcephaly, kyphosis of the thoracic spine with enhanced gap of the pedicles of vertebral arches, epispadias, talipes equinovarus and prominent length of both thumbs in relation to generally long, tapering fingers and toes were noted (Fig. 1A, B, online Supplementary Fig. 2). His parents retrospectively remarked on feeding difficulties with poor suck. At the age of ten months, the infant presented with failure to thrive (height 12 cm below 3rd centile, weight 3 kg below 3rd centile, head circumference 1 cm below 3rd centile) and generalized muscular hypotonia. A unilateral narrow auditory canal and a conductive hearing loss were noted. Endocrinological examinations revealed normal growth hormone and thyroid function. At this age, magnetic resonance imaging (MRI) of the brain and spine illustrated a marked leukencephalopathy with no myelisation in the T2 sequence and a dilatation of the external and internal subarachnoid space (Fig. 1C). Hence, metabolic tests were conducted but showed only a mild increase of lactate and pyruvate with a normal lactate/pyruvate ratio and normal results for amino acids, organic acids, oligosaccharides, glycosaminoglycans and acylcarnitines. Abdominal organ malformations could be excluded by abdominal ultrasound. There was no evidence of cardiac disease. In the following years, the boy repeatedly presented to our hospital with only partly infection-associated emesis and ketoadiposis. Neurological examination including Munich functional development screening revealed a global retardation with a delay in developmental milestones: He sat independently at 10 months of age, started walking independently at the age of 18 months and spoke first words at the age of two years. His cognitive ability was measured with the K-ABC test (Kaufman Assessment Battery for Children) at the age of 4 and 5; 9 years and showed mild underperforming with pronounced deficiency of the acoustic memory, fine motor skills and concentration (mental processing composite 80, sequential processing scales 71, simultaneous processing scales 88 and achievement scales 75; normal scales 85–115). The developmental test of visual perception 2 performed at the age of 7 years revealed results below average. At the age of 11 years, the boy is attending third grade in an integrated school. His language development has been good, he can count up to 100, but he still has marked difficulties with writing and reading and lacks any sense of time.

3.2. Genetic results

The result of a chromosome analysis of the index patient was normal. Array CGH analysis revealed a de novo triple imbalance of 18q: (i) an interstitial duplication of 827,42 bp in the 18q22.1 region, (ii) a triplication of 1.54 Mb in the region 18q22.1q22.2 and (iii) a deletion of 9.92 Mb in the 18q22.2q23 region. His karyotype was arr 18q22.1(65,727,264–66,554,628)×3 de novo, 18q22.1q22.2(66,554,687–68,093,381)×4 de novo and 18q22.2q23(68,093,837–78,010,032)×1 de novo (GRCh37/ hg19) (Fig. 2, Table 1). The duplicated region contains one and parts of a further gene (thioredoxin-related transmembrane protein 3, coiled-coil domain containing 102B), both not listed in the Online Mendelian Inheritance in Man (OMIM). The triplicated region contains four genes that are listed in OMIM (docking protein 6, CD226, rotatin and suppressor of cytokine signaling 4), and a part of the partially duplicated gene coiled-coil domain containing 102B. The deleted region contains 25 known genes of which 14 are listed in OMIM. This genetic result was confirmed by a second array CGH.

4. Discussion

Here we describe a first case of a combined interstitial duplication of 18q22.1 (0.82 Mb), triplication of 18q22.1q22.2 (1.54 Mb) and terminal deletion of 18q22.2q23 (9.92 Mb) (Figs. 1 and 2). Cytogenetic results were attained by array CGH analysis and confirmed by a second assay (BlueGnome) (Fig. 2). Further FISH analysis revealed normal genotypes in the parents as evidence for a de novo occurrence of chromosomal aberrations in the index patient. Deletions and duplications of chromosome 18 rank among the most common autosomal anomalies with abnormalities of chromosome 18q being the most frequent and deletions of 18q being reported in 1/40,000 live births (Cody et al., 1999). Partial and complete duplications of chromosome 18 are described to be as frequent as 1/3600–1/10,000 (Cereda and Carey, 2012). The chromosomal breakpoint identified in our patient lies within a fragility site on chromosome 18, which has been described by Debacker et al. (2007) but not by other authors (Feenstra et al., 2007;
Fig. 1. Clinical and radiological phenotype of patient. (A) Synopsis of the patient’s phenotype. (B) X-ray of the left foot at age 7 demonstrating talipes. (C) Axial T2 cerebral MRI demonstrates leukencephalopathy and dilatation of the external and internal cerebrospinal fluid spaces at the age of 10 months. The axial T1 at the age of 10 months does not reveal dysmyelination.

Fig. 2. Genes located within the duplication 18q22.1 (0.82 Mb), the triplication 18q22.1q22.2 (1.54 Mb) and the deletion 18q22.2q23 (9.92 Mb). (A) Array CGH results using Agilent’s Microarray Kit 244A and BlueGnome’s CytoChip Oligo ISCA 180K. (B) Array CGH results with a proximal duplication 18q22.1 (log2 = 0.58) followed by a triplication 18q22.1q22.2 (log2 = 1) and a terminal deletion 18q22.2q23 (log2 = −1). Genes localized within the (C) duplicated (D) triplicated and (E) deleted region are depicted in our index patient according to UCSC genome browser on human GRCh37/hg19 including OMIM numbers. See Tables 1–3 for abbreviations.
4.1. Deletion 18q22.2–q23 and the deletion 18q phenotype

The terminal deletion 18q22.2–q23 in our patient does not include previously suggested haplolethal genes (Heard et al., 2009). In contrast to rare terminal deletions, more than 300 patients with terminal deletions of 18q have been described with a varying degree of cognitive impairment, hypotonia, short stature, ear canal abnormalities, abnormal genitalia, limb deformities (long, tapered fingers, proximally placed thumbs, talipes equinovarus, vertical talus, pes planus or cavus and overriding toes) and delayed myelination, a phenotype summarized as the deletion 18q syndrome (18q− syndrome) (Cody et al., 1999; Feenstra et al., 2007; Linnankivi et al., 2006; Strathdee et al., 1995; Wertelecki and Gerald, 1971). On the basis of these reports, candidate regions for specific features of the syndrome have been suggested (Fig. 3). Within the candidate region 18q22.3–q23 these phenotypic features have been linked to specific genes: aural atresia is caused by mutations in TSHZ1 (Core et al., 2007; Feenstra et al., 2006; Strathdee et al., 1995; Wertelecki and Gerald, 1971). The interstitial deletion 18q22.1 and triplication 18q22.1q22.2 in our patient presents with a deletion 18q22.2q23 corresponding with the above described candidate region and the typical phenotypic anomalies dysmyelination, short stature and aural atresia of 18q− syndrome but absence of a kidney malformation. The absence of the latter feature may be explained by its incomplete penetrance of 25% compared to a high penetrance of the other features of 78–100% (Cody et al., 2009). The dysmyelination in 18q− syndrome, characteristic associated with poor differentiation of gray and white matter on T2-weighted images, has been ascribed to haploinsufficiency of the myelin basic protein gene (MBP), the second most abundant CNS myelin protein involved in the compaction of central nervous system myelin (Gay et al., 1997; Linnankivi et al., 2006; Miller et al., 1990; Ono et al., 1994; Vogel et al., 1990; Weiss et al., 1991). Our report of a deletion of the MBP gene associated with dysmyelination supports this hypothesis. Likewise a dysmyelination has been described in association with an MBP gene deletion in mice (Griffiths, 1996; Roch et al., 1986). Finally, a microcephaly may be associated with 18q− syndrome and critical regions for its appearance have been defined on 18q21.2−18q21.3 (Kline et al., 1993) or 18q21.33 (Feenstra et al., 2007). Our report of a patient with microcephaly and interstitial deletion 18q22.2q23 without deletion of 18q21 may compromise the suggested critical region for microcephaly.

4.2. Duplication 18q22.1/triplication 18q22.1q22.2

The interstitial duplication 18q22.1 and triplication 18q22.1q22.2 in our patient have not been described so far. The location of the duplication proximal to the triplication in our patient is in line with previous reports of intrachromosomal duplications and supports the theory of a common mechanism in the genesis of interstitial intrachromosomal triplications (Giorda et al., 2011). The duplication/triplication in our patient furthermore corresponds to a proposed critical region on chromosome 18q for appearance of the Edwards syndrome phenotype (Fig. 3) (Turleau and de Grouchy, 1977). Still, this duplication did not induce the characteristic phenotype with distinct craniofacial dysmorphism, clenched hands and overlapping digits and internal organ malformations

<table>
<thead>
<tr>
<th>Table 1</th>
<th>List of genes within duplication 18q22.1.</th>
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<tbody>
<tr>
<td>Gene</td>
<td>Protein</td>
</tr>
<tr>
<td>TMX2</td>
<td>Thioredoxin-related transmembrane protein 3</td>
</tr>
<tr>
<td>CCDC102B</td>
<td>Coiled-coil domain containing 102B</td>
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<th>Table 2</th>
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<td>Protein</td>
</tr>
<tr>
<td>CCDC102B</td>
<td>Coiled-coil domain containing 102B</td>
</tr>
<tr>
<td>DOK5</td>
<td>Docking protein 6</td>
</tr>
<tr>
<td>CD226</td>
<td>CD226 antigen</td>
</tr>
<tr>
<td>RTTN</td>
<td>Rotatin</td>
</tr>
<tr>
<td>SOCS4</td>
<td>Suppressor of cytokine signaling 4</td>
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</tbody>
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<th>Table 3</th>
<th>List of genes within deletion 18q22.2q23.</th>
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<tbody>
<tr>
<td>Gene</td>
<td>Protein</td>
</tr>
<tr>
<td>CBLN2</td>
<td>Preicerebellin 2</td>
</tr>
<tr>
<td>NETD1</td>
<td>Neurin- and tolloid-like 1</td>
</tr>
<tr>
<td>FBXO15</td>
<td>F-box only protein 15</td>
</tr>
<tr>
<td>CYB5A</td>
<td>Cytochrome b5, type A</td>
</tr>
<tr>
<td>PEPD</td>
<td>Peptidase A</td>
</tr>
<tr>
<td>CDND1</td>
<td>Carnosine dipeptidase 1</td>
</tr>
<tr>
<td>ZNF236</td>
<td>Zinc finger protein 236</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>GALR1</td>
<td>Galanin receptor 1</td>
</tr>
<tr>
<td>SALL3</td>
<td>Sal-like 3</td>
</tr>
<tr>
<td>NFATC1</td>
<td>Nuclear factor of activated T cells</td>
</tr>
<tr>
<td>CTDPI</td>
<td>C-terminal domain of RNA polymerase II subunit A</td>
</tr>
<tr>
<td>KCNG2</td>
<td>Potassium channel, voltage-gated, subfamily G, member 2</td>
</tr>
<tr>
<td>PARDD6G</td>
<td>Parititioning-defective protein 6</td>
</tr>
</tbody>
</table>
in our patient. Our findings and few further case reports suggest that a
distal or terminal duplication of chromosome 18q is not sufficient for
the appearance of the Edwards syndrome phenotype (Isidor et al.,
2008; Quiroga et al., 2011), but that in fact a combined duplication
of a specific proximal and distal region of 18q is necessary to induce
this phenotype. These regions may comply with a combination of
18q11 and 18q22-qter (Turleau and de Grouchy, 1977) or combination
of 18q12.1–q21.2 and 18q22.3-qter (Boghosian-Sell et al., 1994). In
total, approximately 10 patients with Edwards syndrome phenotype
among 50 patients with a partial duplication of chromosome 18 have
been identified and helped to delineate further candidate regions for
this phenotype: (i) 18q21 (Matsuoka et al., 1981), (ii) CRES is proximal
to 18q12.2 (Mucke et al., 1982) and (iii) 18q11–q12 (Fryns et al., 1978).
The possibility of an inconstant Edwards syndrome phenotype in our
index case as a consequence of incomplete penetrance and phenotype
variability needs to be considered.

In conclusion, we describe a patient with a combined interstitial du-
pllication 18q22.1, triplication 18q22.1q22.2 and deletion 18q22.2q23
with phenotypic traits of 18q deletion syndrome without overt clinical
signs of Edwards syndrome. The duplication/deletion identified in
the index patient lies within a previously suggested fragile site on
chromosome 18 (Debaker et al., 2007) and does not contain pos-
posed haplolethal genes (Heard et al., 2009). This report supports
the hypothesis of MBP haploinsufficiency in dysmyelination and allows
for reevaluation of the critical region for appearance of microcephaly
in 18q — syndrome. Even though various authors have attempted to
clarify genotype–phenotype correlations of chromosome 18 for several
decades, the candidate regions for distinct phenotypes remain to be
identified in order to improve patient consultation and potentially
allow for therapeutic consequences for affected patients.

Supplementary data to this article can be found online at http://
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