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RESEARCH NOTE

Atypical microdeletion 22q11.2 in a patient with tetralogy of Fallot

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Abstract. The 22q11.2 microdeletion syndrome (22q11.2 DGS) is characterized by an extreme intrafamilial and interfamilial variability. The main clinical features are congenital heart defects, palatal abnormalities, learning disability, facial dysmorphisms and immune deficiency. In 85–90% of cases, the 22q11.2 DGS is caused by a heterozygous ∼3-Mb deletion, including the *TBX1* gene, considered one of the major genes responsible for heart defects. Individuals with atypical deletions with at least one breakpoint outside low copy repeats have been reported. Our patient is a child presenting tetralogy of Fallot (TOF) with an atypical 22q11.2 deletion proximal to the critical DiGeorge region. The rearrangement was inherited from the healthy mother and spanned ∼642–970 kb, encompassing *DGCR6* and *PRODH*, two novel possible candidate genes for conotruncal heart defects.

Keywords. tetralogy of Fallot; DiGeorge syndrome; 22q11.2 deletion; conotruncal defect.

Introduction

The 22q11.2 recurrent microdeletion is the most frequent microdeletion in humans, with a likely underestimated prevalence of 1:4000–6000 live births (McDonald-McGinn *et al.* 1999). The 22q11.2 chromosomal region contains low-copy repeats (LCRs), namely LCR22A-H, that mediate meiotic nonallelic homologous recombination predisposing to copy number variations (CNV) (Burnside 2015). The 22q11.2 microdeletion syndrome (22q11.2 DGS) is characterized by an extreme intrafamilial and interfamilial variability and includes several phenotypes previously described as DiGeorge syndrome (DGS), velocardiofacial syndrome (VCFS), conotruncal anomaly face syndrome (CTAF), some cases of autosomal dominant Opitz G/BBB syndrome and Cayler cardiofacial syndrome (McDonald-McGinn *et al.* 1999). The main clinical features are congenital heart disease (74% of individuals), palatal abnormalities (69%) (e.g., velopharyngeal incompetence, cleft palate), learning disability, facial dysmorphisms and immune anomalies (77%) (McDonald-McGinn *et al.* 1999). Additional findings may include hypoparathyroidism and hypocalcaemia (50%), structural gastrointestinal anomalies, renal anomalies (31%), hearing loss, laryngotracheoesophageal anomalies, growth hormone (GH) deficiency, autoimmune disorders, seizures, skeletal or ophthalmologic abnormalities, autism spectrum disorder, psychiatric illness, genitourinary tract anomalies (McDonald-McGinn *et al.* 2010).

Congenital heart defects are the major cause of morbidity and mortality in these patients and include conotruncal malformations of the outflow tract as tetralogy of Fallot (TOF), interrupted aortic arch, and septal defects (McDonald-McGinn *et al.* 2010). Although the typical facial appearance is characterized by long face, malar hypoplasia, auricular and

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nasal abnormalities, 'hooded' eyelids, hypertelorism, strabismus, cleft lip and palate, asymmetric crying facies, these features are extremely variable and facial dysmorphisms can be very subtle (McDonald-McGinn *et al.* 2010). Most patients with 22q11.2 DGS (85%) have a heterozygous 3-Mb deletion encompassing LCR22A-D and including the *TBX1* gene, which is considered the major gene responsble for heart defects in DGS. Approximately 8–10% of the patients carry a nested 1.5 Mb LCR22A-B deletion, also spanning the *TBX1* gene (McDonald-McGinn *et al.* 1999). Individuals with atypical deletions with at least one breakpoint not in an LCR have been reported (McDonald-McGinn *et al.* 1999; Edelmann *et al.* 1999; Beaujard *et al.* 2009). It is noteworthy that deletions that do not encompass the LCR22A-B region are not usually detectable with commercially available fluorescence *in situ* hybridization (FISH) probes (McDonald-McGinn *et al.* 1999), posing the hypothesis that some of these atypical 22q11 deletion are not currently diagnosed. A classification in proximal, central and distal 22q11.2 deletions has been proposed. Proximal deletions have the proximal breakpoint in LCR22A; central deletions span either B–D or C–D and do not include *TBX1*; distal deletions extend to LCR22E or F (Burnside 2015). Patients with central deletions have a lower incidence of immune deficiency, hypotonia, palatal abnormalities and behavioural problems. However, given the extreme variability of clinical presentation, genotype–phenotype correlation cannot be predicted (Burnside 2015). So far, haploinsufficiency of two genes, *TBX1* and *CRKL*, has been proposed as the main molecular player in the pathogenesis of the neural crest dysfunction and the anomaly of the anterior heart field, potentially affected during the morphogenesis of the syndrome (Yagi *et al.* 2003; Moon *et al.* 2006; Keyte *et al.* 2014). *MAPK1* and *HIC2*, located distally to the formers, may also be implicated in the phenotype (Newbern *et al.* 2008; Dykes *et al.* 2014)

Here, we present two patients, mother and son, with an atypical 22q11.2 deletion spanning outside the critical DiGeorge region.

Clinical report

The proband was the first child of nonconsanguineous Caucasian parents. The mother, 29 years old, is affected by lupus tumidus, with normal echocardiogram and normal immunological status. The father presents duplicated ureter and is otherwise healthy. During pregnancy, maternal cholestasis was diagnosed. Foetal DNA screening by noninvasive prenatal testing was negative for aneuploidy and for microdeletion/duplication syndromes, including 22q11.2 syndrome. The obstetric ultrasound at 20 weeks of gestational age revealed a foetal heart defect, subsequently characterized as TOF, with over-riding of the aorta, interventricular defect and hypoplastic pulmonary artery. The proband was born at 39+1 weeks of gestational age by caesarean section due to nonreassuring cardiotocography. Birth weight was 3.090 g (28° centile, −0.58 SDS) and Apgar score was 8 at 1st and 5th min. He did not present hypocalcaemia. The neonatal echocardiogram confirmed the diagnosis of TOF. Electrocardiogram was normal. Abdominal ultrasound revealed bilateral renal pyelectasis with duplicated left ureter. Cranial ultrasound was morphologically normal for age. At 20 days after birth, he underwent heart surgery. The corrective infundibulotomy was not possible because of the intraoperative finding of a coronary anomaly, consisting of a double anterior descending coronary artery with the major one originating from the right coronary artery and running on the infundibulum. Therefore, a palliative systemic-pulmonary right shunt was performed while total TOF correction with an infundibular patch was postponed at 16 months of age. At 45 days of age, the clinical examination of the proband revealed weight 3.080 g (<3° centile, −2.60 SDS), length 55.5 cm (33° centile, −0.44 SD), OFC 37.0 cm (12° centile, −1.18 SD), no facial dysmorphisms were noted, suggesting an isolated heart defect. At the last clinical evaluation at 16 months of age, length was 74 cm (5° centile), weight 8.075 g (1° centile), neuromotor development normal for age. He presented gastroesophageal reflux and laryngomalacia, the latter causing nocturnal desaturation and roncopathy. Surgical correction of the laryngeal defect was scheduled. Immunological status, evaluated by serum immunoglobulin measurement and lymphocyte count and immunophenotyping was normal, and all scheduled vaccines were regularly administered.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent to participate was obtained from all individual participants included in the study.

Array-CGH

We performed array-CGH with a 60K whole-genome oligonucleotide microarray on chorionic villi extracted DNA, which revealed an atypical 22q11.2 microdeletion centromerically proximal to the DiGeorge critical region (chr22:17950020-18919941x1, hg19) (Decipher case 402772) (figure 1). The microdeletion spanned ∼642–970 kb. Segregation analysis by MLPA (SALSA MLPA Probemix P250 DiGeorge; https://www.mrcholland.com/) showed a maternal inheritance. Standard karyotype on amniocytes was normal [46,XY].

Discussion

The patient we describe carries an atypical 22q11.2 deletion, containing five autosomal recessive disease-causing genes: *ATP6V1E1*, causing autosomal recessive cutis laxa, type IIC (MIM: 617402); *PEX26*, causing peroxisome biogenesis

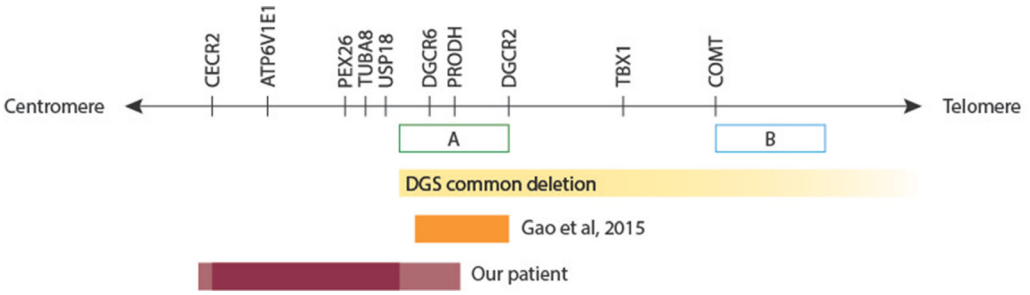

Figure 1. Patient's 22q11.2 deletion. The region is proximal to the canonical 22q11.2 DGS region and partially overlaps the deletion/duplication described by Gao *et al.* (2015), which encompasses *DGCR6* and *PRODH.* The A and B boxes show the LCRA and LCRB sequences. The typical DGS region, the deletion described by Gao *et al.* and the patient's deletion are shown.

disorder 7B (MIM: 614873) and a form of Zellweger syndrome (MIM: 614872); *TUBA8*, causing a form of cortical dysplasia (MIM: 617401) and *USP18*, causing the pseudo-TORCH syndrome 2 (MIM: 617397) and *PRODH*, causing hyperprolinaemia, type I (MIM: 239500). Given the clinical presentation, the low carrier prevalence of these diseases and the recessive transmission, it is unlikely that the phenotype could be related to these disorders.

Gao *et al.* (2015) reported two patients with conotruncal heart defects with overlapping proximal 22q11.2 deletion and duplication respectively, within the DiGeorge critical region not encompassing *TBX1* (Gao *et al.* 2015). The authors also reviewed four more cases of patients with heart defects and a microdeletion/duplication in the proximal critical DGS region, not comprehensive of *TBX1* but including two new candidate gene, *DGCR6* and *PRODH*, potentially involved in phenotype pathogenesis either directly or through *TBX1* interaction (Amati *et al.* 1999; Lu *et al.* 2001; Gao *et al.* 2015). The deletion found in our familial case partially overlaps the deletion/duplication described by Gao *et al.* encompassing *DGCR6* and *PRODH* (figure 1). Altered expression of *DGCR6* was reported as responsible for cardiac malformations in chicken models of DGS, supporting the hypothesis proposed by Gao *et al.* (Hierck *et al.* 2004). These deletions lie outside the critical region for DGS, proximally to LCR22A, implicating that other mechanisms, beside the *TBX1* related, could be involved in the development of conotruncal heart defect. It can be hypothesized that genes dysregulation could be related to loss of long-range regulatory sequences and could affect developmental pathways (Zeitz *et al.* 2013). The possibility of gene expression dysregulation due to presence of allele specific interaction can also be hypothesized and *DGCR6* has been reported to be more variably expressed among 22q11.2 DGS patients, especially on the maternally inherited alleles (Das Chakraborty *et al.* 2012; Lonfat *et al.* 2013).

Recently Zhao *et al.* (2020) tested whether variants in the hemizygous LCR22A-D region are associated with risk for conotruncal heart defects and found a significant association in a cluster of common single nucleotide variants in a 350 kb region on the conserved allele. Interestingly this region contains *CRKL*, one of the candidate genes responsible for congenital heart defects in DiGeorge syndrome (Zhao *et al.* 2020). It would be interesting to look for single nucleotide variants proximally to the DiGeorge region, especially in *DGCR6,* associated with the risk of heart defects in these patients. MicroRNA (miRNA) regulation has also been proposed to be a contributor to the 22q11.2 DGS phenotype (de la Morena *et al.* 2013) and Merico *et al.* (2014) showed that miRNAs in the proximal A–B region are involved in the regulation of expression of genes in different developmental pathways (Merico *et al.* 2014). Nonetheless, we cannot rule out the possibility that the cause of TOF in our patients is yet unknown and that the 22q11.2 deletion is just an occasional finding.

The present case further demonstrates that, yet, much has to be learned about 22q11.2 DGS pathogenesis and associated atypical microdeletion. Future studies on long-range interaction regulatory elements and reporting of further atypical CNVs could be helpful in explaining the wide clinical variability and the genotype–phenotype correlation in this disorder. While further data on genotype–phenotype correlation will be acquired, atypical proximal deletion of the 22q11.2 region containing *DGCR6* and/or *PRODH* should be considered a potential risk factor for complex or isolated conotruncal heart defect, with yet unknown mechanism.

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