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RESEARCH NOTE

'Distal 16p12.2 microdeletion' in a patient with autosomal recessive deafness-22

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Abstract. The 16p12.2 chromosome band contains three large segmental duplications: BP1, BP2 and BP3, providing a substrate for recombination and recurrent chromosomal rearrangements. The '16p12.2 microdeletion' is a recurrent deletion comprised between BP2 and BP3, associated with variable clinical findings. We identified a heterozygous 16p12.2 microdeletion spanning between BP1 and BP2 in a child evaluated for short stature and mild dyslexia. Unexpectedly, the mother carried the same deletion in the homozygous state and suffered from severe hearing loss. Detailed family history revealed consanguinity of the maternal grandparents. The 16p12.2 microdeletion is a rare condition and contains only three genes: *METTL9*, *IGSF6* and *OTOA* of which the *OTOA* is considered responsible for DFNB22 hearing loss (MIM: 607039) under its homozygous condition. A number of *OTOA* mutations have been described, whereas very few cases of a 16p12.2 microdeletion similar to that observed in our family have been reported. In conclusion, we describe a rare 'distal 16p12.2 microdeletion' widening the phenotypic spectrum associated with the recurrent 16p12.2 microdeletion and support the causative role of *OTOA* microdeletion in hearing impairment.

Keywords. segmental duplications; chromosomal rearrangements; hearing loss; *OTOA* gene.

Introduction

Several novel submicroscopic microdeletion and duplication syndromes have been described since the implementation of array analysis. The majority of these known recurrent rearrangements result from nonallelic homologous recombination (NAHR) or unequal crossing-over between large and highly identical segmental duplications (>10 kb) (Lupski 1998). Chromosome 16 is enriched for interspersed segmental duplications, especially the short arm. In particular, the 16p12.2 chromosome band contains three large segmental duplications: BP1, BP2 and BP3. The recurrent 16p12.2 microdeletion is flanked by two large blocks of segmental duplications: BP2 and BP3, and extends over about 520 kb (Girirajan *et al.* 2010). This rearrangement is associated with variable clinical findings that do not constitute a recognizable syndrome. The estimated incidence of 16p12.2 microdeletion is ~1:15,000 live births. Subjects carrying this deletion might present with a variable phenotype including developmental delay, cognitive impairment, growth impairment, cardiac malformations, epilepsy and psychiatric and/or behavioural problems. In some cases, hearing loss, dental abnormalities, renal and male genital abnormalities, cleft palate and/or cleft lip were found (Girirajan *et al.* 2010, 2012; Pizzo *et al.* 2017).

Rarely microdeletions occur distally to the 'recurrent 16p12.2 microdeletion' between the segmental duplications BP1 and BP2 including only three genes: *METTL9*, *IGSF6* and *OTOA*.

OTOA (MIM 607038) is one of the gene responsible for hearing loss. This gene encodes otoancorin, a 120-kDa glycoprotein of the acellular gels of the inner ear expressed on the surface of the cochlear spiral limbus. The defective stimulation of the inner hair cells resulting from abnormal otoancorin function is responsible for the hearing impairment of autosomal recessive deafness-22 (DFNB22; MIM: 607039) (Zwaenepoel *et al.* 2002; Shahin *et al.* 2010; Lukashkin *et al.* 2012; Lee *et al.* 2013). Here we report a family study of a rare distal 16p12.2 deletion of ~165

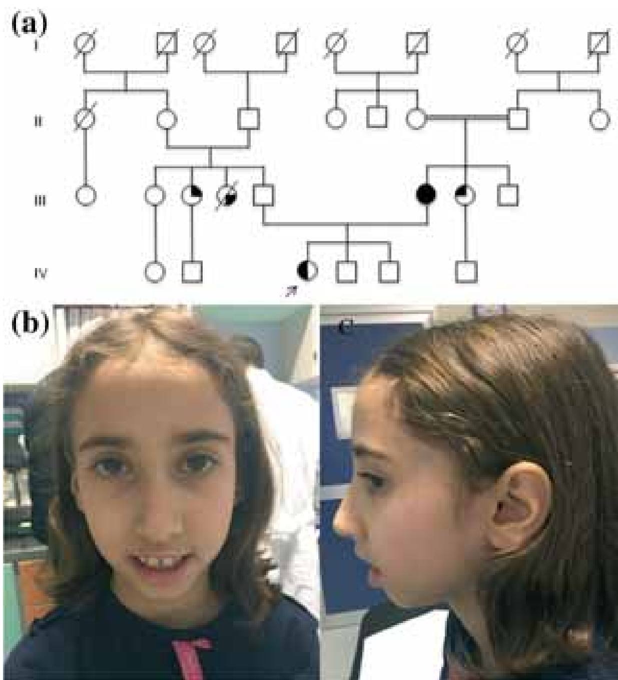

Figure 1. (a) Pedigree structure of the family. Closed symbols indicate affected family members whereas unaffected members are represented by open symbols. Partially filled symbols indicate family members affected by medical conditions other than the one studied in the proband. Deceased members are indicated by the slash symbol. (b) Images of the subjects. Facial dysmorphic features of the proband include: high frontal hairline, wide frontal region, high nasal root and diastema of central incisors, retrognathia and bilateral hypoplasia of the helix, combined with bilateral agenesis of the crus helices.

kb, identified by array-comparative genomic hybridization (CGH).

Materials and methods

A 10-year-old child was referred to our Pediatric Endocrinology Centre for short stature, mild dyslexia and dysmorphic facial features (figure 1). She is the second child of unrelated parents. When the child was presented, her weight was 19.9 kg (−3.4 SD), height of 123.2 cm (−2.3 and 2.5 SD below the target height) and head circumference of 50.7 cm (−1.2 SD). She had pectus excavatum, kyphosis and mild facial dysmorphisms including high nasal root, retrognathia, bilateral agenesis of the crus helices and bilateral hypoplasia of the helix. She was born small in size for gestational age after an uncomplicated full-term pregnancy. She was delivered vaginally after an uncomplicated full-term pregnancy. Her birth weight was 2.270 kg (−2.85 SD, small for gestational age).

Her three brothers and her father were healthy. Detailed family history revealed consanguinity of maternal grandparents, who were first-degree cousins (figure 1). Interestingly, her mother suffered from congenital bilateral deafness and severe speech impairment. She received binaural cochlear implants during infancy. Her reproductive history revealed a previous first trimester spontaneous abortion. Her past medical history was unremarkable. She reported that her sister suffered from isolated speech difficulties, without any associated hearing impairment. We have diagnosed a16p12.2 deletion in the proband and her mother using a whole-genome 180 K Agilent array with ∼13 kb overall median probe spacing (Human Genome CGH Microarray, Agilent Technologies, Santa Clara, USA), according to the manufacturer's protocol. Written consent was obtained from the parents for publication of this case report.

Results

Array-CGH analysis of the child revealed a heterozygous interstitial deletion at 16p12.2 spanning about ∼165 kb of genomic DNA: arr[GRCh37] 16p12.2(21,574,908–21,739,885) according to UCSC Genome Browser (hg19; GRCh Build 37.1, February 2009). Her mother carried the same homozygous deletion whereas her father had a normal array-CGH.

The deleted region contains only three OMIM genes: *METTL9* (MIM: 609388; methyltransferase-like 9: or *DORA, DREV*), *OTOA* (MIM: 607038; otoancorin) and *IGSF6* (MIM: 606222; immunoglobulin superfamily, member 6). Interestingly, the deleted region is comprised between two large blocks of segmental duplications (BP1 and BP2) predisposing to NAHR events resulting in microdeletions/microduplications. BP1 spans ∼216 kb (chr16:21,353,862–21,570,112) while BP2 spans ∼204 kb (chr16:21,741,217–21,946,145). The identity between these two loci is greater than 99%. Finally, BP3 spans ∼194 kb (chr16:22,430,867–22,624,823) (figure 2).

Discussion

Segmental duplications and the flanking unique regions are sites of both rare and common copy number variations (CNVs). Segmental duplications or low copy repeats (LCRs) are blocks of DNA occurring at many sites within the genome and share a high level (>90%) of sequence identity. LCRs may be substrates for NAHR. Chromosome 16 is particularly rich in LCRs, three of which named BP1, BP2 and BP3 are located in 16p12.2 (figure 2). Our subjects carry a microdeletion spanning ∼165 kb between BP1 and BP2. This rearrangement is very rare whereas a number of studies reported a ∼520 kb microdeletion commonly named '16p12.1 or 16p12.2 microdeletion' (Antonacci *et al.* 2010; Girirajan *et al.* 2012; Pizzo *et al.* 2017). This 520 kb deletion is comprised between BP2 and BP3 and contains four genes with no OMIM (*PDZD9*, *C16orf52*, *VWA3A* and *POLR3E*) and

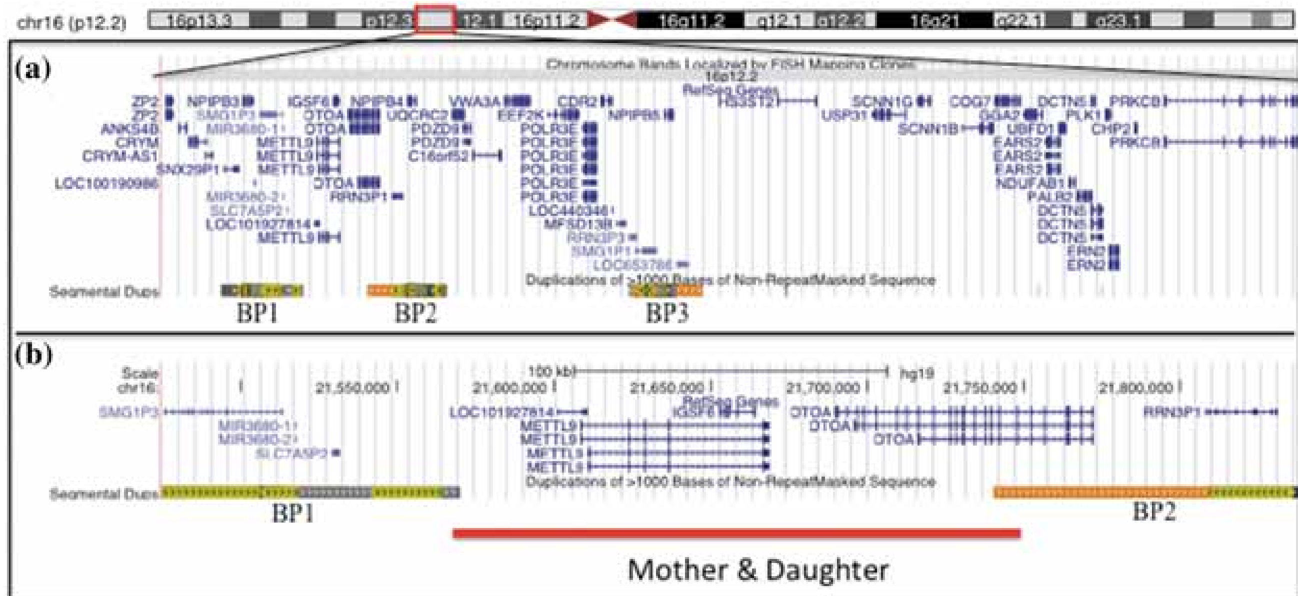

Figure 2. Extract from the UCSC genome browser (http://genome.ucsc.edu/) GRCh37/hg19 of the 16p12.2 region. (a) The 16p12.2 region with the gene content and segmental duplications. (b) The 16p12.2 deletion region in our family encompassing between the segmental duplication BP1 and BP2.

three OMIM genes *UQCRC2* (MIM: 191329), *EEF2K* (MIM: 606968) and *CDR2* (MIM: 117340). Differently, the 165 kb microdeletion that we identified in our family is situated distally and comprised between BP1 and BP2. The deleted region contains only three OMIM genes: *METTL9, IGSF6* and *OTOA*. *OTOA* is responsible for DFNB22 hearing loss (MIM: 607039) in its homozygous condition (Zwaenepoel *et al.* 2002). Hearing loss is a public health concern affecting 1–3 per 1000 live births (Kemper and Downs 2000). More than 50% of the cases with congenital hearing loss are of genetic origin and many of them show autosomal recessive inheritance. Over 50 causative genes have been identified. *OTOA* encodes otoancorin, which belongs to a group of noncollagenous glycoproteins of the acellular gels of the inner ear, specifically located at the interface between the apical surface of the sensory epithelia and the overlying acellular gel (Zwaenepoel *et al.* 2002). Studies on the mouse model of human DFN22B have shown that loss of function mutations of *OTOA* results in a failure of inner ear hairy cell stimulation and consequently hearing impairment (Lukashkin *et al.* 2012). A number of *OTOA* mutations have been described, but very few reports exist in the literature regarding 16p12.2 microdeletions similar to that observed in the proband's family. In addition, a comprehensive analysis of the phenotypes and comorbidities of the subjects is not available since these studies were conducted with a focus on hearing impairment. In a Palestinian family (DO) described by Shahin *et al.* (2010) five deaf individuals were homozygous for the deletion and their consanguineous parents were heterozygous. The deleted region included *OTOA* and spanned 5.4 Mb. In another family, three sibs with mild to moderate sensorineural hearing loss carried a microdeletion containing the *OTOA* gene (Bademci *et al.* 2014).

In the current study, we unexpectedly identified a homozygous distal 16p12.2 microdeletion encompassing *OTOA* in the deaf mother of a child evaluated for short stature, mild facial dysmorphisms and dyslexia. This finding further supports the evidence of a pathogenic role of *OTOA* in autosomal recessive hearing loss.

We propose to define this microdeletion as 'distal 16p12.2 microdeletion' to distinguish it from the other 16p12.2 microdeletions comprised between BP2 and BP3 or between BP1 and BP3.

Searches in the DECIPHER database showed 17 cases with a 'distal 16p12.2 microdeletion' as unique CNV and three cases with a second CNV (DECIPHER n. 293777, 324179, 339473) (table 1). These microdeletions spanned from ~140 to ~411 kb, contained essentially the same genes (*METTL9*, *IGSF6* and *OTOA*), and were flanked by BP1 and BP2 segmental duplications.

Clinical findings associated with the 16p12.1–16p12.2 microdeletion are variable with a nontypical facial gestalt, suggesting that this rearrangement is nonsyndromic. Cases carrying this deletion may suffer from developmental delay, cognitive impairment and psychiatric disorders with uncertain pathogenesis (Antonacci *et al.* 2010; Girirajan *et al.* 2012; Pizzo *et al.* 2017).

Almost all the cases reported in the DECIPHER database with 'distal 16p12.2 microdeletion', inherited the deletion from a parent. Sixteen cases carried a heterozygous microdeletion while one carried a homozygous

Table 1. Patients with 'distal 16p12.2 microdeletion' reported in the DECIPHER database.

DECIPHER	UCSC co-ordinates	Size (kb)	Heredity	Phenotype	Genes
4073	chr16:21574908–21739885 (−1)	164.98	Inherited	Autism, brachycephaly, delayed speech and language development	*METTL9*, *IGSF6*, *OTOA*
251445	chr16:21475060–21837551 (−1)	362.49	Inherited	Cleft palate, non-midline cleft lip, preauricular skin tag	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
267149	chr16:21599627–21739945 (−1)	140.32	Inherited	Absence seizures, apraxia, generalized tonic-clonic seizures, myoclonus, optic atrophy, sensorineural hearing impairment	*METTL9*, *IGSF6*, *OTOA*
269365	chr16:21513975–21799607 (−1)	285.63	Inherited	Sensorineural hearing impairment	*METTL9*, *IGSF6*, *OTOA*
266291	chr16:21574938–21837522 (−1)	262.58	ND	Attention-deficit hyperactivity disorder, cognitive impairment, cryptorchidism, delayed fine motor development, joint hypermobility, protruding ear, ptosis, short attention span, strabismus, wide nasal bridge	*METTL9*, *IGSF6*, *OTOA*, *RRN3P*
269680	chr16:21513975–21799607 (−1)	285.63	Inherited	Clinodactyly of the fifth finger, deep palmar crease, sensorineural hearing impairment	*METTL9*, *IGSF6*, *OTOA*
276011	chr16:21569216–21754953 (−1)	185.74	Inherited	Bilateral sensorineural hearing impairment, cognitive impairment, congenital sensorineural hearing impairment, enlarged vestibular aqueduct, oromotor apraxia	*METTL9*, *IGSF6*, *OTOA*
276830	chr16:21576792–21841364 (−1)	264.55	Inherited	Morphological abnormality of the central nervous system	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
291432	chr16:21574908–21739885 (−1)	164.98	Maternal	Abnormal facial shape, intellectual disability, scoliosis	*METTL9*, *IGSF6*, *OTOA*
291437	chr16:21328537–21739885 (−1)	411.35	Paternal	Laryngomalacia, mild global developmental delay, Pierre-Robin sequence, stenosis of the external auditory canal	*CRYM-AS1*, *SNX29P1*, *NPIPB3*, *METTL9*, *IGSF6*, *OTOA*
293777	chr16:21475060–21837551 (−1) +chr8	362.49	ND	Autism, intellectual disability	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
300202	chr16:21599687–21739885 (−1)	140.20	Paternal	Cognitive impairment	*METTL9*, *IGSF6*, *OTOA*
322729	chr16:21576957–21837522 (−1)	260.57	Maternal	Bilateral cryptorchidism, decreased body weight, global developmental delay, narrow mouth, short stature, wide nasal bridge	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
322840	chr16:21574908–21951438 (−1)	376.53	Paternal	Developmental regression, developmental stagnation at onset of seizures	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*, *NPIPB4*
324179	chr16:21574908–21837551 (−1) +chr13	262.64	ND	Global developmental delay, intellectual disability, microcephaly	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
327478	chr16:21631625–21837551 (−1)	205.93	ND	Intellectual disability, short stature	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
339473	chr16:21631625–21837551 (−1) +chr6	205.93	ND	Global developmental delay, hemihypertrophy, synophrys	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
349809	chr16:21596412–21776157 (−1)	179.75	Maternal	Short stature	*METTL9*, *IGSF6*, *OTOA*
350273	chr16:21475060–21837551 (−1)	362.49	Maternal	Long-segment aganglionic megacolon, non-midline cleft lip, unilateral cleft lip	*METTL9*, *IGSF6*, *OTOA*, *RRN3P1*
350436	chr16:21599687–21739885 (−3)	140.20	ND	Bilateral conductive hearing impairment	*METTL9*, *IGSF6*, *OTOA*

ND, not defined.

microdeletion (DECIPHER n. 350436). Clinical features were variable, but six cases suffered from sensorineural hearing impairment even though they had a heterozygous microdeletion. Only the case n.350436 had bilateral conductive hearing impairment and carried the homozygous microdeletion as expected. The 'two-hit' model hypothesis formulated by Girirajan *et al.* (2012) might explain the variable expressivity of phenotypes associated with the 16p12.2 microdeletion. Indeed, according to this hypothesis, the occurrence of a secondary event (e.g. another CNV, a disruptive single base pair mutation in a phenotypically related gene, or an environmental event) is associated with a more severe phenotype. Our child did not exhibit mental retardation and suffered from only mild dyslexia. In addition, the communication problems of her mother might be attributed to her severe hearing impairment rather than to a primary intellectual disability. In this regard, we cannot consider the 'distal 16p12.2 microdeletion' as an independent risk factor for intellectual disability in our family. However, it is not possible to rule out that this rearrangement might contribute to the phenotype of our cases. Indeed, the clinical evidence of this family goes against the claim that 16p12.2 microdeletion was considered nonpathogenic by Antonacci *et al.* (2010). Further studies will be necessary to shed some light on the pathogenic role of this rearrangement in intellectual disability.

In conclusion, we report a rare familial 'distal 16p12.2 microdeletion' expanding the phenotypic spectrum associated with the recurrent 16p12.2 microdeletion. Our findings support the pathogenic role of *OTOA* in autosomal recessive hearing impairment DFN22B and strengthen the idea that this gene should be investigated when nonsyndromic genetic deafness is suspected. The presence of sensorineural hearing impairment in individuals with heterozygous 16p12.2 microdeletion and the possible pathogenic role of the other genes present in this region still remain to be explained.

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