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Oculo-auriculo-vertebral spectrum: Clinical and molecular analysis of 51 patients

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ABSTRACT

Introduction: Oculo-auriculo-vertebral spectrum (OAVS OMIM 164210) is a craniofacial developmental disorder affecting the development of the structures derived from the 1st and the 2nd branchial arches during embryogenesis, with consequential maxillary, mandibular, and ear abnormalities. The phenotype in OAVS is variable and associated clinical features can involve the cardiac, renal, skeletal, and central nervous systems. Its aetiology is still poorly understood.
Methods: We have evaluated the clinical phenotypes of 51 previously unpublished patients with OAVS and their parents, and performed comparative genomic hybridization microarray studies to identify potential causative loci.
Results: Of all 51 patients, 16 (31%) had a family history of OAVS. Most had no relevant pre-natal history and only 5 (10%) cases had a history of environmental exposures that have previously been described as risk factors for OAVS. In 28 (55%) cases, the malformations were unilateral. When the involvement was bilateral, it was asymmetric. Ear abnormalities were present in 47 (92%) patients (unilateral in 24; and bilateral in 23). Hearing loss was common (85%), mostly conductive, but also sensorineural, or a combination of both. Hemifacial microsomia was present in 46 (90%) patients (17 also presented facial nerve palsy). Ocular anomalies were present in 15 (29%) patients. Vertebral anomalies were confirmed in 10 (20%) cases; 50% of those had additional heart, brain and/or other organ abnormalities. Brain abnormalities were present in 5 (10%) patients; developmental delay was more common among these patients. Limb abnormalities were found in 6 (12%) patients, and urogenital anomalies in 5 (10%). Array-CGH analysis identified 22q11 dosage anomalies in 10 out of 22 index cases screened.
Discussion: In this study we carried out in-depth phenotyping of OAVS in a large, multicentre cohort. Clinical characteristics are in line with those reported previously, however, we observed a higher

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incidence of hemifacial microsomia and lower incidence of ocular anomalies. Furthermore our data suggests that OAVS patients with vertebral anomalies or congenital heart defects have a higher frequency of additional brain, limb or other malformations.

We had a higher rate of familial cases in our cohort in comparison with previous reports, possibly because these cases were referred preferentially to our genetic clinic where family members underwent examination. We propose that familial OAVS cases show phenotypic variability, hence, affected relatives might have been misclassified in previous reports. Moreover, in view of its phenotypic variability, OAVS is potentially a spectrum of conditions, which overlap with other conditions, such as mandibulofacial dysostosis.

Array CGH in our cohort identified recurrent dosage anomalies on 22q11, which may contribute to, or increase the risk of OAVS. We hypothesize that although the 22q11 locus may harbour gene(s) or regulatory elements that play a role in the regulation of craniofacial symmetry and 1st and 2nd branchial arch development, OAVS is a heterogeneous condition and many cases have a multifactorial aetiology or are caused by mutations in as yet unidentified gene(s).

1. Introduction

Oculoauriculovertebral spectrum (OAVS; OMIM 164210) is a phenotypically, and aetiologically heterogeneous disorder of craniofacial morphogenesis (Hennekam et al., 2010; Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009) with a reported prevalence in Europe of 3.8 per 100,000 births; this incidence has based from data from EUROCAT, a large network of population-based congenital anomaly registries in Europe (Barisic et al., 2014). The term OAVS, suggested by Gorlin and colleagues (Beleza-Meireles et al., 2014), encompasses different overlapping diagnoses such as hemifacial microsomia, 1st and 2nd branchial arches syndrome, otomandibular dysostosis, facioauriculovertebral syndrome and Goldenhar syndrome, all representing a phenotypic continuum of the same entity.

OAVS includes a group of malformations primarily involving the structures derived from the 1st and 2nd branchial arches and the intervening first pharyngeal pouch and branchial cleft, in particular the ear, mouth, mandible, eye and cervical spine. The craniofacial anomalies are generally asymmetrical (unilateral or bilateral). OAVS can range from mild to severe and includes hemifacial microsomia, bilateral or unilateral ear anomalies (preauricular tags and pits, ear dysplasia, anotia, microtia), hearing loss (conductive and/or sensorineural), ocular defects (epibulbar dermoids, microphthalmia, coloboma of upper eyelid), orofacial clefts and vertebral abnormalities. According to a recent report (Barisic et al., 2014), there is a high rate of associated anomalies of other organs/systems (up to 69.5%), most commonly congenital heart defects (in about in 27.8% of patients), but also renal and cerebral malformations (Barisic et al., 2014; Tasse et al., 2005; Rooryck et al., 2010a; Figueroa and Pruzansky, 1982; Melnick, 1980; Rollnick et al., 1987). Most patients with OAVS do not usually present with all the common features, hence there has been no universal agreement upon minimal diagnostic criteria for OAVS, but an ear anomaly has been suggested by some authors as the mildest form (Tasse et al., 2005; Rooryck et al., 2010a; Figueroa and Pruzansky, 1982).

OAVS usually occurs sporadically, however, segregation analysis has suggested genetic transmission in some familial cases (Kaye et al., 1992). Moreover, clinical studies have shown that, following careful history and clinical examination of the relatives of probands with OAVS, up to 45% of "unaffected" relatives do have minor OAVS manifestations (Rollnick and Kaye, 1983). Reports of familial cases following Mendelian inheritance (Mastroiacovo et al., 1995; Tasse et al., 2007; Vendramini-Pittoli and Kokitsu-Nakata, 2009; Tsai and Tsai, 1993; Goodin et al., 2009), as well as evidence for genetic linkage in two families (Huang et al., 2010a; Kelberman et al., 2001), and the presence of OAVS features in patients with various chromosomal aberrations and genomic imbalances (Callier et al., 2008; Huang et al., 2010b; Ala-Mello et al., 2008; Rooryck et al., 2009; Abdelmoity et al., 2011; Ballesta-Martínez et al., 2013; Brun et al., 2012; Verloes et al., 1991; Herman et al., 1988; Xu et al., 2008; Digilio et al., 2009a; Tan et al., 2011; Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013; Rao et al., 2005; Garavelli et al., 1999; Poonawalla et al., 1980; Rooryck et al., 2010b; Wilson and Barr, 1983), all suggest that some cases of OAVS have a genetic basis. Environmental causes have also been suggested, particularly twinning, assisted reproductive techniques and maternal pre-pregnancy diabetes (Hennekam et al., 2010; Barisic et al., 2014).

To advance studies into OAVS we have carried out a detailed clinical evaluation of 51 previously unreported patients with OAVS and also collated data on published cases. We provide a comprehensive assessment of the OAVS phenotype and revaluation of the minimal diagnostic criteria for clinical diagnosis and counselling purposes. Comparative genomic hybridization array screening (aCGH) of DNA samples was performed to identify recurrent copy number variations (CNVs) and identify candidate genes for mutation screening in our OAVS population.

2. Patient data/material and methods

2.1. Patients

Fifty-one patients were re-examined after a search for all cases of OAVS, Goldenhar syndrome and Hemifacial Microsomia in our clinical archives in Manchester and in Coimbra Clinical Genetics Centres. Details of family and medical histories were collected on all patients. Each patient from the cohort underwent a detailed physical examination by the clinical authors. Clinical data was entered into a comprehensive OAVS phenotype database designed in house.

As the minimal inclusion criteria for patients in our study, we selected the presence of: (i) hemifacial microsomia or facial asymmetry together with (ii) microtia or milder ear malformations, such as preauricular tags. These minimal inclusion criteria were agreed after a critical literature review and agreement between the co-authors with expertise in Clinical Genetics (Barisic et al., 2014; Tasse et al., 2005; Rooryck et al., 2010a; Figueroa and Pruzansky, 1982). For familial cases, we accepted as affected individuals those with isolated hemifacial microsomia, facial asymmetry or microtia/preauricular tags, as long as the index patient was a 1st

degree relative fulfilling the above minimal diagnostic criteria for OAVS. We excluded OAVS patients with gross chromosomal abnormalities and abnormal karyotypes, or patients OAVS features plus another recognisable pattern of clinical malformations.

Ethics for the study was obtained from the appropriate ethical committees, and followed the Ethical Principles for Medical Research Involving Human Subjects of the WMA Declaration of Helsinki.

2.2. DNA analysis

2.2.1. DNA extraction

Genomic DNA was extracted from peripheral blood lymphocytes cells using Jetquick blood and cell culture DNA Midi Spin kit (Genomed, Löhne, Germany) or Chemagic MSM I with extraction kits (Chemagic DNA Blood Kit special, Perkin Elmer) according to the manufacturers' instructions. DNA concentration and purity were evaluated using a NanoDrop1000 Spectrophotometer (Thermo Scientific, Waltham, USA).

2.2.2. Array-comparative genome hybridization (aCGH)

The patient DNA and gender-match normal controls DNA were labelled with Cyanine 3-dCTP and Cyanine 5-dUTP, respectively, using the Agilent Genomic DNA enzymatic labelling kit (Agilent Technologies, Santa Clara, CA, USA) followed by a purification step with Amicon ultra 0.5 ml centrifugal filters (Millipore, Billerica, MA, USA) according to manufacturer's instructions. DNA labelling efficiency was measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Waltham, USA). After labelling quality control, the patient Cy5-labelled DNA and Cy3-labelled reference DNA were mixed together and combined with 2× hybridization buffer, 10× blocking agent, human Cot-1 DNA and hybridized onto a Agilent SurePrint G3 Human Genome microarray 4 × 180K* (Agilent Technologies, Santa Clara, CA, USA). After 24 h in a hybridization oven at 65 °C, arrays were washed and scanned on an Agilent scanner following manufacturer's instructions. The generated images were processed with Feature Extraction software (v10.7) and imported into Agilent Genomic Workbench (v6.5) for analysis according to Human Genome build 19 (hg19). All the aberrations considered included at least three consecutive probes with abnormal log2 ratios and were interpreted by databases consultation: UCSC genome browser, Decipher (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources), ISCA (The International Standards for Cytogenomic Arrays), OMIM (Online Mendelian Inheritance in Man) and DGV (Database of Genomic Variants). OGT CytoSure ISCA v2 (8 × 60k) were used for some of the samples using the methodology described in the manufacturer's instructions (Oxford Gene Technology), and analysed using CytoSure Interpret Software using the analysis criteria described above. (* *only samples 16, 17, 19 and 20 were analysed using the Agilent SurePrint G3 180K arrays*).

Consent was available to screen 22 patients in our cohort. Inheritance was determined where parents were available.

2.2.3. Review of the literature and construction of a map of OAVS loci

In reviewing the relevant literature for this topic, we retrieved peer reviewed articles from Pubmed, US National Library of Medicine National Institutes of Health, 1985–2013 using as search terms "Oculo-auriculo-vertebral", "Oculoauriculovertebral", "Hemifacial Microsomia", "First and Second Pharyngeal Arch syndrome", "Otomandibular Dysostosis", "Facio-auriculo-vertebral syndrome" and "Goldenhar syndrome". In order to propose putative mechanisms of OAVS, the authors also searched for recent publications on craniofacial morphogenesis and on cranial neural crest cell migration and patterning in craniofacial development. We constructed a map of OAVS candidate loci across the human genome based on published reports of cytogenetic and genomic abnormalities identified in OAVS patients and from genome-wide scans performed in OAVS families (Beleza-Meireles et al., 2014; Tasse et al., 2005; Rooryck et al., 2010a).

3. Results

3.1. Clinical characterisation of 51 OAVS patients

We included 51 patients, 23 (45%) female and 28 (55%) male, with a diagnosis of OAVS in this study (see Table 1 for a summary of the clinical features). Sixteen cases from seven different families (31% of all cases) had a confirmed family history of OAVS compatible with an autosomal dominant (14) or recessive (2) mode of inheritance. The remaining individuals (69%) were unrelated sporadic cases. Fig. 1 shows some of the characteristic craniofacial features seen in our OAVS patients. All these patients presented at least hemifacial microsomia or facial asymmetry together with microtia or milder ear malformations, such as preauricular tags. Familial cases presented at least isolated hemifacial microsomia, facial asymmetry or microtia/preauricular tags, and a 1st degree relative fulfilling the minimal diagnostic criteria for OAVS described under the methods section.

In 34 cases (67%), there was an unremarkable pregnancy history, with no apparent environmental exposure that could increase the risk for OAVS. In the remaining 17 cases (33%), there was an event during pregnancy that was considered relevant. However, only 5 (10%) had a history of environmental exposures that have previously been described as risk factors for OAVS: Diabetes in two sporadic cases, twinning in two sporadic cases, and exposure to Thalidomide (although this case was familial). Otherwise, in the other cases, there were reports of minor, sporadic vaginal bleeding during the pregnancy in 4 cases; and one case of: preterm delivery due to premature rupture of membranes; possible *in utero* compression by pelvic cysts; maternal hypothyroidism; maternal celiac disease; sickness, and threatened early miscarriage.

Twenty-three patients (45%) presented with bilateral, but asymmetric, involvement; in 28 cases (55%), the manifestations were unilateral (left sided in 17 and right-sided in 11). Hemifacial microsomia was observed in 46 patients (90%), 31 left-sided and 15 right-sided. Facial nerve weakness was observed in 17 cases (33% of the total number of patients; 37% of the cases with hemifacial microsomia), 10 on the left and 7 right-sided. Cleft lip and/or cleft palate were described in 4 patients (8%). Transverse facial clefts (giving the appearance of macrostomia) were seen in 3 patients (6%). Additional craniofacial features included bilateral, micrognathia (symmetrical mandibular hypoplasia), palate hypoplasia and an extra maxillary incisor (each found in only 1 case).

External ear abnormalities were present in nearly all patients (47 cases, 92%) and were unilateral in half of the cases: 24 patients, 13 left-sided and 11 right-sided. Twenty-three patients had bilateral external ear anomalies, which were always asymmetrical. Microtia, defined as the underdevelopment of the pinna, or anotia, complete absence of the pinna, were present in 31 (61%) of the patients. And in at least 26 patients, atresia of external auditory canal was confirmed. Preauricular skin tags were seen in 27 (53%) cases; half of the cases with microtia or anotia also had preauricular skin tags and/or pits. Some degree of hearing loss was confirmed in 30 (59%) of the cases (we were not able to check auditory acuity in 9 patients). It is worthwhile noting that only one of the patients with confirmed hearing loss did *not* have an external ear anomaly.

Ocular involvement was present in 15 patients. Epibulbar dermoids, the most common anomaly, were present in 8 patients

Table 1
Summary of the clinical features observed in 51 OAVS patients included in our study. F-Feminine; M-Masculine L-Left; R-Right; B-Bilateral; +-Observed/Confirmed (in the case of "Familial OAVS"). Blank boxes indicate absence of the phenotype.

					Craniofacial anomalies						Ear anomalies				Eye anomalies				Vertebral anomalies				Other anomalies			
Patient	Familial OAVS	Sex	Prenatal factors	auriculo-craniofacial involvement	hemifacial microsomia	cleft lip/palate	facial nerve weakness	mandibular hypoplasia	frontal bossing	other craniofacial feature	preauricular skin tags/pits	atresia of external auditory canal	microtia/anotia	hearing loss	epibulbar dermiod	micropthalmos	coloboma upper eyelid	other eye anomaly	hemivertebrae	fusion	scoliosis	kyphosis	cardiovascular	brain	developmental delay	other organs/ systems anomalies
1		M		L	L		L	L				L	L	+												
2	+	F	+	B	L						L	L	B								+					
3	+	F	+	L	L										L				+							
4		F	+	L	L						L	L	L													
5		F		R	R			R				R	R	+												
6		M	+	R	R		R				R	R	R											+		+
7		M		R	R							R	R	+									+			+
8	+	M		B	L			L			B	B	B	+	R						+	+		+	+	
9	+	M		B	L			L			B	B	B	+	L									+	+	
10		M		L	L		L	L		+		L	L	+												
11		M	+	B	L			L		+	B															+
12		M	+	R	R		R	R				R	R	+												
13		F		B	L		L	L		+		B	L	+											+	
14		M		B	L			L				R	B	+								+		+		
15	+	M		L	L			L			L			+				+								
16	+	F		R	R			R			R	R			R			+								+
17	+	F		B	L			L			L															
18	+	F		L	L						L															
19	+	F		B	L			L			L															
20	+	M		R	R						R				R			+								+
21		M	+	B	L		L	L			B	L	L	+				+								
22		M		R	R		R	R				R	R	+												
23		M	+	L	L		L			+																+
24		F		R	R			R			R		R	+					+	+	+		+		+	
25		F	+	R			R				R	R														+
26		F		B									B	+						+	+		+		+	+
27		F		R	R			R			B	R	R	+												
28		F	+	B										+												
29		M	+	L	L																		+			+
30		M	+	R	R		R					R	R	+		L	L								+	
31		F		B	L		L	L			B		L												+	
32		M		R	R		R	R					R	+												
33		F		B	L		L				L	L	L	+						+						
34	+	M		B	L			L		+	L			+												+
35	+	M		L	L			L		+					L											
36		F		L	L			L	+														+			
37		F		L	L			L						+						+						
38		F		L	L			L				L	L	+					+	+	+		+		+	+
39		M	+	L	L	+	L				L		L										+			+
40		F	+	B	R				+		R			+												
41		M		B	L						B	L			B											
42		M	+	B	R	+	R		+	+	B	B	B	+		R							+	+		+
43		M		L							L				L											
44		M		L		+	L	+				L	L	+									+			
45		F		R	R					+	B		L				R	+								
46		M		L	L							L	L	+				+								
47		M		L	L		L						L		L											
48		F	+	L	L					+											+					
49		M	+	L	L					+		L	L	+												+
50		M		B	L	+				+	R	L	L	+												+
51		F		B	R						B		B	+				+							+	+

(16%); microphthalmia was seen in 2 (4%); orbital dystopia in 2 cases (4%); and a coloboma of the upper eyelid in 2 cases (4%). Additionally, we noted epicanthic folds in 3 and dystopia canthorum in 2 cases. In most cases, the ocular involvement was unilateral (in 7 cases only the left eye, and in 5 cases only the right eye); in the remaining cases, the involvement was bilateral.

Vertebral anomalies were identified in 10 patients (20%): hemivertebrae in 3 patients (2 in cervical and 1 in thoracic vertebrae), vertebral fusions in the cervical spine in 5 patients, scoliosis or kyphoscoliosis in 5. In 5 (50%) of the patients with vertebral anomalies, there were additional heart, brain and/or other organ abnormalities.

Brain abnormalities were observed in 5 patients (10%). These included microcephaly in 2 patients (4%), a small pituitary gland and interrupted pituitary stalk associated with single central incisor (holoprosencephaly spectrum) in 1 patient (2%); history of small subependymal haemorrhage associated with mild hypotonia in 1 patient (2%); and epilepsy in 1 case (2%). Developmental delay was present in 9 patients (18%). However, in the cases with brain abnormalities, 40% had developmental delay or intellectual disability.

Eight patients had a congenital heart defect (16%). The most common abnormality was a ventricular septal defect in 4 cases; in one of these patients, an atrial septal defect was also observed and in another there was a patent ductus arteriosus. A patent foramen ovale was observed in a patient who also had mild aortic coarctation and dysplastic mitral valve. Additional anomalies were right pulmonary artery stenosis and an isolated patent foramen ovale. It was interesting to note that 2/3 of these patients also had abnormalities in other organs and systems.

Sixteen patients had disorders in other organs and systems (31%). Limb abnormalities were found in 6 cases (12%). In 5 of these cases a hypoplastic thumb or other radial ray defect was observed; the other patient had a pre-axial polydactyly in one hand. Inability to fully extend elbows was observed in one patient. Urogenital anomalies were present in 5 cases (10%), and included pelvicalyceal dilation (1 cases; 2%), absence of ovaries and uterus associated with persistent Müllerian structures (1 case; 2%), chordee (1 case; 2%), hypospadias (1 case; 2%), and ambiguous genitalia (1 case; 2%). Interestingly, 3 of the patients (6%) had both limb and urogenital anomalies.

Additional abnormalities included inguinal hernias (2 cases; 4%); umbilical hernia (2 cases; 4%), mild chest asymmetry (2 cases; 4%) and congenital hip dysplasia (2 cases; 4%). We also observed imperforate anus associated with sacral dysgenesis and leg length discrepancy, hypoplasic rib, hydrocele, short stature and macrosomia, each found in 1 patient (2%).

A comparison of the prevalence of abnormalities found in our OAVS cohort and collated published studies (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a) are summarised in Table 2.

3.2. Genetic analysis

Using oligonucleotide a-CGH screening we identified dosage anomalies in 12 out of 22 OAVS patients tested (54.5%) (see Table 3).

3.2.1. CNVs on 22q11.21

Ten patients have CNVs involving the 22q11.21 region (chr22: 16495650-21661435, hg19). Four of these have 2 or more non-overlapping genomic duplications in the 22q11 region. All of the 22q11 CNVs are polymorphic and seen in the DGV database, however the frequency seen within our OAVS cohort and reported cases in the literature (Beleza-Meireles et al., 2014; Rooryck et al., 2010a) warrant further investigation. Patient 37 has two duplications on 22q11, one involving the Cat-Eye syndrome region inherited from his father (patient 38) who was less severely affected (mild facial asymmetry and epibulbar dermoids). Patient 51 had a larger duplication (~5.1 Mb) overlapping both the 22q11 del/dup and Cat-Eye syndrome regions (the latter encompasses the duplication seen in patient 37 and his father).

When analysing the pathogenicity of the CNVs, we have identified reports of patients with OAVS features and CNVs within the Cat Eye region (Klopocki and Mundlos, 2011; Zeitz et al., 2013 Nov–Dec). Moreover, chromosome 22q has been repeatedly reported in association with OAVS (Beleza-Meireles et al., 2014; Tasse et al., 2005; Rooryck et al., 2010a).

Apart from the 22q11 changes, there were no other CNVs in common between patients in our cohort or those in the published literature (Beleza-Meireles et al., 2014) (see Fig. 2 for summary).

3.2.2. Incidental CNVs unlikely to cause the OAVS phenotype

A *de novo* 0.961 Kb deletion involving *FOXG1* on 14q12 was detected in one patient (#16) with OAVS and mild developmental delay. *FOXG1* encodes a developmental transcription factor, which plays an important role in embryonic brain development, particularly the telencephalon, and *de novo* heterozygous point mutations in this gene have been reported in patients with congenital forms of Rett syndrome (Santen et al., 2012). Deletions involving chromosome 14q13 have also been linked to variable phenotypes, and their severity seems directly related to *FOXG1* haploinsufficiency (van Nunen et al., 2014). The majority of patients with *FOXG1* deletions present with severe psychomotor delay, postnatal microcephaly, stereotypic movements, and a dyskinetic movement disorder. However, patient 16 is more mildly affected. Alongside OAVS features, this patient has some degree of learning difficulties (extra support needed at school but otherwise capable of leading an independent life), and does not have epilepsy or microcephaly. This suggests that there is clinical variability/penetrance associated with *FOXG1* loss of function mutations. Alternatively, it is also possible that our patient has a mosaic mutation; however, this was not tested.

The Xp11.21 195.27 kb duplication detected in patient 6 encompasses the *FAAH2 gene*, a fatty acid amide hydrolase, which may play a role in fatty acid catabolism and is not a common genomic variation. Although the consequences of haploinsufficiency for this gene are not known, it is unlikely to be involved in OAVS.

A10p15.3 169.8 kb duplication was detected in patient 25 involving non-coding RNA as well the promoter region of *DIP2C*, which encodes a member of the disco-interacting protein homolog 2 family expressed in the central nervous system. Although our patient has no brain malformations or developmental delay, larger deletions and duplications encompassing this region have been found in patients with developmental delay and dysmorphisms in the Decipher database. However since partially overlapping CNVs also occur in the normal population, the phenotypic consequences of this duplication are not known.

The maternally inherited 588.6 Kb deletion on 19q13.3 in patient #14 encompasses many genes and contains many polymorphic CNVs, but may be pathogenic. This patient has moderate craniofacial involvement and a global IQ of 41 (psychomotor evaluation with WISCIII). The maternal carrier does not have OAVS but was diagnosed with mild cognitive difficulties and Parkinson's disease, therefore the deletion could contribute towards these latter phenotypes.

4. Discussion

4.1. Clinical characterisation of OAVS

The spectrum of phenotypic features in OAVS is variable, ranging from subtle facial asymmetry with a small skin tag in front of an

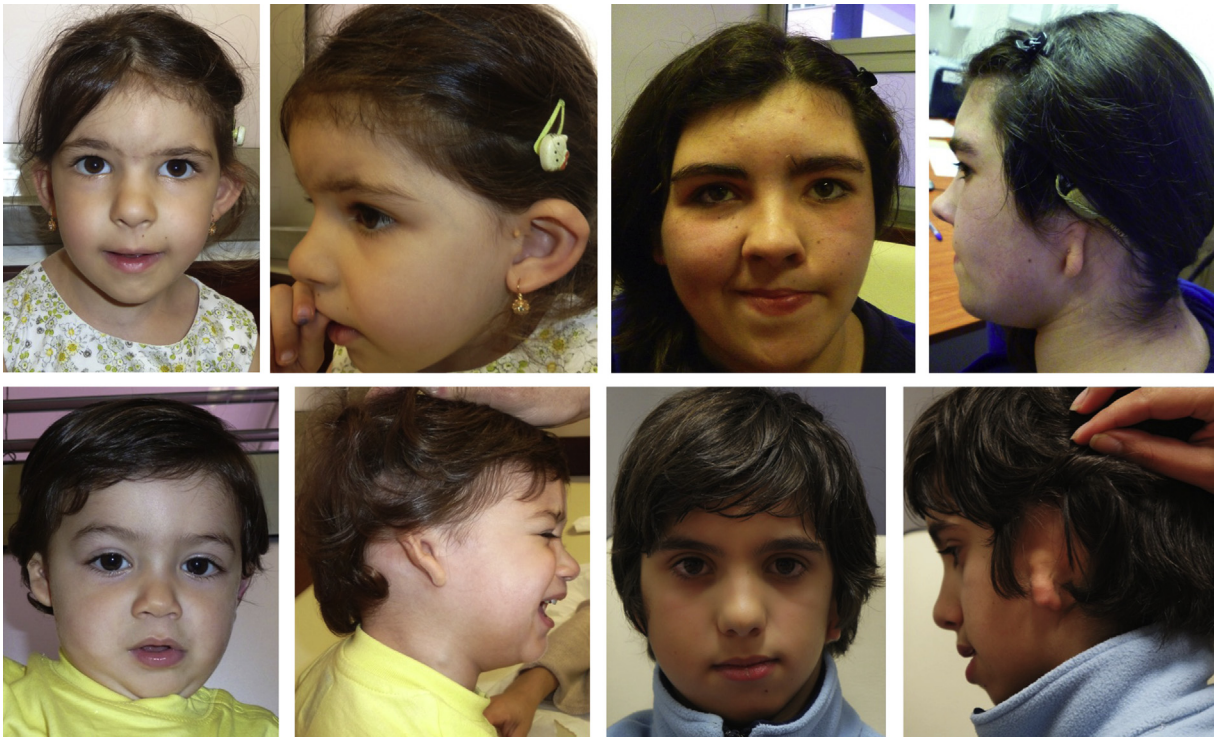

Fig. 1. Characteristic clinical features of OAVS. Patients with OAVS at different ages, presenting facial asymmetry/hemifacial microsomia of different severities; and microtia and/or other ear anomalies.

otherwise normal-appearing ear, to a complex phenotype comprising multiple congenital abnormalities. Due to the variable expressivity, it has been difficult to reach a general consensus regarding the minimum diagnostic criteria for OAVS. In view of this phenotypic variability, OAVS might be a spectrum of conditions, which overlap with other conditions such as mandibulofacial dysostosis. However, based on our study and the literature, we agree that the minimal diagnostic criteria for OAVS should be microtia; or hemifacial microsomia together with mild ear malformations. An associated family history of OAVS should also be a component of the diagnostic criteria. Differential diagnosis should be considered and excluded as appropriate.

External ear malformations such as microtia, anotia, aural atresia, preauricular tags or hillocks and pre-auricular pits are the most common features in our OAVS cohort. Interestingly, a retrospective study of all patients referred for reconstructive surgery of the auricle over the period 1990–2012 (van Nunen et al., 2014), has demonstrated that atresia of the acoustic meatus, preauricular skin tags, hemifacial microsomia and facial nerve paralysis were present in 76%, 30.5%, 27.5% and 8.3%, respectively, of these patients. This suggests that many common anomalies of the external ear might be part of an overlooked or undiagnosed OAVS phenotype. These findings are consistent with previous reports (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a) indicating that the presence of congenital ear abnormalities should prompt clinicians to search for additional OAVS related anomalies in patients displaying these features (Hennekam et al., 2010; Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995). Although OAVS is generally associated with unilateral ear and face involvement, we have frequently observed that bilateral asymmetric involvement. A reasonable question to ask is whether we might be in the presence of different aetiological mechanisms. An additional important fact highlighted by our study is the presence of hearing loss detected in about 85% of OAVS patients. The referral of all OAVS patients for audiology screening should therefore be mandatory to improve clinical management.

Nearly all patients with OAVS in our cohort also have some degree of hemifacial microsomia, resulting from maxillary and/or mandibular hypoplasia. The craniofacial involvement was most commonly unilateral, but bilateral anomalies were also frequently seen, and were always asymmetrical. Facial palsy, asymmetric palatal elevation, impairment of extraocular movements and trigeminal anaesthesia have been described in OAVS (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a). A recent report suggests a wide variety of facial nerve disorders in OAVS, with the involvement of either all facial nerve branches or only the lower branches (Cline et al., 2014). Facial clefts, or cleft lip and/or palate were also observed which is consistent with a recent study that reported a frequent association of OAVS and cleft lip and/or palate (Suutarla et al., 2014 Jan).

A variety of ocular abnormalities are observed in our OAVS cases, with epibulbar dermoids being the most common finding (8%). However, in our cohort the frequency of ocular features is lower than previously described (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a). We also found a relatively lower prevalence of coloboma of the upper eyelid. Microphthalmia or other severe eye malformations are uncommon. It is worthwhile noting that the lower frequency of ocular anomalies observed in our study might be due to an ascertainment bias and an ophthalmological assessment is warranted in all OAVS patients.

Vertebral defects, impaired mobility of the spine, restricted neck movements and torticollis may also be present in OAVS and x-rays of the spine, ideally antero-posterior and lateral views of the whole spine, or only of the cervical spine if no anomalies are observed or

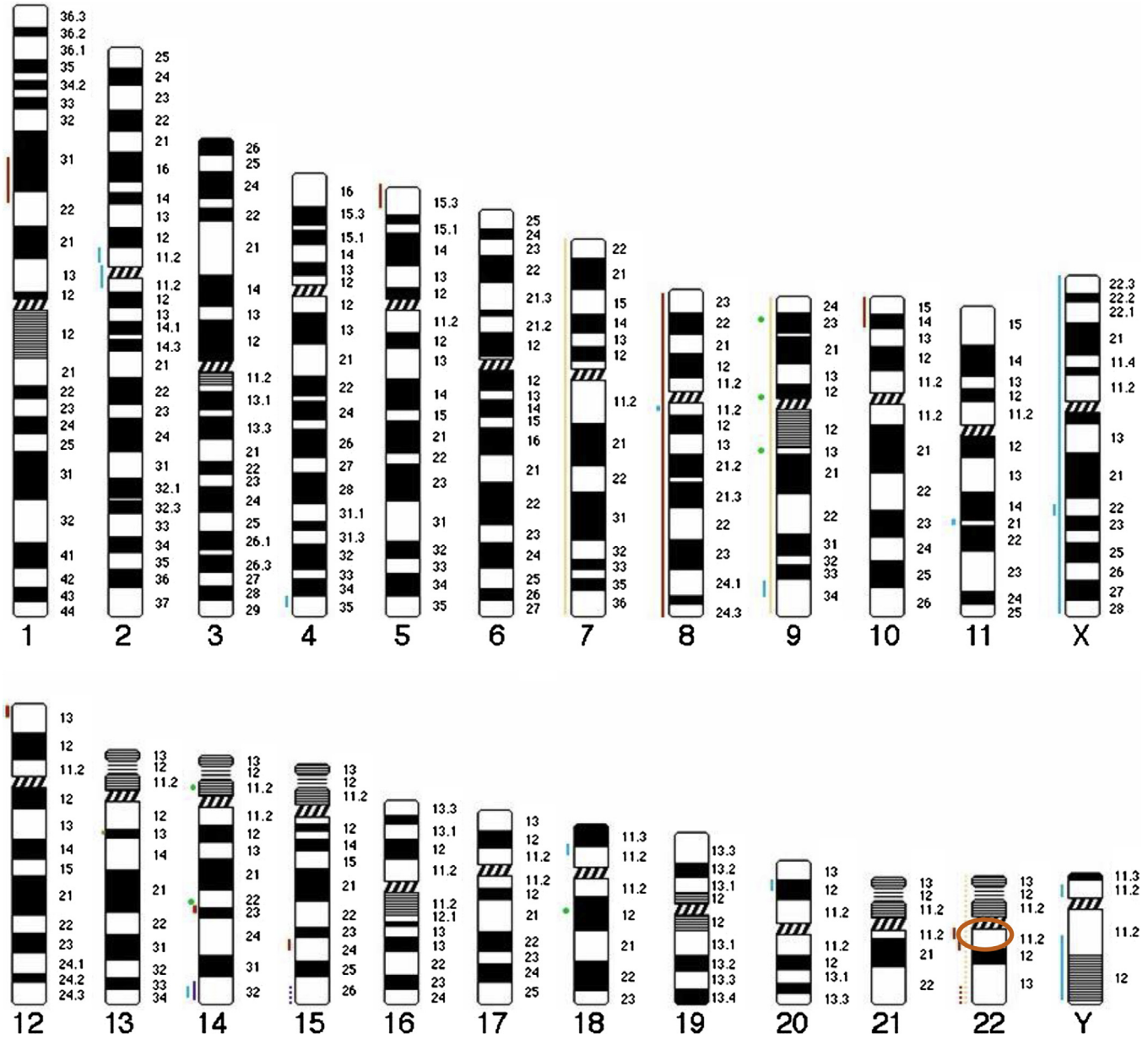

Fig. 2. Map of OAVS loci. Candidate genetic loci for OAVS that been suggested by the identification of chromosomal anomalies in patients with phenotypic characteristics of this spectrum (in red); by the identification of the breakpoints in apparently balanced chromosomal rearrangements (in green); by the presence of chromosomal mosaicism in individuals with OAVS features (in orange); by genome wide search for linkage in families (in purple — full and, less convincingly due to a non-significant LOD score, in purple - - - dashed); and by high density oligonucleotide array-CGH (blue) (Beleza-Meireles et al., 2014; Tasse et al., 2005; Rooryck et al., 2010a; Kaye et al., 1992; Rollnick and Kaye, 1983; Tasse et al., 2007; Vendramini-Pittoli and Kokitsu-Nakata, 2009; Tsai and Tsai, 1993; Goodin et al., 2009; Huang et al., 2010a; Kelberman et al., 2001; Callier et al., 2008; Huang et al., 2010b; Ala-Mello et al., 2008); chromosome 22 was also repeatedly reported. Circled in orange is the region highlighted by our study.

suspected on clinical examination, should be performed as part of clinical investigations. Interestingly, in our cohort, patients with vertebral anomalies we detected a higher frequency of additional heart, brain, limb or other malformations, which warrants at least an echocardiogram and a renal ultrasound examination. A brain scan may be considered if there are neurological signs or significant microcephaly or developmental delay.

Congenital heart defects (tetralogy of Fallot, septal defects, transposition of the great vessels, aortic arch anomalies, situs inversus, dextrocardia) are not uncommon in patients with OAVS; and in our cohort, patients with heart defects had a higher frequency of associated abnormalities in other organs and systems, which has also been suggested in several other publications (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a). Hence, the presence of congenital heart defects, as well as vertebral anomalies, appears to increase the risk of additional malformations being present, which should then be screened for.

Limb (particularly radial ray) defects, renal malformations (unilateral kidney agenesis, double ureter, renal ectopia, hydronephrosis, hydroureter) and anomalies of the central nervous system (developmental delay, microcephaly, encephalocele, hydrocephaly, hypoplasia of the corpus callosum, Arnold-Chiari

Table 2
Comparison of the prevalence of phenotypes found in this study and the published literature (Barisic et al., 2014; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a).

Principal anomalies		A	B	C	D	E
Cranio-facial[a]	Hemifacial microsomia[k]	–	83%	84%	49%[l]	90%
	Macrocephaly	–	–	5%		–
	Microcephaly	–	8%	–		4%
	Cleft lip/palate	15%–22%		18%	17.4%	11%
	Macrostomia/facial cleft	17%–62%		13%	2.3%	6%
	Facial nerve palsy	10%–45%		–	3.5%	33%
Ear	Anotia/microtia[k]	66%–99%	100%[b]	70%	25%/88.8%	61%
	Preauricular tags[k]	34%–61%		67%	44.4%	53%[h]
	Preauricular sinus/pit	6%–9%		7%	2.3%	–
	Hearing loss	50%-66%[c]	85%	68%		59%
Ocular	Epibulbar dermoids	4%–35%	22%	31%	7.7%	8%
	Coloboma of the upper eyelid	12%–25%	8%	11%	3.9%	4%
	Microphthalmia	–	10%	12%	5.4%	4%
	Orbital dystopia	15%–43%	–	–	–	4%
	Lacrimal duct atresia/stenosis	11%–14%	–	–	–	–
Velopharyngeal insufficiency		35%–55%	–	–	–	–
Vertebral anomalies		16%–60%[d]	53%	35%	24.3%	20%
Congenital heart defects		4%–33%	15%	27%	27.8%	16%
Limb defects		3%–21%	12%	–	11.6%	12%
Developmental delay		–	9%[e]	14%	–	18%
Central nervous system anomalies		5%–18%	17%[f]	2%[f]	10.4%	10%[f]
Genitourinary anomalies		4%–15%	18%	7%[g]	15.8%	10%
Pulmonary anomalies		1%–15%	–	–	3%	2%[i]
Gastrointestinal anomalies		2%–12%	–	–	7.7%	2%[j]

A-prevalence rates from 19 reports published between 1983 and 1996 (Heike et al., 2009); B-Clinical evaluation of 53 patients (Tasse et al., 2005); C-Analysis of a cohort of 86 patients in 2010 (Rooryck et al., 2010a); D-Data from (Barisic et al., 2014); E-Data from this study.
[a] Mandibular, malar, maxillary, or facial muscular hypoplasia.
[b] Microtia/preauricular tag.
[c] Conductive hearing loss.
[d] Vertebral/rib.
[e] Delay of speech development.
[f] Brain anomalies.
[g] Renal anomalies.
[h] We included pre-auricular skin tags with or without pits.
[i] Two patients with asthma, but none with congenital pulmonary abnormalities.
[j] One patient with imperforate anus. - Not documented.
[k] minimal diagnostic criteria for OAVS.
[l] The authors include asymmetry of the face, micrognathia, mandibular hypoplasia and anomalies of jaw size.

malformation, holoprosencephaly) have been observed in OAVS. We had a higher prevalence of developmental delay in our cohort (18%), as compared with previous reports (Beleza-Meireles et al., 2014; Barisic et al., 2014; Mastroiacovo et al., 1995; Cousley and Calvert, 1997; Heike et al., 2009; Tasse et al., 2005; Rooryck et al., 2010a). However, this, again, might have been due to biased ascertainment as only more severe patients may have been referred to the genetic clinics. The differences may also indicate different methodologies used for psychological assessment. It is worthwhile noting that the prevalence of developmental delay in our cohort was higher in patients with brain abnormalities.

OAVS is usually described as sporadic, with no relevant family history and a low recurrence risk. However, a genetic predisposition has been proposed based on growing evidence from the literature (Kaye et al., 1992; Rollnick and Kaye, 1983; Tasse et al., 2007; Vendramini-Pittoli and Kokitsu-Nakata, 2009; Tsai and Tsai, 1993; Goodin et al., 2009; Huang et al., 2010a; Kelberman et al., 2001; Callier et al., 2008; Huang et al., 2010b; Ala-Mello et al., 2008; Rooryck et al., 2009; Abdelmoity et al., 2011; Ballesta-Martínez et al., 2013; Brun et al., 2012; Verloes et al., 1991; Herman et al., 1988; Xu et al., 2008; Digilio et al., 2009a; Tan et al., 2011; Quintero-Rivera and Martinez-Agosto, 2013). A recent report by Rooryck et al., 2010b (Rooryck et al., 2010a), identified 12% of familial cases in a cohort of 95 patients. The percentage of familial cases is higher in our cohort (31% of all cases). We, therefore, propose that, because of the variable expressivity of OAVS, affected relatives might have been misclassified in previous reports. Our experience indicates that, on further examination of presumed unaffected relatives, we identified minor facial asymmetry and/or mild ear anomalies, a history of preauricular tags or epibulbar dermoid removed in childhood, and asymmetric crying face as babies, in approximately 28% of assessed cases. In the mother of two affected sibs, we observed bilateral external ear dysmorphisms (posteriorly rotated, somewhat cupped, protruding ear with underdevelopment of the antihelix), as the sole feature. These facts are important for genetic counselling. Based on our study, guidelines for counselling these families should include thorough parental examination and clinical history evaluation for procedures such as surgical interventions that might have removed ear tags or pits, corrected micrognathia or mandibular asymmetry. This might include also questioning grandparents.

4.2. The involvement of 22q11 in OAVS

We identified a variety of non-overlapping and partially overlapping CNVs encompassing the 22q11 region, with a higher prevalence than expected when compared to frequencies reported in the Database for Genomic Variants (DGV), or within our in-house cytogenetics private databases. Moreover, the likelihood of finding recurrent variations in such small number of patients was very low (we found 22q11 duplication CNVs in 10/22 cases). Hence we cannot exclude a potential pathogenic role of these variants either through haploinsufficiency for a gene within these loci or a position effect impacting a gene outside the CNV. The 22q11 locus contains regions of low copy repeat (LCR) sequences that mediate non-allelic homologous recombination and predispose the locus to copy

Table 3
aCGH screening of OAVS patients in this study. Common polymorphic CNVs were excluded. F = familial; P = paternal; M = maternal; S = sporadic *de novo*; n/a = not available; del = deletion; dup = duplication. IHF = in house frequency of CNV from our cytogenetics database.

Patient	Array CGH	Dosage imbalance	Coordinates (hg19)	Min. size (kb)	Inherited?	Genes in interval	Region associated with a disorder	Comment
2	OGT8*60k	dup	**Xp11.21** 57367786-57563057	195.27	n/a	*FAAH2*	unknown significance	IHF = 0.01%
3	OGT8*60k	dup	**22q11.21** 20402618-20659614	257	S	*RIMBP3*	CNV seen in 2.6% of samples in MCGM database	IHF = 2.6% (mainly gains)
7	OGT8*60k	dup	**22q11.21** 18628147-18894879	266.73	S	*USP18; GGT3P;DGCR6 (partial)*	Overlaps with 22q11 del/dup syndrome	IHF = 7.5% (del/dup). Region rich in segmental duplications
		dup	**22q11.21** 21440456-21661435	220.98	S	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13,FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del/dup). Region rich in segmental duplications
		dup	**22q13.32-13.33** 49010019-49416258	406.24	F (P)	*MIR4535, FAM19A5*	unkown significance	IHF = 0%
		del	**14q12** 29236278-29237238	0.961	S	*FOXG1*	Rett syndrome, congenital variant 613454	IHF = 0% loss; 0.05% gains.
10	OGT8*60k	dup	**22q11.21** 18661699-18848020	186.32	n/a	*AK129567;AK302545;GGT3P*	Overlaps with 22q11 del/dup syndrome	IHF = 7.5% (del/dup). . Region rich in segmental duplications
		dup	**22q11.21** 21468352-21661435	193.08	n/a	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13,FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del/dup). . Region rich in segmental duplications
11	OGT8*60k	dup	**22q11.21** 18628147-18848020	219.87	n/a	*USP18; AK129567;AK302545;GGT3P*	Overlaps with 22q11 del/dup syndrome	IHF = 7.5% (del/dup). . Region rich in segmental duplications
		dup	**22q11.21** 21468352-21661435	193.08	n/a	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13,FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del&dup). . Region rich in segmental duplications
12	OGT8*60k	dup	**10p15.3** 647272-817076	169.8	n/a	*PRR26 (ncRNA), DIP2C*	unkown significance	IHF = 0.02%
14	OGT8*60k	dup	**22q11.21** 21468352-21661435	193.08	S	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13,FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del&dup). . egion rich in segmental duplications
		del	**19q13.3** 2068443-2657043	588.6	F (M)	*MIR1227; U6;AC004490.1;MIR4321;AC004152.5 AC004152.6;SPPL2B;AC005258.3;AC005624.2 AC104537.2;CTC-265F19.2;CTC-265F19.3;CTC-265F19.1; MOB3A;IZUMO4;AP3D1;DOT1L;PLEKHJ1;SF3A2;AMH;JSRP1; OAZ1;C19orf35;LINGO3;LSM7;TMPRSS9;TIMM13; LMNB2;GADD45B;GNG7*	PERSISTENT MULLERIAN DUCT SYNDROME, TYPE I	IHF = 0%
15	Agilent 180K	dup	**22q11.1** 17,068,186-17,290,334	222.1	F (P)	CCT8L2 (partial), FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1, XKR3(partial)	Overlaps with Cat-Eye syndrome	IHF = 0.08% region rich in segmental duplications
16	OGT8*60k & Agilent 4 × 180K	dup	**22q11.21** 21468352-21722313	253.96	F (P)	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13, FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del/dup). Region rich in segmental duplications
	Agilent 180K	dup	**22q11.1** 17,068,186-17,290,334	222.1	F (P)	CCT8L2 (partial), FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1, XKR3(partial)	Overlaps with Cat-Eye syndrome	IHF = 0.08% region rich in segmental duplications
17	OGT8*60k & Agilent 180K	dup	**22q11.1** 17068186-17290334		n/a	*CCT8L2 (partial), XKR3, FABP5P11, TPTEP1, SLC25A15P5, PARP4P3, ANKRD62P1-PARP4P3, ANKRD62P1, VWFP1*	Overlaps with Cat-Eye syndrome	IHF = 0.08% region rich in segmental duplications
18	OGT8*60k	dup	**22q11.21** 21468352-21661435	193.08	n/a	*POM121L7,GGT2,BCRP2,KB-1592A4.15, KB-2A4.13, FAM230B, KB-1592A4.14,KB-1183D5.9,POM121L8P,BCRP6*	Overlaps with 22q11 del/dup syndrome	IHF = 7% (del/dup). Region rich in segmental duplications
51	Affymetrics SNP6	dup	**22q11.1q11.21** 16495650-21616784	5121.135	n/a	*many including TBX1*	Overlaps with Cat-Eye & 22q11 del/dup syndrome	IHF = 0.14% IHF = 0.047% (dups involving regions 19009792-21452445 and 16940617-18848020, respectively)

number abnormalities (Edelmann et al., 1999). Susceptibility of the chromosome 22q11 region to rearrangements has been recognised on the basis of recurrent clinical disorders such as 22q11 deletion/duplication syndromes and Cat-Eye syndrome, which are associated with either decreased or increased gene dosage (Edelmann et al., 1999; McDermid and Morrow, 2002).

Skeletal and soft tissue asymmetries of craniofacial structures, as well as dynamic facial asymmetries, have been observed in patients with genomic imbalances on the 22q11 region (Herman et al., 1988; Xu et al., 2008; Digilio et al., 2009a; Tan et al., 2011; Quintero-Rivera and Martinez-Agosto, 2013; Torti et al., 2013). The asymmetry may include different areas of the head and neck, including the larynx, velum and pharynx. A characteristic pattern of auricular abnormalities is common in 22q11.2 deletion syndrome: overfolding of the helix, cup ear, constricted auricle and protruding ears, attachment of the lobules and narrow external auditory canals. More severe microtia has been reported, but is not common in 22q11.2 deletion syndrome. Additionally, many patients initially diagnosed with asymmetric crying facies (ACF)/Calyer syndrome had 22q11 deletions (Butts, 2009; Ryan et al., 1997). The phenotypic variability of atypical 22q11.2 deletions, excluding *TBX1,* also includes similar craniofacial features (Verhagen et al., 2012). Furthermore, 22q11.2 duplication syndrome, a recently identified condition with significant phenotypic variability (Ensenauer et al., 2003; Portnoï, 2009), appears to be associated with an increased frequency of minor ear malformations, such as dysplastic ears and preauricular pits/tags, as well as with facial asymmetry and hypoplasia of the mandible. Cat-Eye Syndrome, usually caused by partial tetrasomy 22q11, or inverted duplication (commonly as a bisatellited supernumerary chromosome representing an inv dup22q11) is another recognisable 22q11 disorder, with large phenotypic variability. It is usually associated with the occurrence of pre-auricular skin tags and/or pits, as well as facial asymmetry (Rosias and et al., 2001). In addition, a number of OAVS cases have been reported with chromosome abnormalities and CNVs involving 22q11 (Beleza-Meireles et al., 2014; Digilio et al., 2009b).

In conclusion, although the 22q11 CNVs detected are located within regions of segmental duplications and are present in the normal population, the high frequency detected within our OAVS cohort and the published literature warrants further investigation. We hypothesize that the 22q11 locus may harbour genes that are important in aspects of the regulation of craniofacial symmetry and 1st and 2nd branchial arch development. Interestingly, a central role of Crkl, a gene located in the 22q11 syndrome region, has been demonstrated in regulating signalling events in the developing pharyngeal arches, with potential to contribute to craniofacial dysmorphism. In fact, an altered retinoic acid and endothelin signalling has been an evidenced in a Crkl mutant mouse. These two signalling pathways play an important role in the migrating and differentiation of neural crest cells in the branchial arches during embryogenesis (Miller et al., 2014).

These 22q11 CNVs might furthermore have a direct or a positional effect (Haraksingh and Snyder, 2013; Klopocki and Mundlos, 2011). Zeitz et al. in 2013 suggested that regulatory interactions with elements outside the 22q11 deletion loci are disrupted in the disease state and modulate the resulting spectrum of symptoms seen in 22q11 deletion disorder. In fact, the authors show that chromosomal rearrangements on 22q11 can have widespread effects on chromatin organization, and may contribute to the inherent phenotypic variability associated with those rearrangements (Zeitz et al., 2013 Nov–Dec). The expression of genes on 22q might be disturbed during development by the presence of genomic imbalances (CNVs), increasing the susceptibility for OAVS craniofacial abnormalities.

We also propose that the phenotype is affected by other genetic and non-genetic factors, in line with a oligogenic or even a multifactorial etiology (Huang et al., 2010a). It is seems clear to us that there are other genetic loci involved in OAVS and using a genome-wide exome or whole genome sequencing approach would be important to identify the gene(s) involved in its etiology.

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References

Abdelmoity, A.T., Hall, J.J., Bittel, D.C., Yu, S., 2011. 1.39 Mb inherited interstitial deletion in 12p13.33 associated with developmental delay. Eur. J. Med. Genet. 54 (2), 198–203.

Ala-Mello, S., Siggberg, L., Knuutila, S., von Koskull, H., Taskinen, M., Peippo, M., 2008. Further evidence for a relationship between the 5p15 chromosome region and the oculoauriculovertebral anomaly. Am. J. Med. Genet. A 146A (19), 2490–2494.

Ballesta-Martínez, M.J., López-González, V., Dulcet, L.A., Rodríguez-Santiago, B., Garcia-Miñaúr, S., Guillen-Navarro, E., 2013. Autosomal dominant oculoauriculovertebral spectrum and 14q23.1 microduplication. Am. J. Med. Genet. A 161 (8), 2030–2035.

Barisic, I., Odak, L., Loane, M., Garne, E., Wellesley, D., Calzolari, E., Dolk, H., Addor, M.C., Arriola, L., Bergman, J., Bianca, S., Doray, B., Khoshnood, B., Klungsoyr, K., McDonnell, B., Pierini, A., Rankin, J., Rissmann, A., Rounding, C., Queisser-Luft, A., Scarano, G., Tucker, D., 2014. Prevalence, prenatal diagnosis and clinical features of oculo-auriculo-vertebral spectrum: a registry-based study in Europe. Eur. J. Hum. Genet. 22 (8), 1026–1033.

Beleza-Meireles, A., Clayton-Smith, J., Saraiva, J.M., Tassabehji, M., 2014. Oculo-auriculo-vertebral spectrum: a review of the literature and genetic update. J. Med. Genet. 51 (10), 635–645.

Brun, A., Cailley, D., Toutain, J., Bouron, J., Arveiler, B., Lacombe, D., Goizet, C., Rooryck, C., 2012. 1.5 Mb microdeletion in 15q24 in a patient with mild OAVS phenotype. Eur. J. Med. Genet. 55 (2), 135–139.

Butts, S.C., 2009. The facial phenotype of the velo-cardio-facial syndrome. Int. J. Pediatr. Otorhinolaryngol. 73 (3), 343–350.

Callier, P., Faivre, L., Thauvin-Robinet, C., Marle, N., Mosca, A.L., D'Athis, P., Guy, J., Masurel-Paulet, A., Joly, L., Guiraud, S., Teyssier, J.R., Huet, F., Mugneret, F., 2008. Array-CGH in a series of 30 patients with mental retardation, dysmorphic features, and congenital malformations detected an interstitial 1p22.2-p31.1 deletion in a patient with features overlapping the Goldenhar syndrome. Am. J. Med. Genet. A 146A (16), 2109–2115.

Cline, J.M., Hicks, K.E., Patel, K.G., 2014. Characterization of facial paresis in hemifacial microsomia. Otolaryngol. Head. Neck Surg. 150 (2), 188–193.

Cousley, R.R., Calvert, M.L., 1997. Current concepts in the understanding and management of hemifacial microsomia. Br. J. Plast. Surg. 50, 536–551.

Digilio, M.C., McDonald-McGinn, D.M., Heike, C., Catania, C., Dallapiccola, B., Marino, B., Zackai, E.H., 2009. Three patients with oculo-auriculo-vertebral spectrum and microdeletion 22q11.2. Am. J. Med. Genet. A 149A (12), 2860–2864.

Digilio, M.C.1, McDonald-McGinn, D.M., Heike, C., Catania, C., Dallapiccola, B., Marino, B., Zackai, E.H., 2009. Three patients with oculo-auriculo-vertebral spectrum and microdeletion 22q11.2. Am. J. Med. Genet. A 149A, 2860–2864.

Edelmann, L., Pandita, R.K., Spiteri, E., Funke, B., Goldberg, R., Palanisamy, N., Chaganti, R.S., Magenis, E., Shprintzen, R.J., Morrow, B.E., 1999. A common molecular basis for rearrangement disorders on chromosome 22q11. Hum. Mol. Genet. 8, 1157–1167.

Ensenauer, R.E., Adeyinka, A., Flynn, H.C., Michels, V.V., Lindor, N.M., Dawson, D.B., Thorland, E.C., Lorentz, C.P., Goldstein, J.L., McDonald, M.T., Smith, W.E., Simon-Fayard, E., Alexander, A.A., Kulharya, A.S., Ketterling, R.P., Clark, R.D., Jalal, S.M., 2003. Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. Am. J. Hum. Genet. 73 (5), 1027–1040.

Figueroa, A.A., Pruzansky, S., 1982. The external ear, mandible and other components of hemifacial microsomia. J. Maxillofac. Surg. 10 (4), 200–211.

Garavelli, L., Virdis, R., Donadio, A., Sigorini, M., Banchini, G., Balestrazzi, P., Fryns, J.P., 1999. Oculo-auriculo-vertebral spectrum in Klinefelter syndrome. Genet. Couns. 10 (3), 321–324.

Goodin, K., Prucka, S., Woolley, A.L., Kohlhase, J., Smith, R.J., Grant, J., Robin, N.H., 2009. Familial transmission of oculoauriculovertebral spectrum (Goldenhar syndrome) is not due to mutations in either EYA1 or SALL1. Am. J. Med. Genet. A 149A (3), 535–538.

Haraksingh, R.R., Snyder, M.P., 2013. Impacts of variation in the human genome on gene regulation. J. Mol. Biol. 425 (21), 3970–3977.
Heike, C.L., Hing, A.V., 2009. Craniofacial microsomia overview. In: Pagon, R.A., Adam, M.P., Ardinger, H.H., Bird, T.D., Dolan, C.R., Fong, C.T., Smith, R.J.H., Stephens, K. (Eds.), GeneReviews® [Internet]. University of Washington, Seattle, Seattle (WA).
Hennekam, R.C.M., Krantz, I.D., Allanson, J.E., 2010. Syndromes of the Head and Neck, fifth ed., vol. 20. Oxford University Press, Oxford, UK, pp. 879–887.
Herman, G.E., Greenberg, F., Ledbetter, D.H., 1988. Multiple congenital anomaly/mental retardation (MCA/MR) syndrome with Goldenhar complex due to a terminal del(22q). Am. J. Med. Genet. 29 (4), 909–915.
Huang, X.S., Li, X., Tan, C., Xiao, L., Jiang, H.O., Zhang, S.F., Wang, D.M., Zhang, J.X., 2010. Genome-wide scanning reveals complex etiology of oculo-auriculo-vertebral spectrum. Tohoku J. Exp. Med. 222 (4), 311–318.
Huang, X.S., Xiao, L., Li, X., Xie, Y., Jiang, H.O., Tan, C., Wang, L., Zhang, J.X., 2010. Two neighboring microdeletions of 5q13.2 in a child with oculo-auriculo-vertebral spectrum. Eur. J. Med. Genet. 53, 153–158.
Kaye, C.I., Martin, A.O., Rollnick, B.R., Nagatoshi, K., Israel, J., Hermanoff, M., Tropea, B., Richtsmeier, J.T., Morton, N.E., 1992. Oculoauriculovertebral anomaly: segregation analysis. Am. J. Med. Genet. 43, 913–917.
Kelberman, D., Tyson, J., Chandler, D.C., McInerney, A.M., Slee, J., Albert, D., Aymat, A., Botma, M., Calvert, M., Goldblatt, J., Haan, E.A., Laing, N.G., Lim, J., Malcolm, S., Singer, S.L., Winter, R.M., Bitner-Glindzicz, M., 2001. Hemifacial microsomia: progress in understanding the genetic basis of a complex malformation syndrome. Hum. Genet. 109, 638–645.
Klopocki, E., Mundlos, S., 2011. Copy-number variations, noncoding sequences, and human phenotypes. Annu. Rev. Genomics Hum. Genet. 22 (12), 53–72.
Mastroiacovo, P., Corchia, C., Botto, L.D., Lanni, R., Zampino, G., Fusco, D., 1995. Epidemiology and genetics of microtia-anotia: a registry based study on over one million births. J. Med. Genet. 32 (6), 453–457.
McDermid, H.E., Morrow, B.E., 2002. Genomic disorders on 22q11. Am. J. Hum. Genet. 70, 1077–1088.
Melnick, M., 1980. The etiology of external ear malformations and its relation to abnormalities of the middle ear, inner ear, and other organ systems. Birth Defects Orig. Artic. Ser. 16 (4), 303–331.
Miller, K.A., Tan, T.Y., Welfare, M.F., White, S.M., Stark, Z., Savarirayan, R., Burgess, T., Heggie, A.A., Caruana, G., Bertram, J.F., Bateman, J.F., Farlie, P.G., 2014. A mouse splice-site mutant and individuals with atypical chromosome 22q11.2 deletions demonstrate the crucial role for crkl in craniofacial and pharyngeal development. Mol. Syndromol. 5 (6), 276–286.
Poonawalla, H.H., Kaye, C.I., Rosenthal, I.M., Pruzansky, S., 1980. Hemifacial microsomia in a patient with Klinefelter syndrome. Cleft Palate J. 17 (3), 194–196.
Portnoï, M.F., 2009. Microduplication 22q11.2: a new chromosomal syndrome. Eur. J. Med. Genet. 52 (2–3), 88–93.
Quintero-Rivera, F., Martinez-Agosto, J.A., 2013. Hemifacial microsomia in cat-eye syndrome: 22q11.1-q11.21 as candidate loci for facial symmetry. Am. J. Med. Genet. A 161 (8), 1985–1991.
Rao, V.A., Kaliaperumal, S., Subramanyan, T., Rao, K.R., Bhargavan, R., 2005. Goldenhar's sequence with associated juvenile glaucoma in Turner's syndrome. Indian J. Ophthalmol. 53 (4), 267–268.
Rollnick, B.R., Kaye, C.I., 1983. Hemifacial microsomia and variants: pedigree data. Am. J. Med. Genet. 15 (2), 233–253.
Rollnick, B.R., Kaye, C.I., Nagatoshi, K., Hauck, W., Martin, A.O., 1987. Oculoauriculovertebral dysplasia and variants: phenotypic characteristics of 294 patients. Am. J. Med. Genet. 26 (2), 361–375.
Rooryck, C., Stef, M., Burgelin, I., Simon, D., Souakri, N., Thambo, J.B., Chateil, J.F., Lacombe, D., Arveiler, B., 2009. 2.3 Mb terminal deletion in 12p13.33 associated with oculoauriculovertebral spectrum and evaluation of WNT5B as a candidate gene. Eur. J. Med. Genet. 52 (6), 446–449.
Rooryck, C., Souakri, N., Cailley, D., Bouron, J., Goizet, C., Delrue, M.A., Marlin, S., Lacombe, F.D., Arveiler, B., 2010. Array-CGH analysis of a cohort of 86 patients with oculoauriculovertebral spectrum. Am. J. Med. Genet. A 152A (8), 1984–1989.
Rooryck, C., VuPhi, Y., Souakri, N., Burgelin, I., Saura, R., Lacombe, D., Arveiler, B., Taine, L., 2010. Characterization of a de novo balanced translocation t(9;18)(p23;q12.2) in a patient with oculoauriculovertebral spectrum. Eur. J. Med. Genet. 53, 104–107.
Rosias, P.R., et al., 2001. Phenotypic variability of the cat eye syndrome. Case report and review of the literature. Genet. Couns. 12 (3), 237–282.
Ryan, A.K., Goodship, J.A., Wilson, D.I., Philip, N., Levy, A., Seidel, H., Schuffenhauer, S., Oechsler, H., Belohradsky, B., Prieur, M., Aurias, A., Raymond, F.L., Clayton-Smith, J., Hatchwell, E., McKeown, C., Beemer, F.A., Dallapiccola, B., Novelli, G., Hurst, J.A., Ignatius, J., Green, A.J., Winter, R.M., Brueton, L., Brøndum-Nielsen, K., Scambler, P.J., et al., 1997. Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J. Med. Genet. 34 (10), 798–804.
Santen, G.W., Sun, Y., Gijsbers, A.C., Carré, A., Holvoet, M., Haeringen, Av, Lesnik Oberstein, S.A., Tomoda, A., Mabe, H., Polak, M., Devriendt, K., Ruivenkamp, C.A., Bijlsma, E.K., 2012. Further delineation of the phenotype of chromosome 14q13 deletions: (positional) involvement of FOXG1 appears the main determinant of phenotype severity, with no evidence for a holoprosencephaly locus. J. Med. Genet. 49 (6), 366–372.
Suutarla, S., Rautio, J., Klockars, T., 2014 Jan. Cleft lip and/or palate and auricular malformations. J. Craniofac. Surg. 25 (1), 177–183.
Tan, T.Y., Collins, A., James, P.A., McGillivray, G., Stark, Z., Gordon, C.T., Leventer, R.J., Pope, K., Forbes, R., Crolla, J.A., Ganesamoorthy, D., Burgess, T., Bruno, D.L., Slater, H.R., Farlie, P.G., Amor, D.J., 2011. Phenotypic variability of distal 22q11.2 copy number abnormalities. Am. J. Med. Genet. A 155A (7), 1623–1633.
Tasse, C., Böhringer, S., Fischer, S., Lüdecke, H.J., Albrecht, B., Horn, D., Janecke, A., Kling, R., König, R., Lorenz, B., Majewski, F., Maeyens, E., Meinecke, P., Mitulla, B., Mohr, C., Preischl, M., Umstadt, H., Kohlhase, J., Gillessen-Kaesbach, G., Wieczorek, D., 2005. Oculo-auriculo-vertebral spectrum (OAVS): clinical evaluation and severity scoring of 53 patients and proposal for a new classification. Eur. J. Med. Genet. 48 (4), 397–411.
Tasse, C., Majewski, F., Böhringer, S., Fischer, S., Lüdecke, H.J., Gillessen-Kaesbach, G., Wieczorek, D., 2007. A family with dominant oculoauriculovertebral spectrum. Am. J. Med. Genet. 78, 345–349.
Torti, E.E., Braddock, S.R., Bernreuter, K., Batanian, J.R., 2013. Oculo-auriculo-vertebral spectrum, cat eye, and distal 22q11 microdeletion syndromes: a unique double rearrangement. Am. J. Med. Genet. A 161 (8), 1992–1998.
Tsai, F.J., Tsai, C.H., 1993. Autosomal dominant inherited oculo-auriculo-vertebral spectrum: report of one family. Zhonghua Min. Guo Xiao Er Ke Yi Xue Hui Za Zhi 34 (1), 27–31.
van Nunen, D.P., Kolodzynski, M.N., van den Boogaard, M.J., Kon, M., Breugem, C.C., 2014. Microtia in the Netherlands: clinical characteristics and associated anomalies. Int. J. Pediatr. Otorhinolaryngol. 78 (6), 954–959.
Vendramini-Pittoli, Kokitsu-Nakata, 2009. Oculoauriculovertebral spectrum: report of nine familial cases with evidence of autosomal dominant inheritance and review of the literature. Clin. Dysmorphol. 18, 67–77.
Verhagen, J.M., Diderich, K.E., Oudesluijs, G., Mancini, G.M., Eggink, A.J., Verkleij-Hagoort, A.C., Groenenberg, I.A., Willems, P.J., du Plessis, F.A., de Man, S.A., Srebniak, M.I., van Opstal, D., Hulsman, L.O., van Zutven, L.J., Wessels, M.W., 2012. Phenotypic variability of atypical 22q11.2 deletions not including TBX1. Am. J. Med. Genet. A 158A (10), 2412–2420.
Verloes, A., Seret, N., Bernier, V., Gonzales, M., Herens, C., Koulischer, L., 1991. Branchial arch anomalies in trisomy 18. Ann. Genet. 34 (1), 22–24.
Wilson, G.N., Barr Jr., M., 1983. Trisomy 9 mosaicism: another etiology for the manifestations of Goldenhar syndrome. J. Craniofac. Genet. Dev. Biol. 3 (4), 313–316.
Xu, J., Fan, Y.S., Siu, V.M., 2008. A child with features of Goldenhar syndrome and a novel 1.12 Mb deletion in 22q11.2 by cytogenetics and oligonucleotide array CGH: is this a candidate region for the syndrome? Am. J. Med. Genet. A 146A (14), 1886–1889.
Zeitz, M.J., Lerner, P.P., Ay, F., Van Nostrand, E., Heidmann, J.D., Noble, W.S., Hoffman, A.R., 2013 Nov-Dec. Implications of COMT long-range interactions on the phenotypic variability of 22q11.2 deletion syndrome. Nucleus 4 (6), 487–493.