CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

A De Novo Proximal 3q29 Chromosome Microduplication in a Patient with Oculo Auriculo Vertebral Spectrum

Valentina Guida,[1] Lorenzo Sinibaldi,[1] Mario Pagnoni,[2] Laura Bernardini,[1] Sara Loddo,[1] Katia Margiotti,[3,4] Maria Cristina Digilio,[5] Maria Teresa Fadda,[2] Bruno Dallapiccola,[5] Giorgio Iannetti,[2] and De Luca Alessandro[1]*

[1]IRCCS-Casa Sollievo della Sofferenza, Mendel Institute, Rome, Italy

[2]Department of Maxillo-Facial Surgery, Policlinico Umberto I, Rome, Italy

[3]Laboratory of Molecular Medicine and Biotechnology, CIR, Campus Bio-Medico University of Rome, Rome, Italy

[4]Department of Experimental Medicine, Policlinico Umberto I, Sapienza University of Rome, Rome, Italy

[5]Bambino Gesù Pediatric Hospital, Piazza S. Onofrio 4, Rome, Italy

Manuscript Received: 26 May 2014; Manuscript Accepted: 21 December 2014

Oculo auriculo vertebral spectrum (OAVS; OMIM 164210) is a clinically and genetically heterogeneous disorder originating from an abnormal development of the first and second branchial arches. Main clinical characteristics include defects of the aural, oral, mandibular, and vertebral development. Anomalies of the cardiac, pulmonary, renal, skeletal, and central nervous systems have also been described. We report on a 25-year-old male showing a spectrum of clinical manifestations fitting the OAVS diagnosis: hemifacial microsomia, asymmetric mandibular hypoplasia, preauricular pits and tags, unilateral absence of the auditory meatus, dysgenesis of the inner ear and unilateral microphthalmia. A SNP-array analysis identified a de novo previously unreported microduplication spanning 723 Kb on chromosome 3q29. This rearrangement was proximal to the 3q29 microdeletion/microduplication syndrome region, and encompassed nine genes including *ATP13A3* and *XXYLT1*, which are involved in the organogenesis and regulation of the Notch pathway, respectively. The present observation further expands the spectrum of genomic rearrangements associated to OAVS, underlying the value of array-based studies in patients manifesting OAVS features.

Key words: Oculo auriculo vertebral spectrum; 3q29 duplication; SNP-array analysis

How to Cite this Article:
Guida V, Sinibaldi L, Pagnoni M, Bernardini L, Loddo S, Margiotti K, Digilio MC, Fadda MT, Dallapiccola B, Iannetti G, Alessandro DL. 2015. A de novo proximal 3q29 chromosome microduplication in a patient with oculo auriculo vertebral spectrum.
Am J Med Genet Part A 167A:797–801.

INTRODUCTION

Oculo auriculo vertebral spectrum (OAVS; OMIM 164210), also known as Goldenhar syndrome or hemifacial microsomia, (HFM) is a complex developmental disorder involving the first and second branchial arch derivatives occurring in ~1 of 5,600–26,000 live births [Gorlin et al., 2001]. OAVS is mainly characterized by facial asymmetry caused by unilateral mandibular and maxillary hypoplasia, microtia or anotia, preauricular skin tags, conductive hearing loss, epibulbar dermoids and vertebral defects, prevalently in the cervical region, including fusion of the vertebral bodies, segmentation abnormalities, and 'butterfly' vertebrae. Cardiac, renal, pulmonary, and central nervous system anomalies have been also associated to this disorder. The phenotype is typically unilateral, although bilateral involvement with more severe manifestations of one side can occur [Gorlin et al., 2001]. Maternal diabetes or exposure to teratogens have been suspected to be causative for OAVS. However, the identification of both autosomal dominant and recessive patterns of inheritance in some families, suggest that

Conflict of interest: none.
Grant sponsor: Italian Ministry of Health; Grant numbers: RF-2010-2310935, RC-2013.
*Correspondence to:
Alessandro De Luca, PhD, Istituto CSS-Mendel, V.le Regina Margherita 261, 00198 Rome - ITALY.
E-mail: a.deluca@css-mendel.it
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 3 March 2015
DOI 10.1002/ajmg.a.36951

OAVS can be genetically determined [Tasse et al., 2005]. Several chromosomal rearrangements have been associated with OAVS, including del(5p), 6q monosomy, del(8q), dup(14q), trisomy 18, ring chromosome 21, del(22q), dup(22q), trisomy 22 and 47,XXY [Rooryck et al., 2010; Tan et al., 2011; Ballesta-Martinez et al., 2013]. In addition, mosaic aneuploidies, such as mosaic trisomy 7 and trisomy 9, has been proposed to explain the confined unilateral features and the low recurrence risk [reviewed in [Josifova et al., 2004]]. The majority of reported rearrangements are patient-specific, except for those of chromosome 5p [Ala-Mello et al., 2008] and chromosome 22q that have been detected in multiple patients [Xu et al., 2008; Digilio et al., 2009a; Torti et al., 2013].

Here, we report on a patient with a de novo non-canonical 3q29 microduplication showing a full blown OAVS phenotype.

CLINICAL REPORT

The patient, a 25-year-old male, was born from Caucasian non-consanguineous parents. The mother and sister had a linearization of spinal column verified by radiographic analysis, whereas no cervical spine defects were found in the proband (Fig. 1a). The mother, as well as her grandmother and her brothers, had borderline glycemia levels, but no gestational diabetes was recorded during the pregnancy. At birth the mother was 38 years old, the father 46. The baby was born by vaginal delivery after an uneventful pregnancy. Birth weight was 2,800 g (15th centile), length and head circumference were not reported. Right ear audiologic screening was normal while conductive hearing loss was demonstrated on the left side where preauricular tags and a cervical pit were recorded at birth. A computed tomography (CT) scan demonstrated the absence of the left external auditory meatus with bony atresia of the canal and dysgenesis of the inner ear system (Fig.1b). Ophthalmologic examination showed unilateral left microphthalmia. ECG was normal.

On clinical examination at 5 months left hemifacial microsomia, asymmetric mandibular hypoplasia, unilateral left preauricular tags and microtia with aural atresia of the left ear were reported. At age 9 the patient was evaluated at the Departement of Maxillo-Facial Surgery, Policlinico Umberto I, Rome, Italy (G.I.). He presented with hypoplasia of the left mandible with vertical and transversal defect, shifting of the chin to the affected side, tilting of the occlusal plane and maxillary canting; functional examination revealed mandibular deflection on mouth opening so he started a functional therapy with oral appliance to stimulate mandibular growth. At the end of skeletal growth he underwent surgical correction of the maxillofacial residual asymmetry through maxillary and mandibular osteotomies. Cognitive development in adult age was normal. A 3-D reconstruction of CT scan images showing clear evidence of the cranial bones asymmetry is shown in Figure 1c. Standard chromosome analysis of peripheral blood lymphocytes revealed a normal male karyotype.

RESULTS

Affymetrix SNP-array 6.0 analysis revealed a microduplication of 723 Kb on chromosome 3q29 (from 194,135,222 bp to 194,858,400 bp; hg19) involving nine genes (*ATP13A3*, *LINC00884*, *AX746839*, *U6*, *TMEM44*, *LSG1*, *FAM43A*, *LOC100507391*, *TMEM44-AS1*, *XXYLT1*), proximally located in respect to the common 3q29 duplicated syndrome region (Table I). This rearrangement encompasses several CNVs also present in the general population (see Database of genomic variants in Iafrate et al., 2004 and Fig. 2), though the majority of these CNVs are very small, representing losses of one copy rather than gains, as in the present case. qPCR analysis on DNA of the proband's parents and sister demonstrated de novo origin of this rearrangement. Paternity was confirmed by STR analysis using the PowerPlex ESX 17 System (Promega, Madison, WI). The identified microduplication was absent in 52 OAVS patients and in a control population of 80 non-OAVS Caucasian subjects (L.B. unpublished observation).

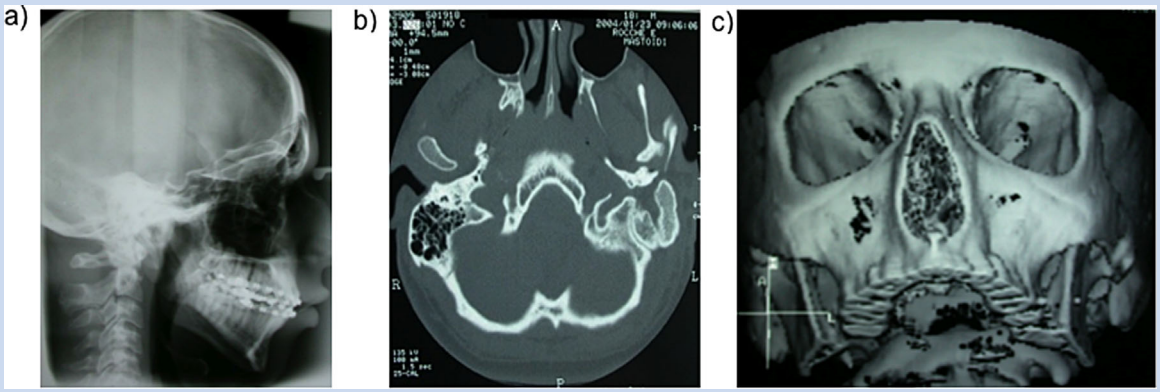

FIG. 1. Radiography and CT scan images of the patient with OAVS features and the 3q29 microduplication. (a) Lateral cervical spine radiograph showing no alteration of cervical vertebrae; (b) Axial cranial CT scan at the level of auricles and external auditory canals showing the absence of the left external auditory meatus and dysgenesis of the inner ear system; (c) 3-D reconstruction of CT scan images showing the facial asymmetry.

TABLE I. Genes Present in the Distal 3q29 Duplicated Region

Gene	Function	OMIM
ATP13A3	*ATP13A3* is a member of the P-type ATPase family of proteins that transport a variety of cations across membranes.	610232
LINC00884	Unknown	
AX746839	Unknown	
U6	Unknown	
TMEM44	*TMEM44* is predicted to encode a seven transmembrane domain protein with an extracellular N-terminus and an intracellular C-terminus, but with no homology to the family of G-protein-coupled receptors	
LSG1	*LSG1* encodes for a member of the novel Ylqf-related GTPase family. LSG1 shuttles between nuclear Cajal bodies and the endoplasmic reticulum and is required for cell viability. Yeast *Lsg1* is involved in ribosome biogenesis	610780
FAM43A	*FAM43A* (family with sequence similarity 43, member A) is a protein-coding gene. An important paralog of this gene is *FAM43B*.	
LOC100507391	Unknown	
TMEM44-AS1	Unknown	
XXYLT1	*XXYLT1* encodes for Alpha-1,3-xylosyltransferase, which elongates the O-linked xylose-glucose disaccharide attached to EGF-like repeats in the extracellular domain of Notch proteins by catalyzing the addition of the second xylose	614552

DISCUSSION

In this study, we describe a patient presenting with OAVS features associated with a 3q29 microduplication. Interstitial duplications of chromosome 3q29, now described as 3q29 duplication syndrome, are associated with a variable phenotype characterized by mild-to-moderate cognitive disability, microcephaly, low-set ears, downturned corners of the mouth, bushy eyebrows and long eyelashes, along with eye, palate, renal and cardiac anomalies [Aqua et al., 1995; Steinbach et al., 1981]. The phenotype spectrum in larger duplications may also include ocular defects, such as microphthalmia and anterior segment anomalies [Rooms et al., 2006; Rosenberg et al., 2006; Lisi et al., 2008; van Essen et al., 1991]. Cardiac defects are also reported, as clinical feature in common to patients with OAVS [Rollnick et al., 1987; Digilio et al., 2008]. Some patients share a typical 3q29 duplication of 1.63 Mb that appears to be the reciprocal duplication product of the 3q29 microdeletion, which in turn is characterized by a variable phenotype including

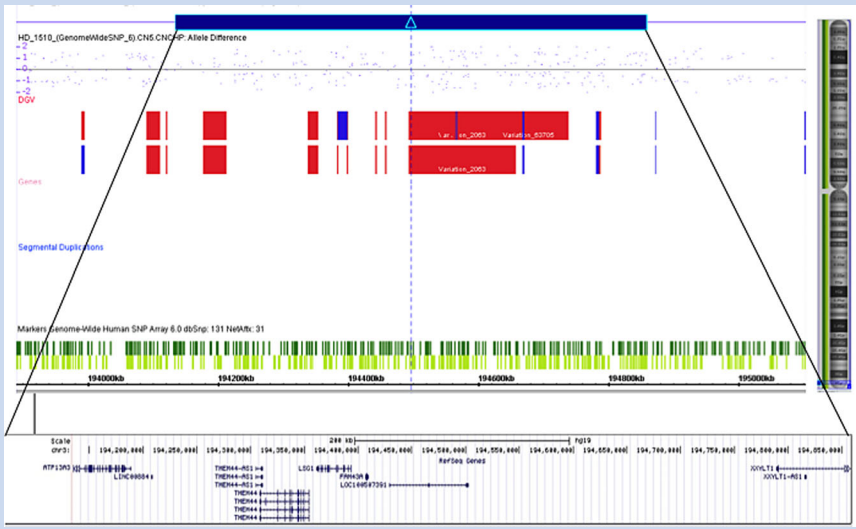

FIG. 2. Graphical view of SNP array analysis generated by Chromosome Analysis Suite Software (Affymetrix, Santa Clara, CA) showing the 3q29 duplication found in the present patient and UCSC graphical view of genes mapping into the 3q29 duplication.

mild-to-moderate intellectual disability, slightly dysmorphic facial features, chest-wall and fingers deformities, autism and gait ataxia [Willatt et al., 2005; Ballif et al., 2008; Lisi et al., 2008; Digilio et al., 2009b]. The presence of low copy repeats (LCRs) flanking this chromosomal region suggests that both 1.63 Mb deletions and duplications of 3q29 arise via non-allelic homologous recombination between LCRs on either side of the breakpoint [Willatt et al., 2005]. In addition to the typical 1.63 Mb rearrangements a few individuals with both atypical deletions and reciprocal 3q29 duplications have been described [Ballif et al., 2008; Goobie et al., 2008]. In the case of duplications, these may be flanking, spanning, or partially overlapping the commonly duplicated region. Ballif et al. [2008] described 19 individuals with duplications of 3q29. Of these, five appeared to be the reciprocal duplication product of the 3q29 microdeletion and 14 were found to be flanking, spannning, or partially overlapping the common deletion region. Interestingly, two atypical duplications overlapped the 3q29 duplication found in the present patient. One was shown to be de novo whether the other was maternally inherited. Both were much larger than the duplication reported here. The authors also described the phenotype of four individuals from this cohort with atypical 3q29 duplications, which included craniosynostosis, high palate, seizures, and ventricular septal defect as recurrent clinical features. Unfortunately, they did not specify the breakpoints of the atypical duplications harbored by the patients from whom clinical data were obtained, which makes it difficult to establish any clear genotype-phenotype correlation with present 3q29 microrearrangement. Clinical description of further subjects with 3q29 duplications will help to further define the phenotypic characteristics associated to these chromosomal anomalies.

The present de novo 3q29 duplication spans 723 Kb of genomic DNA and includes nine UCSC genes (Table I). Differently from the common duplicated 3q29 region, no LCR elements are included in this rearrangement, suggesting a different etiological mechanism, such as non-homologous end joining, or fork stalling and template switching [Zhang et al., 2009; Coughlin 2nd et al., 2012]. The pathogenicity of this CNV might be either related to altered dosage of a single gene or a contiguous set of genes located into the duplicated segment or more indirectly, through a position effect by altering gene expression of a gene or genes that map outside the rearrangement, including those mapping to the adjacent typical 3q29 duplication of 1.63 Mb [Zhang et al., 2009; Coughlin 2nd et al., 2012]. Of the nine genes included within the duplicated region, four have a known function and two could be considered candidates for OAVS, namely *ATP13A3* and *XXYLT1* (alias *C3ORF21*). *ATP13A3* is a member of the P-type ATPase family of proteins. It is implicated in transportation of a variety of cations across the membrane and it is significantly expressed in a variety of organs, including colon, kidney, liver, intestine, stomach, skeletal muscle and brain [Schultheis et al., 2004]. Moreover, few studies showed higher levels of this transcript during first stages of mouse development, suggesting that *ATP13A3* may be involved in early organogenesis [Weingarten et al., 2012]. *XXYLT1* encodes for an endoplasmic reticulum localized xylosiltransferase, which can transfer a second xylose to different epidermal growth factor (EGF)-like domains of the Notch protein [Sethi et al., 2012]. Of note, *drosophila* models with decreased Notch xylosylation indicate that xylose residues on EGF16–20 negatively regulate the surface expression of the Notch receptor [Lee et al., 2013]. This is interesting since animal studies showed that Notch genes are expressed during the development of the inner ear driving the sensory program in nonsensory cells [Pan et al., 2013] and plays an important role during vascular morphogenesis [Roca and Adams, 2007]. Unfortunately no RNA sample or other tissues (bones, cartilage etc,) involved in OAVS were available for the expression analyses.

In conclusion, we report on the first de novo 3q29 microduplication proximal to the 3q29 duplicated syndrome region associated to OAVS. This result further expands the spectrum of cytogenetic anomalies associated with OAVS, underlying the importance of array-based studies in patients with OAVS. Further studies on larger cohorts are necessary to better define the clinical and molecular spectra of microrearrangements associated with OAVS.

ACKNOWLEDGMENTS

We would like to express our gratitude to the patient and his family, which made this study possible. The authors have no conflict of interest to declare. This research was funded by grants from the Italian Ministry of Health RF-2010-2310935 (to A.D.L.) and RC-2013 (to V.G.).

REFERENCES

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:2490–2494.

Aqua MS, Rizzu P, Lindsay EA, Shaffer LG, Zackai EH, Overhauser J, Baldini A. 1995. Duplication 3q syndrome: Molecular delineation of the critical region. Am J Med Genet 55:33–37.

Ballesta-Martinez MJ, Lopez-Gonzalez V, Dulcet LA, Rodriguez-Santiago B, Garcia-Minaur S, Guillen-Navarro E. 2013. Autosomal dominant oculoauriculovertebral spectrum and 14q23.1 microduplication. Am J Med Genet A 161A:2030–2035.

Ballif BC, Theisen A, Coppinger J, Gowans GC, Hersh JH, Madan-Khetarpal S, Schmidt KR, Tervo R, Escobar LF, Friedrich CA, McDonald M, Campbell L, Ming JE, Zackai EH, Bejjani BA, Shaffer LG. 2008. Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. Mol Cytogenet 1:8.

Coughlin CR, 2nd, Scharer GH, Shaikh TH. 2012. Clinical impact of copy number variation analysis using high-resolution microarray technologies: Advantages, limitations and concerns. Genome Med 4:80.

Digilio MC, Calzolari F, Capolino R, Toscano A, Sarkozy A, de Zorzi A, Dallapiccola B, Marino B. 2008. Congenital heart defects in patients with oculo-auriculo-vertebral spectrum (Goldenhar syndrome). Am J Med Genet A 146A:1815–1819.

Digilio MC, McDonald-McGinn DM, Heike C, Catania C, Dallapiccola B, Marino B, Zackai EH. 2009a. Three patients with oculo-auriculo-vertebral spectrum and microdeletion 22q11. 2. Am J Med Genet A 149A:2860–2864.

Digilio MC, Bernardini L, Mingarelli R, Capolino R, Capalbo A, Giuffrida MG, Versacci P, Novelli A, Dallapiccola B. 2009b. 3q29 Microdeletion: A mental retardation disorder unassociated with a recognizable phenotype in two mother-daughter pairs. Am J Med Genet A 149A:1777–1781.

Goobie S, Knijnenburg J, Fitzpatrick D, Sharkey FH, Lionel AC, Marshall CR, Azam T, Shago M, Chong K, Mendoza-Londono R, den Hollander NS, Ruivenkamp C, Maher E, Tanke HJ, Szuhai K, Wintle RF, Scherer SW. 2008. Molecular and clinical characterization of de novo and familial cases with microduplication 3q29: Guidelines for copy number variation case reporting. Cytogenet Genome Res 123:65–78.

Gorlin RJ, Hennekam CMM. 2001. Syndromes of the head and neck. New York: Oxford University Press.

Iafrate AJ, Rivera FL, Listewnik MN, Donahoe ML, Qi PK, Scherer Y, Lee SW. 2004. Detection of large scale variation in the human genome. Nature Genetics 949–951.

Josifova DJ, Patton MA, Marks K. 2004. Oculoauriculovertebral spectrum phenotype caused by an unbalanced t(5;8)(p15.31;p23.1) rearrangement. Clin Dysmorphol 13:151–153.

Lee TV, Sethi MK, Leonardi J, Rana NA, Buettner FF, Haltiwanger RS, Bakker H, Jafar-Nejad H. 2013. Negative regulation of notch signaling by xylose. PLoS Genet 9:e1003547.

Lisi EC, Hamosh A, Doheny KF, Squibb E, Jackson B, Galczynski R, Thomas GH, Batista DA. 2008. 3q29 interstitial microduplication: A new syndrome in a three-generation family. Am J Med Genet A 146A:601–609.

Pan W, Jin Y, Chen J, Rottier RJ, Steel KP, Kiernan AE. 2013. Ectopic expression of activated notch or SOX2 reveals similar and unique roles in the development of the sensory cell progenitors in the mammalian inner ear. J Neurosci 33:16146–16157.

Roca C, Adams RH. 2007. Regulation of vascular morphogenesis by Notch signaling. Genes Dev 21:2511–2524.

Rollnick BR, Kaye CI, Nagatoshi K, Hauck W, Martin AO. 1987. Oculoauriculovertebral dysplasia and variants: Phenotypic characteristics of 294 patients. Am J Med Genet 26:361–375.

Rooms L, Reyniers E, Wuyts W, Storm K, van Luijk R, Scheers S, Wauters J, van den Ende J, Biervliet M, Eyskens F, van Goethem G, Laridon A, Ceulemans B, Courtens W, Kooy RF. 2006. Multiplex ligation-dependent probe amplification to detect subtelomeric rearrangements in routine diagnostics. Clin Genet 69:58–64.

Rooryck C, Souakri N, Cailley D, Bouron J, Goizet C, Delrue MA, Marlin S, Lacombe FD, Arveiler B. 2010. Array-CGH analysis of a cohort of 86 patients with oculoauriculovertebral spectrum. Am J Med Genet A 152A:1984–1989.

Rosenberg CKJ, Bakker E, Vianna-Morgante AM, Sloos W, Otto PA, Kriek M, Hansson K, Krepischi-Santos ACV, Fiegler H, Carter NP, Bijsma EK, van Haeringen A, Szuhai K, Tanke Hj.. 2006. Array CGH detection of micro rearrangements in mentally retarded individuals: Clinical significance of imbalances present both in affected children and normal parents. J Med Genet 43:180–186.

Schultheis PJ, Hagen TT, O'Toole KK, Tachibana A, Burke CR, McGill DL, Okunade GW, Shull GE. 2004. Characterization of the P5 subfamily of P-type transport ATPases in mice. Biochem Biophys Res Commun 3:731–738.

Sethi MK, Buettner FF, Ashikov A, Krylov VB, Takeuchi H, Nifantiev NE, Haltiwanger RS, Gerardy-Schahn R, Bakker H. 2012. Molecular cloning of a xylosyltransferase that transfers the second xylose to O-glucosylated epidermal growth factor repeats of notch. J Biol Chem 287:2739–2748.

Steinbach P, Adkins WN Jr, Caspar H, Dumars KW, Gebauer J, Gilbert EF, Grimm T, Habedank M, Hansmann I, Herrmann J, Kaveggia EF, Langenbeck U, Meisner LF, Najafzadeh TM, Opitz JM, Palmer CG, Peters HH, Scholz W, Tavares AS, Wiedeking C. The dup(3q) syndrome: Report of eight cases and review of the literature Am J Med Genet 10:159–177.

Tan TY, Collins A, James PA, McGillivray G, Stark Z, Gordon CT, Leventer RJ, Pope K, Forbes R, Crolla JA, Ganesamoorthy D, Burgess T, Bruno DL, Slater HR, Farlie PG, Amor DJ. 2011. Phenotypic variability of distal 22q11.2 copy number abnormalities. Am J Med Genet A 155A:16 23–1633.

Tasse C, Bohringer S, Fischer S, Ludecke HJ, Albrecht B, Horn D, Janecke A, Kling R, Konig 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 48:397–411.

Torti EE, Braddock SR, Bernreuter K, Batanian JR. 2013. Oculo-Auriculo-Vertebral Spectrum, Cat Eye, and Distal 22q 11 Microdeletion Syndromes: A Unique Double Rearrangement. Am J Med Genet A 161A:1992–1998.

van Essen AJ, Kok K, van den Berg A, de Jong B, Stellink F, Bos AF, Scheffer H, Buys CH. 1991. Partial 3q duplication syndrome and assignment of D3S5 to 3q25–3q28. Hum Genet 87:151–154.

Weingarten LS, Dave H, Li H, Crawford DA. 2012. Developmental expression of P5 ATPase mRNA in the mouse. Cell Mol Biol Lett 17:153–170.

Willatt L, Cox J, Barber J, Cabanas ED, Collins A, Donnai D, FitzPatrick DR, Maher E, Martin H, Parnau J, Pindar L, Ramsay J, Shaw-Smith C, Sistermans EA, Tettenborn M, Trump D, de Vries BB, Walker K, Raymond FL. 2005. 3q29 microdeletion syndrome: Clinical and molecular characterization of a new syndrome. Am J Hum Genet 77:154–160.

Xu J, Fan YS, Siu VM. 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:1886–1889.

Zhang F, Gu W, Hurles ME, Lupski JR. 2009. Copy number variation in human health, disease, and evolution. Annu Rev Genomics Hum Genet 10:451–481.