Journal of Human Genetics (2015) 60, 199–202

www.nature.com/jhg

SHORT COMMUNICATION

Agnathia-otocephaly complex and asymmetric velopharyngeal insufficiency due to an in-frame duplication in *OTX2*

Panagiotis I Sergouniotis[1,2,6], Jill E Urquhart[1,3,6], Simon G Williams[3], Sanjeev S Bhaskar[3], Graeme C Black[1,3], Simon C Lovell[4], David J Whitby[5], William G Newman[1,3] and Jill Clayton-Smith[1,3]

Agnathia-otocephaly complex is a malformation characterized by absent/hypoplastic mandible and abnormally positioned ears. Mutations in two genes, *PRRX1* and *OTX2*, have been described in a small number of families with this disorder. We performed clinical and genetic testing in an additional family. The proband is a healthy female with a complicated pregnancy history that includes two offspring diagnosed with agnathia-otocephaly during prenatal ultrasound scans. Exome sequencing was performed in fetal DNA from one of these two offspring revealing a heterozygous duplication in *OTX2*: c.271_273dupCAG, p.(Gln91dup). This change leads to the insertion of a glutamine within the OTX2 homeodomain region, and is predicted to alter this signaling molecule's ability to interact with DNA. The same variant was also identified in the proband's clinically unaffected 38-year-old husband and their 9-year-old daughter, who presented with a small mandible, normal ears and velopharyngeal insufficiency due to a short hemi-palate. This unusual presentation of *OTX2*-related disease suggests that OTX2 might have a role in palatal hypoplasia cases. A previously unreported *OTX2* variant associated with extreme intrafamilial variability is described and the utility of exome sequencing as a tool to confirm the diagnosis of agnathia-otocephaly and to inform the reproductive decisions of affected families is highlighted.
Journal of Human Genetics (2015) **60,** 199–202; doi:10.1038/jhg.2014.122; published online 15 January 2015

OTX2 (MIM*600037) encodes a transcription factor with essential functions in embryonic head formation and, at later developmental stages, in eye and brain development.[1] After birth, although its expression decreases, it maintains an important role in the retina.[2] The OTX2 protein is a member of the OTX group of signaling molecules, a highly conserved protein subclass characterized by a DNA-binding homeodomain region.[1]

Defects in *OTX2* can lead to a wide range of phenotypes. OTX2 haploinsufficiency, resulting from heterozygous loss-of-function mutations, is typically associated with structural eye defects (ranging from bilateral anophthalmia to coloboma).[3] Ocular abnormalities are often combined with extraocular features including learning disability and pituitary abnormalities.[1,3] *OTX2*-related disorders that do not primarily involve the eye have also been reported. These include hemifacial microsomia (that is, facial asymmetry due to maxillary and mandibular hypoplasia and ear malformations), and the agnathia-otocephaly complex (AOC).[4–7]

AOC (MIM#202650) is a rare, often sporadic, malformation complex of the first pharyngeal arch that is characterized by agnathia/dysgnathia, microstomia, aglossia/hypoglossia and variable displacement of the ears toward the midline. Prognosis is poor with affected individuals often dying shortly after birth due to respiratory failure secondary to airway obstruction.[7] Nevertheless, a few patients have been reported to survive into childhood. AOC has been attributed to both genetic and teratogenic causes.[5–8] Genetic causes include heterozygous or biallelic *PRRX1* mutations as well as heterozygous, presumed loss-of-function *OTX2* mutations (Table 1).[5,6,8]

This study expands the phenotypic spectrum of *OTX2*-related disease and details the clinical and genetic findings in a family segregating a previously unreported three base-pair duplication in *OTX2* (Figure 1a). The proband is a healthy female with a complicated obstetric history (II:2): during her first pregnancy, a 23-week ultrasound scan revealed a fetus with a small jaw and low-set ears (III:1). There was polyhydramnios and she went into preterm labor; there was immediate neonatal death and the diagnosis of AOC was confirmed on autopsy. A subsequent pregnancy (III:5) was electively terminated after a 17-week scan revealed polyhydramnios and facial

[1]Institute of Human Development, University of Manchester, Manchester, UK; [2]Manchester Royal Eye Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; [3]Manchester Centre for Genomic Medicine, St Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK; [4]Department of Computational and Evolutionary Biology, Faculty of Life Sciences, University of Manchester, Manchester, UK and [5]North West, Isle of Man and North Wales Cleft Lip and Palate Network, Royal Manchester Children's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK
[6]These authors contributed equally to this work.
Correspondence: Professor J Clayton-Smith, Manchester Centre for Genomic Medicine, 6th Floor, St Mary's Hospital, Oxford Road, Manchester M13 9WL, UK.
E-mail: jill.clayton-smith@cmft.nhs.uk
Received 15 October 2014; revised 3 December 2014; accepted 5 December 2014; published online 15 January 2015

Table 1 Subjects with *OTX2* disease-causing variants and agnathia-otocephaly complex reported here and elsewhere

Family ID (case ID); reference	*Mandible and mouth*	*Ears*	*Eyes*	*Other*	*Family history and parental DNA analysis*	*Heterozygous OTX2 mutation detected in family*[a]
B (II-1);[5]	Agnathia; microstomia; aglosia	Low set but normally formed	Astigmatism	12 years old. Long tubular nose.	Sporadic case; mother had 8 miscarriages. *De novo* mutation.	c.130delC, p.(Arg44Glyfs*15)
(III:2); this report	Hypoplastic left soft palate; micrognathia	Normal	Normal, myopic	9 years old. No health problems.	Clinically unaffected father harbors the mutation.	c.271_273dupCAG, p.(Gln91dup)
(III:1); this report	Micrognathia	Large, low set	Normal	Immediate neonatal death after preterm labor at 30 weeks. No genetic testing (AOC-related genes not known when diagnosis was made; sample not collected for testing).		
(III:5); this report	Agnathia	Low set	Normal	Pregnancy terminated at 17 weeks. Prominent nose.		
(case 4);[6]	Aganthia; astomia; aglosia	Low set, posteriorly rotated, paramedian, convergent	Bilateral microphthalmia	Pregnancy terminated at 16 weeks. Absent pharyngeal floor. Optic chiasm and pituitary gland not found. Abnormalities of extremities present.	Mother and two maternal relatives have unilateral microphthalmia. Mother harbors the mutation.	c.289C>T, p.(Arg97*)
A (IV-1);[5]	Agnathia; microstomia	Synotia	Not reported	Immediate neonatal death.	17 members display micro/anophthalmia (2 of which have isolated bilateral anophthalmia and 3 of which have bilateral anophthalmia with mental retardation). Mutation segregates in an autosomal dominant manner.	c.316delC, p.(Gln106Asnfs*11)
A (IV-2);[5]	Prenatal ultrasound scan revealed facial malformations suggestive of AOC			Pregnancy terminated.		
A (IV-3);[5]	Microretrognathia; microglossia	Not reported	Bilateral microphthalmia; absence of anterior chamber; cataract; retinal dysplasia	Immediate neonatal death. Hypoplasia of upper pharynx. Noncommunication between proboscis and hypopharynx. Triangular face. Thymic hyperplasia. 11 ribs. Micropenis.		
A (IV-6);[5]	Immediate neonatal death with probable diagnosis of AOC		Bilateral anophthalmia	Immediate neonatal death.		
A (III-7);[5]	Micrognathia	Normal	Unilateral anophthalmia	Adulthood.		
(case 1);[6]	Agnathia; microstomia	Dysmorphic, anterocaudally positioned.	Normal on external inspection	Immediate neonatal death. Persistent buccopharyngeal membrane.	Clinically unaffected parents. Deletion appeared *de novo*.	~400 kb deletion in 14q23.1 including the whole gene

Abbreviation: AOC, agnathia-otocephaly complex.
[a]Variants annotated according to NCBI Reference Sequences NM_021728.2 and NP_068374.1.

malformations suggestive of recurrence of AOC. DNA was extracted from fetal (III:5) tissue and analyzed using exome sequencing (Supplementary Information). A heterozygous c.271_273dupCAG, p.(Gln91dup) change in *OTX2* was identified. This change was also found in the proband's clinically unaffected 38-year-old husband (II:1) and their 9-year-old daughter (III:2; Figure 2). The latter presented with respiratory problems after birth and was noted to have a small mandible. Intra-oral examination revealed an unusually short left hemi-palate. She failed to thrive because of feeding difficulties and was fed by nasogastric tube for the first year of life. No other malformations were noted and the diagnosis of AOC was excluded. Although she started to speak at the normal time, her speech was severely hypernasal. A videofluoroscopic swallowing study at age 3 years confirmed a hypoplastic soft palate on the left side; there was only a small amount of palate movement on the right side and no movement on the left side. A Furlow palatoplasty was carried out at the age of 3 years followed by a hemi-pharyngoplasty at the age of 6 years, and these resulted in a marked improvement in her speech quality. At the age of 9 years, there are no concerns about her health and development; she is moderately myopic and has a normal eye examination.

The c.271_273dupCAG, p.(Gln91dup) change affects the 3′ end of *OTX2* exon 4 (found in both major OTX2 isoforms (NP_068374.1 and NP_758840.1)). This duplication does not disrupt the sequence of the splice donor site and it was not found to have a significant effect on splicing by *in silico* analysis (Alamut v2.2.1, Interactive Biosoftware,

Figure 2 Clinical photographs of two related individuals harboring a heterozygous c.271_273dupCAG change in *OTX2*. Subject II:1 (**a**) is clinically unaffected while his daughter, subject III:2, (**b**) presented with an unusually short left hemi-palate and severe micrognathia at birth. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

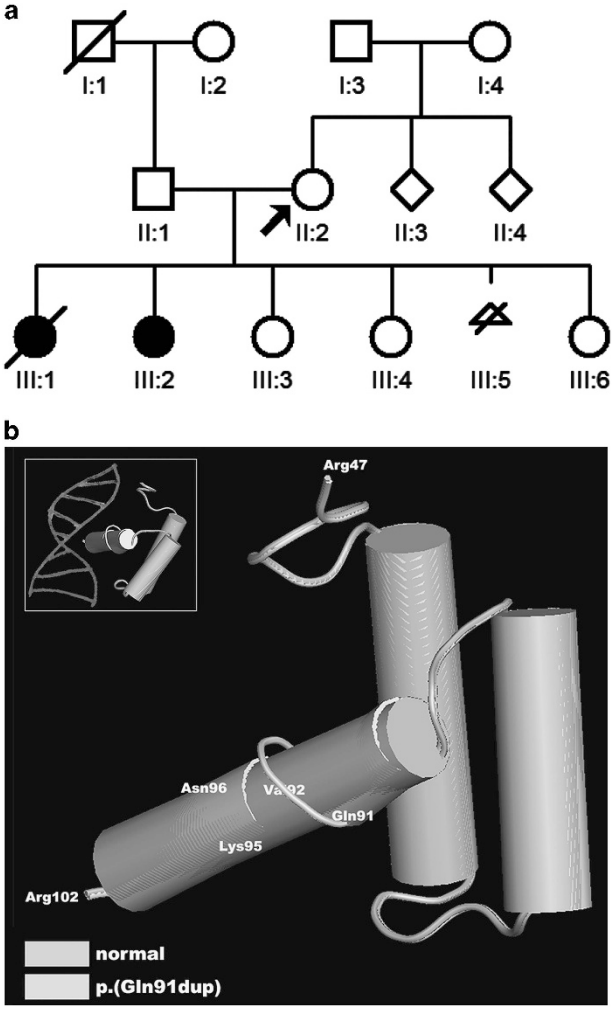

Figure 1 (**a**) Pedigree of the reported family. Subject III:1 was diagnosed with likely agnathia-otocephaly complex during a prenatal ultrasound scan; there was immediate neonatal death after preterm labor at 30 weeks gestation. Subject III:2 has micrognathia and had surgery to correct her hypoplastic left soft palate. For subject III:5, there was medical termination of pregnancy after prenatal ultrasonography revealed recurrence of agnathia-othocephaly complex. Subject II:1 was found to have the c.271_273dupCAG, p.(Gln91dup) mutation but he is unaffected (no palatal abnormalities and just a mild impression of having a small jaw). The proband (II:2) was also unaffected and did not harbor the *OTX2* duplication. (**b**) Predicted protein structure of the OTX2 homeodomain. Overlay of normal (green) and c.271_273dupCAG, p.(Gln91dup) mutant (yellow) protein is presented. Similar to other transcription factors, the OTX2 homeodomain comprises a 60 amino-acid region (corresponding to amino acids 46–105 (NP_068374.1)) in which three α-helices (shown as cylinders) are connected by short loops. The more C-terminal helix of this extremely conserved structure is the longest and lies roughly perpendicular to the other two. It is known as the recognition helix as it confers susceptibility to DNA binding (schematic in top left hand corner panel). Previous functional studies have emphasized the critical role of three residues of the recognition helix in setting sequence-specific protein–DNA contacts.[9] These are valine 92, lysine 95 and asparagine 96 in OTX2 (NP_068374.1; highlighted). The mutation identified in the family adds a glutamine at position 91 and distorts the recognition helix close to these three, critical for DNA recognition, residues (yellow protrusion from helix). It is also possible that an insertion like this will not disturb the helical structure but it will shift all subsequent residues by one position (i.e., one third of the way around the helix). The latter could lead to loss of binding; the side chains will rotate so that the residues pointing out toward the DNA will be pointing in the wrong direction for making interactions. The structural model was generated by using the SWISS-MODEL protein homology modeling server (Swiss Institute of Bioinformatics and the Biozentrum University of Basel, Switzerland). The solution structure of the OTX2 homeobox domain with Protein Data Bank ID 2dms.1 was utilized. PyMOL (PyMOL Molecular Graphics System, Version 1.3r1, Schrödinger, LLC) was used to view the three-dimensional molecular structures. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

Rouen, France). However, it would lead to the insertion of a glutamine residue, within the highly conserved, from fly to human homeodomain region.[9] We have evaluated the physiological significance of this change using homology modeling and the results are presented in Figure 1b.

Over 50 disease-associated variants have been reported in *OTX2* (HGMD, Cardiff, UK, accessed 31 October 2014). These are sparsely distributed throughout the gene and typically result in a protein product with reduced or no function.[1,4] All cases with *OTX2*-related disease reported to date were heterozygotes with 40% known to be *de novo* mutational events. Variable expressivity is common.[3] We report four related cases harboring the same *OTX2* variant and presenting with different phenotypes: one has a normal examination and just a mild impression of having a small jaw (II:1; Figure 2a), one has an abnormally small jaw (Figure 2b) and a hypoplastic left soft palate (III:2) and two were severely affected with AOC (III:5 and III:1; no molecular confirmation in the latter). Significant intrafamilial variability has been previously reported in two families with *OTX2*-related AOC,[5,6] but neither a clinically unaffected individual nor a subject with a phenotype similar to that of subject III:2 have been previously described (Table 1). Notably, incomplete penetrance has been reported in families segregating *OTX2*-associated structural eye defects.[3]

AOC is genetically heterogeneous and mutations in two genes, *PRRX1* (recessive or dominant) and *OTX2* (dominant), explain only a small subset of cases.[5–8] The condition is typically sporadic and often suspected during second trimester ultrasound scans.[7] We have demonstrated the utility of exome sequencing as a means of elucidating the molecular pathology in fetuses with AOC. Knowledge of the genetic diagnosis allows more accurate estimation of the recurrence risk and can guide the reproductive decisions of affected families.

An unusual presentation of *OTX2*-related disease consisting of migrognathia and unilateral soft palate hypoplasia is described. Ongoing work to dissect the major roles and key interactions of this important transcription factor may allow identification of modifier genes and shed light on the mechanisms that underlie variable expressivity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the family for their participation and assistance. This research was supported by the Manchester Biomedical Research Centre and NIHR Greater Manchester: Clinical Research Network.

1 Beby, F. & Lamonerie, T. The homeobox gene Otx2 in development and disease. *Exp. Eye Res.* **111**, 9–16 (2013).
2 Roger, J. E., Hiriyanna, A., Gotoh, N., Hao, H., Cheng, D. F. & Ratnapriya, R. *et al.* OTX2 loss causes rod differentiation defect in CRX-associated congenital blindness. *J. Clin. Invest.* **124**, 631–643 (2014).
3 Williamson, K. A. & FitzPatrick, D. R. The genetic architecture of microphthalmia, anophthalmia and coloboma. *Eur. J. Med. Genet.* **57**, 369–380 (2014).
4 Zielinski, D., Markus, B., Sheikh, M., Gymrek, M., Chu, C. & Zaks, M. *et al. OTX2* duplication is implicated in hemifacial microsomia. *PLoS ONE* **9**, e96788 (2014).
5 Chassaing, N., Sorrentino, S., Davis, E. E., Martin-Coignard, D., Iacovelli, A. & Paznekas, W. *et al. OTX2* mutations contribute to the otocephaly-dysgnathia complex. *J. Med. Genet.* **49**, 373–379 (2012).
6 Patat, O., van Ravenswaaij-Arts, C. M., Tantau, J., Corsten-Janssen, N., van Tintelen, J. P. & Dijkhuizen, T. *et al*. Otocephaly-dysgnathia complex: description of four cases and confirmation of the role of *OTX2*. *Mol. Syndromol.* **4**, 302–305 (2013).
7 Gekas, J., Li, B. & Kamnasaran, D. Current perspectives on the etiology of agnathia-otocephaly. *Eur. J. Med. Genet.* **53**, 358–366 (2010).
8 Donnelly, M., Todd, E., Wheeler, M., Winn, V. D. & Kamnasaran, D. Prenatal diagnosis and identification of heterozygous frameshift mutation in *PRRX1* in an infant with agnathia-otocephaly. *Prenat. Diagn.* **32**, 903–905 (2012).
9 Chatelain, G., Fossat, N., Brun, G. & Lamonerie, T. Molecular dissection reveals decreased activity and not dominant negative effect in human *OTX2* mutants. *J. Mol. Med. (Berl)* **84**, 604–615 (2006).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)