OTX2 microphthalmia syndrome: four novel mutations and delineation of a phenotype

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Abstract

The *OTX2* homeobox-containing transcription factor gene was shown to play a key role in the development of head structures in vertebrates. In humans, *OTX2* mutations result in anophthalmia/ microphthalmia (A/M) often associated with systemic anomalies. We screened fifty-two unrelated individuals affected with A/M and identified disease-causing variants in four families (8%), a higher frequency than previously reported. All four mutations are predicted to result in truncation of normal OTX2 protein sequence consistent with previously reported mechanisms; three changes occurred de novo and one mutation was inherited from an affected parent. Four out of the five *OTX2*-positive patients in our study displayed additional systemic findings, including two novel features, Wolf-Parkinson White syndrome and an anteriorly placed anus. Analysis of the phenotypic features of *OTX2*-positive A/M patients in this study and those previously reported suggests the presence of pituitary anomalies and lack of genitourinary and gastrointestinal manifestations as potential distinguishing characteristics from SOX2 anophthalmia syndrome. Interestingly, pituitary anomalies seem to be more strongly associated with mutations that occur in the second half of *OTX2*, after the homeodomain and SGQFTP motif. *OTX2* patients also show a high rate of inherited mutations (36%), often from mildly or unaffected parents, emphasizing the importance of careful parental examination/testing.

Keywords

Anophthalmia; microphthalmia; OTX2; pituitary

INTRODUCTION

OTX2 belongs to the family of homeobox-containing transcription factors related to the Drosophila gene *orthodenticle* (1). The *Otx* genes were shown to control variable developmental processes and, in particular, to be required for specification of the developing brain (2,3). Complete *Otx2*-deficiency in mice results in early embryonic lethality due to

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abnormal primitive streak formation. *Otx2+/−* mouse embryos show multiple abnormalities in the formation of head structures with phenotypes including anophthalmia/microphthalmia (A/M), micrognathia/agnathia, ethmocephaly, holoprosencephaly, short nose, and acephaly (2,4). Studies in other species also support the conserved head-organizing role of *Otx2* during development (5,6).

Heterozygous *OTX2* mutations in humans were first identified in eight probands affected with anophthalmia/microphthalmia (A/M) along with brain malformations and short stature in some individuals (7); two of these mutations (p.P133T and p.P134A) were later determined unlikely to be pathogenic (8). Since then, thirteen additional families with point mutations in *OTX2* and four patients with gene deletions were reported among individuals affected with A/M and/or pituitary abnormalities (8–15). *OTX2* mutations were determined to explain at least 2–3% of A/M in the first cohorts screened (8). The ocular and other systemic anomalies observed in human patients are consistent with *Otx2* activities during eye and brain development documented in animal models (2,3,16).

Anophthalmia/microphthalmia is observed in 1–3 per 10,000 births and often associated with systemic anomalies (17–19). Besides *OTX2*, several other genetic factors have been implicated in anophthalmia/microphthalmia (19,20) with most genes explaining only a small subset of cases (<1%), the exception being *SOX2* which is mutated in 10–20% of A/M (21–24). Identification of genetic mutations in human patients allows classification of A/M into distinct subtypes based on the specific ocular and/or other systemic features associated with specific genetic causes. Genotype-phenotype correlations, if identified, can be used to target genetic testing and also to guide the diagnosis and management of patients with A/M. In this report, we present four additional *OTX2* mutations identified in syndromic A/M patients and provide analysis of the new and previously reported cases to better define the *OTX2* microphthalmia syndrome.

MATERIALS AND METHODS

Patients were enrolled through the A/M Clinical Registry at Albert Einstein Medical Center, Philadelphia, or the Genetic Studies of Human Developmental Disorders at the Medical College of Wisconsin, Milwaukee. This human study was approved by the Institutional Review Boards of Children's Hospital of Wisconsin and Albert Einstein Healthcare Network. Written informed consent was obtained from all participants and/or parents, as appropriate.

A total of 52 probands were screened; 40 had bilateral A/M and 12 had unilateral A/M. The control population included 96 Caucasian (European Collection of Cell Cultures, Salisbury, UK) and 96 Hispanic (Coriell Institute for Medical Research, Camden, NJ) individuals. The *OTX2* coding region was examined using direct sequencing of genomic DNA, as previously described (25) and using Mutation Surveyor analysis (SoftGenetics, State College, PA); PCR products were obtained with primers: set 1 forward 5'-AAGTTAGTGCTGGAACGTGG-3' and reverse 5'-TCAGTAAATGCCCTGGGACT-3'; set 2 forward 5'-GAAAGAAGCAGCGTGTGTCC-3' and reverse 5'-GATTAAGTGGTGACGGGCAG-3'; set 3 forward 5'-GAGCCTCCCCAACTTTCTTAC-3' and reverse 5'-GAAGCTGGTGATGCATAGGG-3'; set 4 forward 5'-TCAGATCCCTTGTCCACCTC-3' and reverse 5'-TTTGTAGGCCCCTCTAAGGC-3'. Reference sequence NM_172337.1 was used.

RESULTS

Screening of *OTX2* identified novel mutations in four of the 52 probands with anophthalmia or microphthalmia (Table 1 and Figure 1).

Patient 1A is a 25-month-old Caucasian female born at full term with no further details available regarding birth history. She has bilateral microphthalmia and head CT at birth showed very severe optic nerve hypoplasia but normal midline structures and cerebral parenchyma. On physical exam she was noted to have a sacral dimple and an anteriorly placed anus. Her height was normal at the 10th centile, weight was <3rd centile (at the 50th centile for a 13-month-old), and head circumference was at the 3rd centile. Pituitary function studies were not performed. Development was normal with no formal assessment deemed necessary. The patient was found to have a heterozygous duplication mutation, c.136dupA (p.T46NfsX84), resulting in frameshift (Figure 1A). This mutation was not seen in Caucasian controls. Family history revealed that the patient's father (Patient 1B) was affected with unilateral microphthalmia, cataract, optic nerve aplasia, and anterior segment dysgenesis (iris pseudocoloboma and posterior synechiae), lack of vascularization in the retinal periphery and absent papilla of the left eye. His right eye was affected with pallor of the papilla and vascular paucity in the retinal periphery only. His growth and development were normal; head imaging studies were not available. He was found to carry the same mutation seen in his daughter.

Patient 2 is a 5½-year-old Caucasian female born at full term with a birth weight of 3.6 kg (50th–75th centile), length of 50 cm (50th–75th centile), and head circumference of 36 cm (75th centile). She was diagnosed with bilateral clinical anophthalmia. Head CT in the neonatal period identified bilateral dysplastic globes, optic nerve aplasia, and otherwise apparently normal brain structures. The patient's growth was normal, with a height of 110 cm (25th centile), weight of 18.5 kg (25th centile), and head circumference of 49 cm (10th centile). Early developmental milestones were within the normal range: she sat at 6 months, walked independently at 18 months, and spoke three words together at 18 months-2 years. However, at the age of 4 years old she was diagnosed with an autism spectrum disorder by a neurologist and developmental pediatrician due to sensory/tactile issues, echolalia, and lack of functional language. She has severe feeding difficulties, felt to be related to her sensory issues, consisting of choking on solid foods despite normal oral motor cavity and swallowing function. A diagnosis of latent Wolf-Parkinson-White syndrome was made by a pediatric cardiologist due to a history of cold sweats and racing pulse throughout her life. ECG at rest and echocardiogram were normal and there is no family history of similar cardiac events. She was found to have a heterozygous nonsense mutation in exon 5, c. 313C>T (p.Q105X), creating a premature stop codon (Figure 1B). This mutation was not seen in Caucasian or Hispanic controls. Family history was unremarkable; clinical testing of buccal samples did not identify the mutation in either unaffected parent.

Patient 3 is a 20-month-old Caucasian male born at 41 weeks' gestation with a birth weight of 4.1 kg (75th–90th centile), length of 51.5 cm (50th–75th centile), and head circumference of 36 cm (50th centile). He was diagnosed with right clinical anophthalmia and left microphthalmia. Cranial magnetic resonance imaging (MRI) in the neonatal period showed an extremely tiny globe to the right, left severe microphthalmia, bilateral optic nerve hypoplasia, and absent optic chiasms on the right, left severe microphthalmia, bilateral optic nerve hypoplasia, and absent optic chiasms. The patient was diagnosed with hypopituitarism at 9 months of age (treated with thyroxine, hydrocortisone, and growth hormone). Clinical Genetics evaluation at 20 months of age revealed feeding difficulties and failure to thrive, with a eight of 71.5 cm (<3rd centile, 50th centile for a 9-month-old), weight of 8.0 kg (<3rd centile, 50th centile for a 6-month old), and microcephaly with a head circumference of 43

cm (<3rd centile, 50th centile for a 5-month-old). He also had global developmental delay: he was able to sit unsupported and had <10 single words, but was not walking or putting two words together. He was found to have a heterozygous c.456_457delGAinsAT mutation which is predicted to create a premature stop codon (p.W152X) (Figure 1C). This mutation was not seen in Caucasian or Hispanic controls. The family history was unremarkable for similar features, but the parents are 3rd cousins; testing of buccal samples from both parents did not identify this mutation.

Patient 4 is a 4½-year-old Hispanic female born at 41-weeks gestation with a birth weight of 3.2 kg (25th– 50th centile), length of 48 cm (25th centile), and head circumference of 34 cm (25th– 50th centile). Ophthalmology evaluation identified left severe microphthalmia with minimal ocular remnants and right mild microphthalmia with maldevelopment of the retina, choroid, and optic nerve. Cranial MRI at 4 years of age noted bilateral microphthalmia, hypoplastic optic nerves, and a small optic chiasm, along with a small anterior pituitary and absent posterior pituitary glands. Clinical genetics evaluation at this time revealed hypoplastic labia minora, hypotonia, failure to thrive with a height of 91 cm (3rd centile, 50th centile for a 30-month-old) and weight of 13.1 kg (<3rd centile, 50th centile for a 31-month-old), and microcephaly with head circumference of 43 cm (<3rd centile, 50th centile for a 7-month old). She also had significant developmental delay: she sat alone at 8 months, is not yet crawling or walking, and has no meaningful words. The patient was found to have a heterozygous insertion mutation, c.556_557insTATA, resulting in frameshift (p.S186IfsX187) (Figure 1D). This mutation was not seen in Hispanic or Caucasian controls. Family history is non-contributory; clinical testing of buccal samples did not identify this mutation in either parent.

DISCUSSION

In this report, we present four additional families with mutations in *OTX2* which increases the total number of families with *OTX2* alterations to twenty-seven (Table 1). The frequency of *OTX2* mutations in our sample (~8%) is somewhat higher than the previously reported rate (2–3%) (8), which may be due to the inclusion of ocular anomalies other than A/M, such as coloboma, within the initial cohorts. This higher frequency further underscores the importance of clinical testing for *OTX2* mutations in A/M patients. *OTX2* represents the second most common cause of anophthalmia/microphthalmia after *SOX2*, which explains up to 20% of this condition (23–24).

The main phenotype seen in *OTX2*-positive patients in our study is anophthalmia/ microphthalmia associated with additional ocular and non-ocular defects such as optic nerve hypoplasia, brain malformations and pituitary abnormalities; short stature, developmental delay/mental retardation, and feeding difficulties were also observed. Previously unreported features include Wolf-Parkinson White syndrome in one patient and an anteriorly placed anus in another affected individual. These characteristics may represent coincidental findings; it is also possible that they may be low-penetrance features associated with *OTX2* deficiency but support from additional mutation screens and/or expression studies is needed (*OTX2* transcripts were reported in human gastrointestinal tract tissues (Genome Browser: http://genome.ucsc.edu)). All families in our study displayed inheritance consistent with a dominant pattern with three de novo alterations and one mutation inherited from an affected parent.

The OTX2 protein contains a paired type homeodomain (amino acids 38–97); two regions, SGQFTP (128–133) and SIWSPA (150–155), which are highly conserved within OTX family members; and tandemly repeated conserved OTX tail motifs (255–263 and 273–281) which were shown to be required for the transactivation activity of the protein (1,26). The

four novel *OTX2* mutations are predicted to result in truncation of normal OTX2 protein at 16% (p.T46NfsX42), 36% (p.Q105X), 53% (p.W152X) and 64% (p.S186IfsX2) of its sequence (Figure 1). Only the first mutation, p.T46NfsX42, is expected to be subject to nonsense medicated decay (27,28). The three remaining mutant proteins are predicted to lack the OTX tail domains; in addition, p.W152X is predicted to be short of SIWSPA motif and p.Q105X is expected to lack the SIWSPA and SGQFTP motifs.

Overall, the newly identified mutations are consistent with the previously reported disease-causing variants, the majority of which result in truncation or lack of protein product. To date, twenty-five different disease-causing variants have been reported with only two missense mutations. Of the remaining variants, eight were nonsense mutations, eleven resulted in frameshift and four were whole gene deletions. Only two recurrent mutations have been reported: a missense mutation, p.N225S, seen in two unrelated patients with combined pituitary hormone deficiency (12) and a nonsense mutation, p.G188X, seen in two unrelated patients with microphthalmia and pituitary abnormalities in one (15) (Table 1). In 35% of families tested, the proband inherited the mutation from a parent. Family 1 is the third case of an *OTX2* mutation inherited from an affected non-mosaic parent; the majority (~2/3) of parents carrying *OTX2* mutations to date have been unaffected due to mosaicism or incomplete penetrence (Table 1). The frequency of unaffected carriers indicates the importance of parental testing even in cases with apparently de novo phenotypes.

We examined the 37 reported cases with mutations in *OTX2* (Tables 1 and 2) in order to determine if any specific traits can distinguish *OTX2*-positive anophthalmia/microphthalmia from A/M caused by other genetic factors. The spectrum of features associated with the major A/M gene, *SOX2*, has been described and includes genitourinary (micropenis, cryptorchidism, kidney defects), gastrointestinal (tracheoesophageal fistula, esophageal atresia, feeding disorder), endocrine (hypopituitarism, abnormal pituitary structure, hypogonadotropic hypogonadism), and brain defects (cavum/absent septum pellucidum, hypoplastic/agenesis corpus callosum, hippocampal abnormalities, hypothalamic hamartoma), developmental delay, especially gross motor, and anophthalmia/microphthalmia (22,24,29). Review of the anomalies linked with *OTX2* mutations (Table 2) demonstrates overlap with features reported in *SOX2* patients, especially ocular, brain, and endocrine abnormalities, while genitourinary and gastrointestinal structural anomalies seem to be more specific to *SOX2* mutations (Tables 1 and 2). The observed similarities in ocular, brain and endocrine defects may be due to the involvement of *OTX2* and *SOX2* in related developmental processes; recent reports have demonstrated functional interactions between *SOX2/Sox2* and *OTX2/Otx2* in directing tissue development and regulation of downstream target genes (20,30).

The most common extra-ocular feature associated with mutations in *OTX2* is abnormal pituitary structure and/or function. Out of 37 total reported *OTX2*-positive patients (from twenty-seven unrelated families), pituitary structure/function was reported as abnormal in eleven, pituitary structure was reported as normal in nine, and the remaining 17 patients had unknown status (head imaging/endocrine evaluation was not reported). While reported pituitary dysfunction was typically associated with short stature, three additional patients were noted to have short stature; specific endocrine evaluation was not reported for these patients, but two of the three were noted to have normal pituitary structure (Table 1). Given these data, there appears to be a strong association between *OTX2* mutations and pituitary abnormalities with more than half of patients with reported pituitary assessment displaying functional/structural pituitary defects and/or short stature (38% of all patients). This is similar to the frequency seen in patients with *SOX2* mutations (29% with pituitary abnormalities and an additional 10% with short stature) (24).

Interestingly, mutations in the second half of the *OTX2* gene (from amino acid 135) appear more likely to be associated with pituitary dysfunction (or short stature) (p≈0.001 based on chi-squared analysis). Data on pituitary structure/function or stature are available for twelve of the thirteen patients with mutations affecting amino acids 135–225; eight have known pituitary abnormalities, two additional patients have short stature, and only two are reported to have normal stature. Data on pituitary structure/function or stature are available for twelve of the twenty patients with mutations earlier in the protein; only one has a confirmed pituitary dysfunction, one has short stature with normal pituitary structure, and ten have reported normal stature (Table 1). Based on location, all of the mutations in the second portion and at least half of the mutations in the first portion of the gene are not expected to be subject to nonsense mediated decay (NMD) (27,28) making this an unlikely explanation for the observed phenotypic differences. One feature that seems to better differentiate the groups is that mutant proteins associated with mutations in the second half of the gene retain the homeodomain and SGQFTP motif while earlier mutations disrupt one or both of these regions. It may be that retention of these two regions in the absence of other functional domains of OTX2 produces proteins with dominant-negative effects. These dominant-negative mutant proteins may further decrease the functional dosage of OTX2 through interference with wild-type protein activities leading to abnormal pituitary development. While the presence of pituitary anomalies in one patient with a whole gene deletion would argue against a dominant negative explanation, there are several other genes within the deleted region which are also important in pituitary development, suggesting the possibility of an alternate explanation for this patient's pituitary anomalies (9,31). Several studies have investigated the activities of human OTX2 mutations (11–13,15,26) and shown decreased transactivation by the mutant proteins. In terms of dominant-negative effect, although one study identified a dominant-negative effect for the p.N225S mutation associated with pituitary anomalies in two patients (12), most reports describe a lack of dominant-negative effects for studied mutants, including the p.S136LfsX43, p.S135LfsX2 mutations that are associated with pituitary defects (11,13,15,26). The analysis is further complicated by the fact that one mutation, p.G188X, has been found to be associated with a discordant pituitary phenotype in two unrelated patients (15). Identification and study of additional *OTX2* mutations are needed to provide further support for or dispute the association of pituitary defects with mutations in the latter portion of the gene and to determine the explanation for this phenomenon.

Despite the wide overlap, differences between the A/M syndromic phenotypes associated with *OTX2* versus *SOX2* mutations can be seen. Based on the presented analysis, A/M patients with genitourinary and gastrointestinal defects in combination with pituitary anomalies are more likely to have *SOX2* mutations while individuals lacking these associated features and displaying pituitary anomalies are more likely to harbor alterations in *OTX2*. Other associated features do not seem to provide significant aid in discriminating between *OTX2*- and *SOX2*- phenotypes. Ideally, screening of both genes should be performed in a stepwise manner, with the more likely candidate screened first, followed by screening of the second if no mutation is identified. Given the high frequency of inherited mutations in *OTX2*, careful parental examination and testing when a mutation in *OTX2* is detected is critical for accurate counseling.

Acknowledgments

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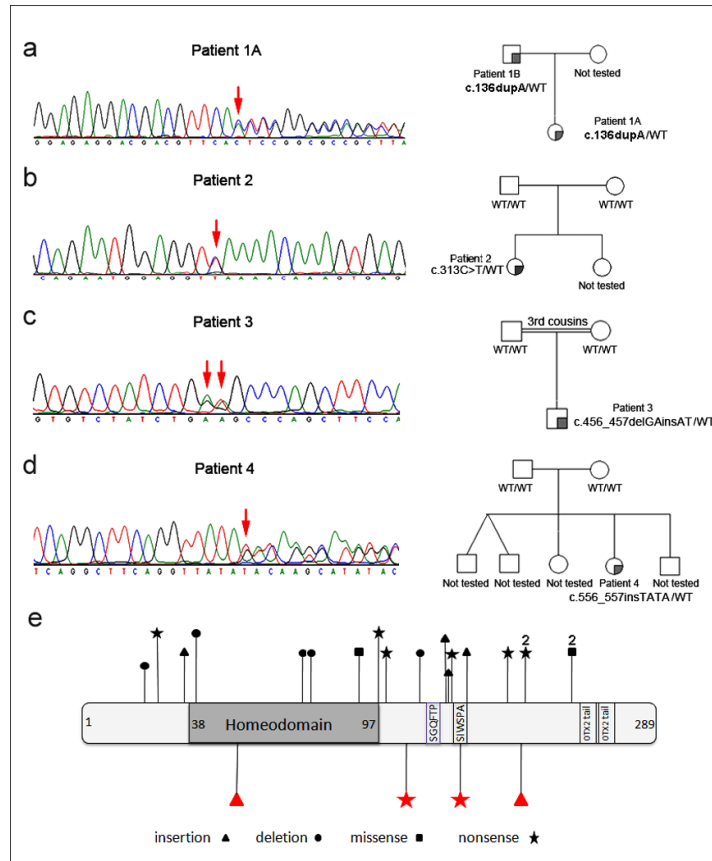
Figure 1.
A–D. Pedigrees and chromatograms corresponding to affected *OTX2* coding sequence for Patients 1 (A and B) through 4. Positions of mutations are indicated with red arrows. *OTX2* genotypes corresponding to tested pedigrees members are shown. **E**. OTX2 diagram showing positions of mutations reported in this manuscript (bottom) as well as the previously reported changes (top); all mutations are unique except for p.N225S and p.G188X, each observed in two unrelated patients (indicated with **2**).

Table 1

Summary of *OTX2* mutations in human patients reported to date. The reference sequence NM_172337.1 was used.

Reference	Nucleotide Mutation*	Amino Acid Mutation	Eye Anomalies					Other Structural Brain Anomaly	Abnormal Pituitary Structure/ Function	SS	DD/ MR	Other features	Family history
			AN	MI	ONH/A	ASD	Other						
Ragge et al. (7)	c.81delC	p.S28PfsX23	-	B	B	-	-	U	U	U	-	-	Unaffected mother mosaic; affected sibling
Ragge et al. (7)	c.81delC	p.S28PfsX23	-	B	U	-	-	Y	U	U	U	Pregnancy terminated	Sibling of above
Wyatt et al. 2008	c.93C>G	p.Y31X	-	L	U	-	R	U	U	U	U	U	Parents DNA normal
Wyatt et al. 2008	c.106dupC	p.R36PfsX52	-	R	U	-	-	U	U	-	-	-	Affected sibling (below), Parents DNA normal; Presumed mosaicism
Wyatt et al. 2008	c.106dupC	p.R36PfsX52	R	-	U	-	L	U	U	-	-	-	Brother of above
Ragge et al. (7)	c.117_118delCC	p.R40GfsX47	B	-	B	-	-	Y	NS	Y	-	-	Unaffected mother carries mutation
Ragge et al. (7)	c.117_118delCC	p.R40GfsX47	-	-	-	-	-	U	U	-	-	-	Mother of above
Patient 1A, this study	c.136dupA	p.T46NfsX42	-	B	B	-	-	-	NS	-	-	Microcephaly, anteriorly placed anus, sacral dimple	Affected father carries mutation
Patient 1B, this study	c.136dupA	p.T46NfsX42	-	L	L	L	B	U	U	-	-	-	Father of above
Dateki et al. 2010	c.214_217delGCACinsCA	p.A72HfsX15	-	B	U	U	U	U	-	-	U	-	Parents' DNA normal
Dateki et al. 2010	c.221_236del	p.K74SfsX30	R	L	U	U	U	-	Y	Y	Y	Retractile testis	Parents' DNA normal
Ragge et al. 2005	c.265C>G	p.R89G	-	B	B	-	B	-	NS	-	-	-	Mother had previous stillbirth; Parents' DNA normal
Wyatt et al. 2008	c.289C>T	p.Q97X	-	B	U	-	-	U	U	U	U	U	Dizygotic twin and father with mutation
Wyatt et al. 2008	c.289C>T	p.Q97X	-	-	U	-	B	U	U	U	U	U	Twin of above

Reference	Nucleotide Mutation*	Amino Acid Mutation	Eye Anomalies					Other Structural Brain Anomaly	Abnormal Pituitary Structure/Function	SS	DD/MR	Other features	Family history
			AN	MI	ONH/A	ASD	Other						
Wyatt et al. 2008	c.289C>T	p.Q97X	U	U	U	U	1	U	U	U	U	"Reduced vision" one eye	Father of above
Ragge et al. (7)	c.295C>T	p.Q99X	B	-	B	-	-	Y	U	U	Y	Seizures	Unaffected father carries mutation
Ragge et al. (7)	c.295C>T	p.Q99X	-	-	-	-	-	U	U	-	-	-	Father of above
Patient 2, this study	c.313C>T	p.Q105X	B	-	B	-	-	-	NS	-	Y	Wolf-Parkinson White syndrome, feeding difficulties	Parents' DNA normal
Wyatt et al. (8)	c.371_372delAG	p.S125Wf sX11	B	-	U	-	-	U	U	U	Y	U	Unaffected mother carries mutation
Wyatt et al. (8)	c.371_372delAG	p.S125Wf sX11	-	-	-	-	-	U	U	-	-	-	Mother of above
Dateki et al. (11)	c.402_403insC	p.S135LfsX2	B	-	B	-	-	-	Y	Y	Y	Cleft palate	Parents' DNA normal
Tajima et al. 2009	c.405_406insCT	p.S136LfsX43	B	-	B	-	-	Y	Y	Y	Y	Microcephaly, small penis, cryptorchidism	Parents' DNA normal
Henderson et al. (10)	c.413C>G	p.S138X	-	-	-	-	B	-	Y	Y	U	Feeding difficulties	Parents' DNA normal
Patient 3, this study	c.456_457delGAinsAT	p.W152X	R	L	B	-	-	-	Y	Y	Y	Microcephaly, feeding difficulties	Parents' DNA normal
Ragge et al. (7)	c.464_465dup	p.S156LfsX23	R	L	B	-	L	Y	NS	-	Y	Microcephaly	Parents' DNA normal
Ragge et al. 2005	c.537T>A	p.Y179X	-	B	U	B	B	U	U	Y	Y	Seizures	Mother mosaic with pigmentary retinopathy; Affected brother and niece
Ragge et al. 2005	c.537T>A	p.Y179X	-	B	-	B	B	-	NS	Y	Y	-	Brother of above, affected daughter (below)
Henderson et al. 2007	c.537T>A	p.Y179X	-	R	U	B	B	Y	U	U	Y	Microcephaly, NF type 1; Mutations in *PAX6* and *NF1*	Daughter of above; mother with NF1 and *PAX6* mutations
Patient 4, this study	c.553_556dupTATA	p.S186IfsX2	-	B	B	-	R	-	Y	Y	Y	Microcephaly, hypotonia, hypoplastic labia minora	Parents' DNA normal
Dateki et al. 2010	c.562G>T	p.G188X	-	B	U	U	U	-	Y	Y	Y	-	Parental testing not reported

Reference	Nucleotide Mutation*	Amino Acid Mutation	Eye Anomalies					Other Structural Brain Anomaly	Abnormal Pituitary Structure/Function	SS	DD/MR	Other features	Family history
			AN	MI	ONH/A	ASD	Other						
Dateki et al. 2010	c.562G>T	p.G188X	-	B	U	U	U	U	-	-	Y	Seizures	Unrelated to above; Parental testing not reported
Diaczok et al. 2008	c.674A>G	p.N225S	U	U	-	U	U	-	Y	U	U	Feeding difficulties	Parental testing not reported
Diaczok et al. 2008	c.674A>G	p.N225S	U	U	-	U	U	-	Y	U	U	Seizures, respiratory distress	Parental testing not reported
Dateki et al. 2010	2.86 Mb deletion incl *OTX2*	whole gene deletion	L	R	U	U	U	-	Y	Y	Y	-	Parents' DNA normal
Wyatt et al. 2008	3.07 Mb deletion incl. *OTX2*	whole gene deletion	-	B	-	-	-	-	NS	-	-	-	Parents' DNA normal
Wyatt et al. 2008	1.28 Mb deletion incl. *OTX2*	whole gene deletion	B	-	U	-	-	U	U	U	Y	Skull fractures, Feeding difficulties	Parents' DNA normal
Nolen et al. (9)	9.66 Mb deletion incl. *OTX2*	whole gene deletion	B	-	B	-	-	Y	Y	Y	Y	Microcephaly, hypotonia, MCA	Parents' DNA normal

AN=anophthalmia, MI=microphthalmia, ONH/A=optic nerve hypoplasia/aplaisa, ASD=anterior segment dysgenesis, SS=short stature, DD/MR=developmental delay/mental retardation, B=bilateral, R=right eye only, L=left eye only, U=unknown/not reported, 1=one eye affected, Y=yes, - = feature not present, NS=normal structure (pituitary), MCA=multiple congenital anomalies

Other' eye anomalies include cataract, coloboma, retinal anomalies, nystagmus, retinal dystrophy, posterior vitreous opacities, persistent pupillary membrane,

*Mutations were numbered based upon *OTX2* cDNA sequence NM_172337.1 transcript variant 2, which is the canonical sequence according to UniProt (http://www.uniprot.org/), with +1 as the A of the ATG start site to allow direct comparison;

No mutations reported by Ragge et al. 2005, p.P133T and p.P134A, were excluded from this table due to their later assertion in Wyatt et al. 2008 (8) that these mutations were unlikely to be pathogenic.

No mutations reported by Dateki et al. 2010, p.T178S and p.A245V, were also excluded due to the authors assertion that they were likely to be rare polymorphisms (15).

Table 2

Frequency of features described in patients with mutations in/deletions of *OTX2*. Only features present in two or more individuals were included.

Feature	Percentage	Frequency/all cases
Anophthalmia/microphthalmia	78%	29/37
Optic nerve hypoplasia	35%	13/37
Anterior segment defects	11%	4/37
Other eye defects	32%	12/37
No eye defects	8%	3/37
Abnormal pituitary structure/function	30%	11/37
Other structural brain anomalies	19%	7/37
Short stature	32%	12/37
DD/MR	46%	17/37
Microcephaly	19%	7/37
Feeding difficulties	14%	5/37
Seizures	11%	4/37
Hypotonia	5%	2/37

Note: not all reported cases were assessed for each feature