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SHORT REPORT

Inherited *PAX6*, *NF1* and *OTX2* mutations in a child with microphthalmia and aniridia

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A girl with aniridia, microphthalmia, microcephaly and café au lait macules was found to have mutations in *PAX6*, *NF1* and *OTX2*. A novel *PAX6* missense mutation (p.R38W) was inherited from her mother whose iris phenotype had not been evident because of ocular neurofibromatosis. Analysis of the *NF1* gene in the proband, prompted by the mother's diagnosis and the presence of café au lait spots, revealed a nonsense mutation (p.R192X). Subsequently an *OTX2* nonsense mutation (p.Y179X) was identified and shown to be inherited from her father who was initially diagnosed with Leber's congenital amaurosis. Since individual mutations in *PAX6*, *OTX2* or *NF1* can cause a variety of severe developmental defects, the proband's phenotype is surprisingly mild. This case shows that patients with complex phenotypes should not be eliminated from subsequent mutation analysis after one or even two mutations are found.
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Introduction

Abnormalities in ocular development can lead to a variety of structural congenital eye defects including anterior segment anomalies and the microphthalmia, anophthalmia and coloboma spectrum.[1–3] These conditions often show reduced penetrance and phenotypic variability and join the growing number of human genetic diseases that cannot easily be classified as single-gene disorders.[4,5] There is increasing recognition that variable penetrance and phenotype are associated with mutations in multiple interacting genes working within common developmental pathways.[5–7] Full clinical assessment and thorough molecular investigation of affected individuals and families are essential for a proper understanding of the underlying developmental biology and genetic interactions.

Here we describe a girl with dominant mutations in three genes that affect eye development, *NF1*, *OTX2* and *PAX6*. *NF1* encodes a multifunctional cytoplasmic signalling protein and is expressed ubiquitously during development, including in early eye tissue.[8] Neurofibromatosis type I (NFI) patients can have a variety of eye anomalies including Lisch nodules, optic gliomas and anterior segment defects.[9] *PAX6* and *OTX2* encode DNA binding proteins that regulate transcription and have highly specific neural and ocular expression patterns.[3] OTX2 has a homeodomain; PAX6 has a homeodomain and a paired domain.[3] *PAX6* mutations typically cause aniridia, but

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some are associated with microphthalmia.[10] *OTX2* mutations cause a range of eye defects varying from relatively mild to bilateral anophthalmia.[11]

Materials and methods

Case reports

The proband (Figure 1) was born at gestational week 38 with a birth weight of 2730 g (90th centile). She was microcephalic (OFC < 3rd centile) and had a microphthalmic right eye and a fixed dilated left pupil. Ophthalmological examination soon after birth revealed right microcornea (3-mm diameter which compares to a normal cornea diameter at birth of approximately 10 mm) with loss of the central portion of the iris, prominent ciliary processes, inferior dislocation of the lens and a cataract. The left eye had a normal sized cornea (10.5-mm diameter), typical aniridia with an iris remnant and a clear lens. The left fundus showed a vertically oval optic disc, foveal hypoplasia, retinal white spots near the macula and a possible temporal epiretinal membrane. Nystagmus was present. Later assessment indicated left hypermetropia with acuity of 6/48 and no vision in the right eye. In the first year, multiple café au lait macules and axillary freckling developed which, together with a family history, provided a clinical diagnosis of NFI.

At age 5 her global developmental age was about 4 years. She attends a mainstream school with a unit for children with special needs. There were no behavioural difficulties and no specific language or motor abnormalities.

On MRI brain scan, which was performed at the age of 3, the pineal gland was not visible and the foramina of Magendie were enlarged. Absence of the pineal gland is associated with *PAX6* mutation.[12]

The proband's mother had NFI (Figure 1) with typical eye defects comprising retinal fibroma, optic nerve glioma and gross Lisch nodules on the iris. Both eyes were of normal size but had small corneas (right 9.5-mm diameter, left 10-mm diameter; these compare to a normal adult cornea diameter of approximately 12 mm), cataracts, optic nerve hypoplasia and nystagmus. There was mild iris stromal hypoplasia with normal-sized pupils. Visual acuity at 10 years of age was 6/24 for each eye although this has deteriorated due to cataracts and optic glioma.

The proband's father (patient 4b in Ragge *et al*[11]) was diagnosed with Leber's congenital amaurosis (LCA). He had typical LCA retinal features of pale optic discs, thin vessels, atrophic maculae, mid-peripheral pigment clumps and nystagmus. The proband's father also had features atypical

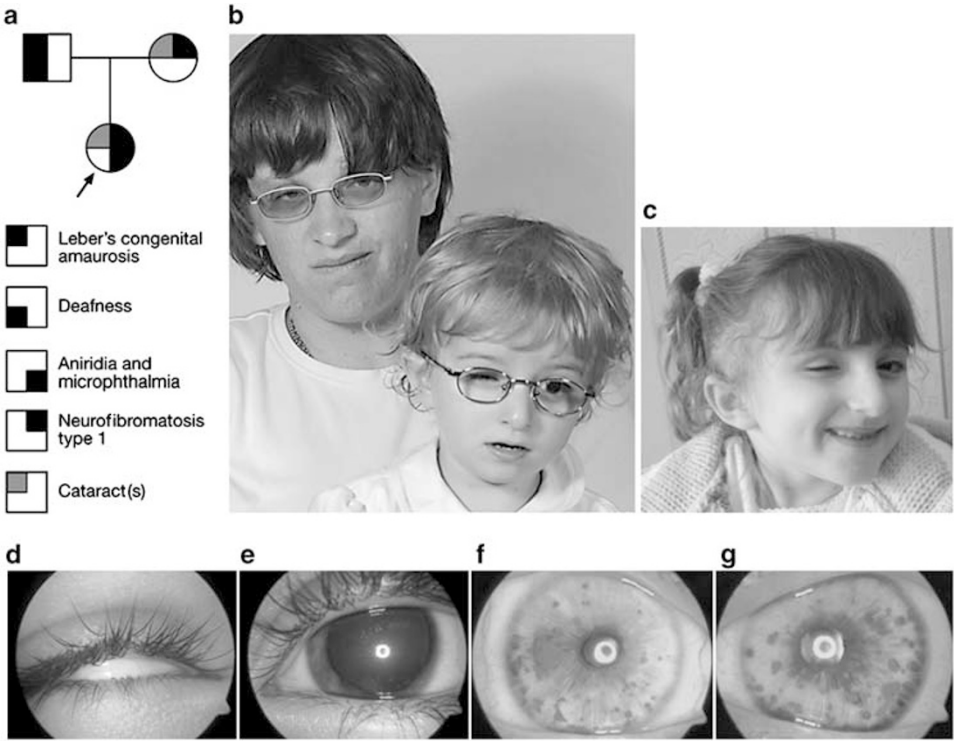

Figure 1 Phenotypes of the proband (indicated with arrow) and her mother. (**a**) The pedigree, showing the proband inheriting neurofibromatosis and cataracts from her mother. (**b**) The mother, with small neurofibroma-like skin lesions, and the proband, aged 2.5 years, with right microphthalmia. (**c**) The proband aged 5 years 3 months. (**d**) Microphthalmic right eye and (**e**) aniridia in the left eye of the proband. (**f**) Right and (**g**) left eye of the mother, with Lisch nodules overlying visible iris tissue.

of LCA including bilateral mild microphthalmia, mild microcornea (11-mm diameters) and iridocorneal synechiae. Intraocular pressure was normal and visual acuity was 6/60. He was deaf in the right ear probably secondary to childhood meningitis.

Molecular analysis

Genomic DNA was extracted as described in Ragge *et al*[11] and screened for point mutations in the entire coding sequences of *PAX6, NF1* and *OTX2*. Pre-screening of *PAX6* by DHPLC[4] (Transgenomic Ltd, Cramlington, UK) revealed heterozygosity for exon 5 which was analysed by direct sequencing and restriction enzyme digestion.[13] *OTX2* and *NF1* were screened by direct sequencing.[11,14] Sequences were bidirectional and generated from duplicate PCRs. Mutation numbering is in accordance with The Human *PAX6* Allelic Variant Database reference sequence (http://pax6.hgu.mrc.ac.uk/) (*PAX6*), GenBank AY796305 (*NF1*) and GenBank NM_172337 (*OTX2*).

Results

On the basis of the proband's eye phenotype, her *PAX6* gene was screened and found to contain a heterozygous exon 5 mutation c.474C>T (p.R38W missense mutation; Figure 2a). Since the proband has café au lait spots and her mother has NFI, the proband's *NF1* gene was sequenced, revealing a heterozygous mutation in exon 4b (c.574C>T; p.R192X nonsense mutation; Figure 2b). The proband's DNA was also included in a large-scale screen for *OTX2* mutations which uncovered a heterozygous exon 3 change c.708T>A (p.Y179X nonsense mutation, Figure 2c). The *OTX2* mutation was not observed in 96 ethnically matched controls. The frequencies of the *PAX6* and *NF1* mutations in the general population are unknown.

From analysis of genotype and phenotype, the *NF1* and *PAX6* mutations were maternally inherited (Figure 2d) and the *OTX2* mutation was paternally inherited. The father, his sister and his mother all have the *OTX2* mutation (family 4 in Ragge *et al*[11]). The father's sister is blind, has severe bilateral microphthalmia, profound developmental delay and seizures. The father's mother, who has retinopathy with pigment clumps, is a gonosomal mosaic, with a low level of the *OTX2* mutant allele in her blood DNA.[11]

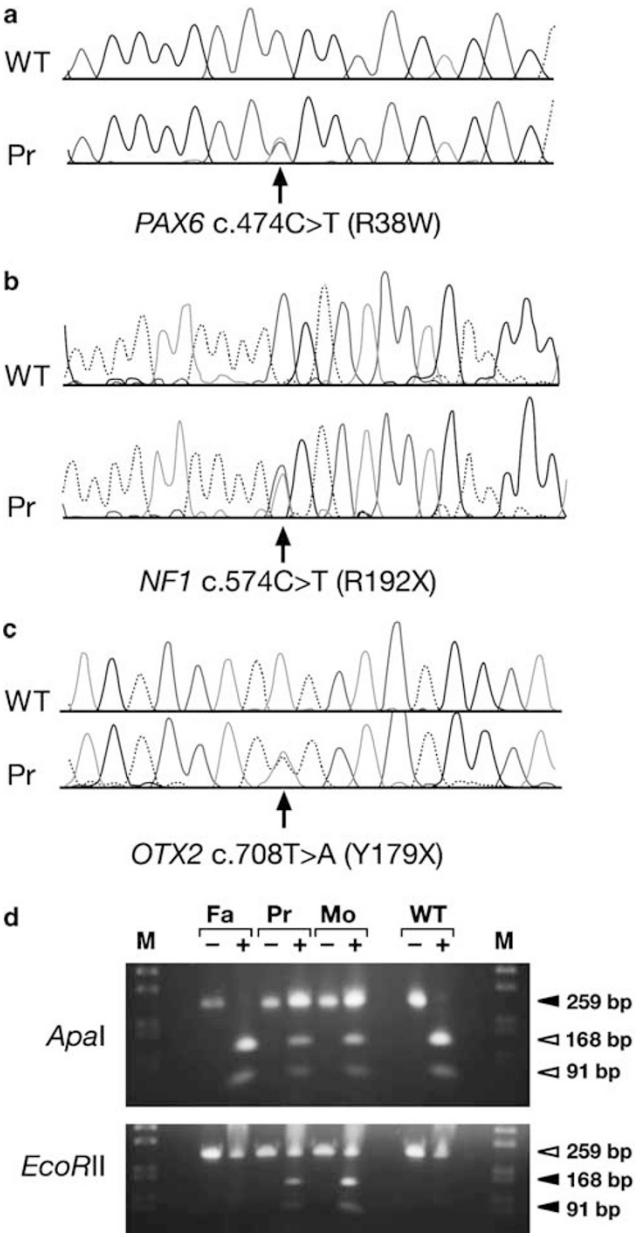

Figure 2 Heterozygous mutations in *PAX6*, *NF1* and *OTX2*. (**a**) *PAX6* exon 5 C>T transition (arrow) in the proband. (**b**) *NF1* exon 4b C>T transition (arrow) in the proband. (**c**) *OTX2* T>A transversion (arrow) in the proband. (**d**) *PAX6* exon 5 PCR products undigested (−) and digested (+) with *Apa*I (top) or *Eco*RII (bottom). Digestion products are indicated as normal (open arrowheads) or mutant (solid arrowheads). The proband and mother have both normal and mutant fragments, whereas the father has only normal fragments. WT, wild type control; Pr, proband; Fa, father; Mo, mother; M, molecular size marker.

Discussion

The PAX6 R38 residue is invariant in all known paired domains and directly contacts DNA.[13] The highly non-conservative substitution of arginine by tryptophan would be predicted to impair PAX6 function. This mutation was also observed in a man with ocular coloboma (unpublished data). The two nonsense mutations may have different consequences. Based on the current model that nonsense-mediated decay (NMD) degrades mammalian mRNAs containing premature stop codons located more than about 50 bases upstream of the last exon–exon junction, NMD is predicted for the *NF1* mutation, creating a functional null, but not the *OTX2* mutation, which is predicted to generate a prematurely truncated protein.[3,11,15]

The proband's mild phenotype is surprising considering the severity of developmental abnormalities associated with single mutations in *PAX6*, *NF1* and *OTX2*. The forebrain patterning gene *Hesx1* is activated by Otx2 and repressed by Pax6,[16] and the reciprocal expression of *Pax6* and *Otx2* in early neural tissue may also indicate mutual regulation.[17] Reduced levels of both proteins may result in amelioration of the associated anomalies.

This family illustrates the importance of rigorous clinical assessment and extensive mutation screening in complex phenotypes.

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