TITLE

An *OTX2* gene mutation causing a more severe retinal phenotype in a female *RPGR* mutation carrier.

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ABSTRACT

This study describes the clinical features of a pedigree with a novel *RPGR* mutation in whom one hemizygous male has a typical manifesting phenotype and three heterozygous females demonstrate a typical carrier phenotype. A forth heterozygous female is described with a strikingly severe retinal phenotype and also harbours an independent disease causing mutation in the *OTX2* gene and an associated systemic phenotype. We hypothesise that the *OTX2* mutation in combination with the familial *RPGR* variant, results in a more severe ocular phenotype than is seen in the other heterozygous females in this pedigree due to a loss of OTX2 mediated photoreceptor protection.

INTRODUCTION

Retinitis Pigmentosa (RP) is known to be the commonest inherited retinal disease leading to significant visual disability by causing nyctalopia, visual field defects and ultimately blindness. The prevalence of RP is about 1:4000 worldwide1. The inheritance of RP can be autosomal recessive, autosomal dominant or X-linked (XLRP). In 2000, Vervoort et al.2 estimated that XLRP represents 11% of all RP patients and suggested that mutations in the retinitis pigmentosa GTPase regulator (*RPGR*) gene( identified in 1996 by Meindle3), account for the disease in over 70% of XLRP cases.

The *OTX2* (Orthodenticle homeobox 2 transcription factor) Homeobox gene4 encodes a transcription factor that is essential for the normal development of the brain, cerebellum, pineal gland, and eye. In the retina, the OTX2 protein is one of the key regulators of photoreceptor genesis and differentiation and is required after birth for bipolar cell terminal maturation.

This study describes the clinical features of a pedigree with a novel *RPGR* mutation in whom one hemizygous male has a typical manifesting phenotype and three heterozygous females demonstrate a typical mild carrier phenotype. A forth heterozygous female is described with a strikingly severe retinal phenotype who also harbours an independent disease causing mutation in the *OTX2* gene and an associated systemic phenotype. We hypothesize that the *OTX2* mutation in combination with the familial *RPGR* variant in our female proband, results in a more severe ocular phenotype than is seen in the other heterozygous females in this pedigree due to a loss of OTX2 mediated photoreceptor protection.

CASE

A 23 year old female born at 32 weeks of gestation was suspected of having Pierre Robin Syndrome due to micrognathia, a cleft palate defect and glossoptosis which resulted in tracheostomy. Genetic testing was performed and a disease causing mutation in the *OTX2* gene was identified and deemed causal (c.130delC). She was subsequently found to have microphthalmia and a possible retinal phenotype as part of her search for associated features. Following identification of a retinal phenotype on examination and by Electroretinography (Great Ormond Street Hospital (GOSH) paediatric protocol) showing rod-cone dysfunction, mainly extramacular, and a family history of suspected RP in her father, further genetic testing targeted towards retinal disease genes identified a heterozygous *RPGR* c.2818G>T p.(Glu940Ter) mutation.

At the age of 2 her visual acuity (VA) measured with Cardiff cards was 0.1 logMAR in both eyes. At 14 her VA in both eyes was 0.2 logMAR, her peripheral and peripapillary retina was noted to be pigmented and nyctalopia was reported. Electrodiagnostic tests (EDTs) performed twice in the last 10 years, revealed marked retinal dysfunction involving both rods and cones. The pattern visual potential evoked potentials (VEPs) were well preserved suggesting that were primarily the extra-macular areas affected.

Segregation studies showed that the *OTX2* mutation was *de novo* and that the *RPGR* variant (*RPGR* c.2818G>T p.(Glu940Ter) Hemizygous) was present in the father (who was known to have retinitis pigmentosa since childhood) and all three sisters were, as expected, obligate carriers. The *RPGR* variant found in this family has not previously been reported in patients with XLRP in the literature, but it is considered to be a likely pathogic variant because the change is predicted to disrupt the normal translation of the *RPGR* gene. Also, as far as the authors are aware, this particular OTX2 mutation has not been reported previously.

Humphrey 120 points screening test at the age of 21 showed a supranasal defect bilaterally, VA was 0.0 logMAR OD and 0.2logMAR OS. Multimodal imaging included colour fundus photographs, fundus autofluorescence (FAF) and spectral domain-optical coherence tomography (SD-OCT). FAF showed symmetrical changes in both fundi with peripapillary hypoautofluorescence extending along the vascular arcades; hyperautofluorescence was noted at the macula surrounding both the isoautofluorescence of the fovea and the perivascular hypoautofluorescence (Figure 1A and 1B; Figure 2A and 2B). SD-OCT of the macula showed fovea plana and loss of the outer retina bilaterally, the left eye also presented an epiretinal membrane (Figure 3B).

The father’s (Figure 1C and 1D) FAF showed a Bull’s eye appearance with a central area of hypoautofluorescence (Figure 2C and 2D), his other 3 daughters (obligate RPGR mutation cariers) were carriers and presented all with excellent VA of 0.0 logMAR, while FAF (Figure 2E to 2J ), SD-OCT and EDTs were all normal despite the mutation and an apparent tapetal-like reflex at fundoscopy (Figure 1E and 1F).

DISCUSSION

SD-OCT and FAF show changes in RP that correlate to clinically evident retinal atrophy and bone spicule pigmentation. SD-OCT in particular demonstrates a clear demarcation where there is loss of the outer nuclear layer (ONL) thickness and loss of the ellipsoid zone (EZ) which correlates with lack of FAF signal due to retinal pigmented atrophy5. Liu et al6 proved that in progressive RP the EZ disappeared from the peripheral part toward the fovea. Our case presents similar features at SD-OCT (Figure 3A and 3B) maintaining normal outer retinal structure only at the fovea.

In 2004 Weigscheider et al7 described FAF in carriers of XLRP associated with mutations in *RPGR*.They found that six of the carriers showed a specific patchy, obvious radial orientation in FAF. On the contrary, none of our female carriers presented this characteristic radial pattern at FAF.

Phenotypic presentation of retinal changes in *RPGR* carrier female patients and their corresponding FAF abnormalities were also described by Nanda et al8. They noticed 4 FAF different patterns: normal (N) representing normal or near-normal FAF appearance; radial (R) pattern reflex without pigmentary retinopathy; focal (F) pigmentary retinopathy and; male (M) phenotype.

Other cases have been reported in the literature with other genetic abnormalities in the *RPGR* gene. A proband female was found to harbour a heterozygous donor splice site mutation in intron 1 (IVS1 + 1G4A) of the *RPGR* gene9. This case and relatives were found to have abnormalities in the perifoveal ring with hyperautofluorescence and with mottled hyperautofluorescence in the peripheral retina of both eyes. This hyperautofluorescent perifoveal ring was also present in our patient, but not in the other 3 sisters.

In a case series10 on X-linked Progressive Cone-Rod Dystrophy, XLCORD, an older affected male showed decreased FAF corresponding to areas of atrophy seen ophthalmoscopically. They described the detailed phenotype of two XLCORD families with novel disease causing *RPGR ORF15* mutations. Clinical findings were consistent with previous reports of CORDX1 phenotypes associated with mutations in *RPGR* ORF15. Important novel FAF data were described that suggests that, in addition to electrophysiology, FAF imaging may be helpful in establishing affected status at an early asymptomatic stage.

Also ultra-wide field (UWF) FAF imaging has been found useful in the diagnosis and monitoring of RP by Hairi11. Specific UWF AF characteristics in RP patients were found to correlate strongly with patient age and stage of the disease. Particular UWF AF characteristics were found to be more prominent in a unique genotype.

A more comprehensive report on phenotipic variation in RPGR carriers has been studied by Talib et al12.

Our patient is the only one among 4 sisters presenting advanced disease and FAF changes. A previous report by Shifera et al9 described an aggressive form of the disease with early onset showing how some carrier females may manifest a more severe phenotype. However, our proband harbours 2 disease causing genotypes in separate genes (heterozygous *RPGR* and *OTX2* mutations) involved in the ocular genesis and vision process and has a significantly more severe phenotype than her sisters who do not harbour the *OTX2* mutation. Therefore, we suggest that it is possible that the *OTX2* mutation acts in consort with the RPGR variant to result in this more deleterious retinal phenotype.

There have been a few reports in the literature associating *OTX2* mutation with retinal dystrophy. One of the earliest *OTX2* gene deletion leading to photoreceptor degeneration and retinal pigment epithelium (RPE) dystrophy was in 2009 by Henderson13 et al.They reported a single de novo mutation in the OTX2 that led to early onset retinal dystrophy and pituitary dysfunction.

Another report in 200914, identified number of genes preferentially expressed in the RPE that are associated with retinal degenerative disease. One of these, BEST1, encodes bestrophin-1, which is a protein that when mutated causes Best macular dystrophy.In this report they also identified a link between reduced promoter activity in OTX2 linked to the dystrophy and suggest that OTX2 may act as positive modulators of the BEST1 promoter in the RPE.

In 2011, while studying OTX2 in medulloblastoma15, OTX2 mutations were found to be associated with structural abnormalities of the pituitary gland and early onset retinal dystrophy.

Beby et al16 used conditional self-knockout in adult mice retina to alter the effect of the *OTX2* gene expression and function and found that the mice that had loss of OTX2 protein developed slow degeneration of photoreceptor cells. This supports the theory that mutations in this gene lead to severe retinal atrophy.

Similarly, some studies have attempted to overexpress the *OTX2* in retinal pigment epithelial cells before their transplantation into the eye of a model of retinitis pigmentosa. The *OTX2* over expression was shown to significantly increase the protection of the photoreceptors. The beneficial effects were noted in the study by Kole17.

In the human, *OTX2* translates into eye malformations of variable expressivity (even between the two eyes of the same individual) and incomplete penetrance18, suggesting the existence of subtle thresholds in OTX2 activity overexpressed OTX2 in retinal pigmented epithelial cells before their transplantation in the eye of a model of retinitis pigmentosa carrying a mutation in *Mertk*, a gene specifically expressed by retinal pigmented epithelial cells. OTX2 significantly increases the protection of photoreceptors as seen by histological and functional analyses. They observed that the beneficial effect of OTX2 is non-cell autonomous, and it is at least partly mediated by unidentified trophic factors. Transplantation of OTX2-genetically modified cells may be medically effective for other retinal diseases involving the retinal pigmented epithelium as age-related macular degeneration.

Furthermore, OTX2 mutations have been proven to cause autosomal dominant pattern dystrophy19 resembling conditional mice models that show slow photoreceptor degeneration secondary to loss of OTX2 function in the adult RPE.

Another report of maculopathy associated with a heterozygous mutation in OTX220 was responsible of a case of atypical hereditary maculopathy. An early onset retinal dystrophy and maculopathy in a 17 year old boy where OTX2 mutations were found. The authors suggested testing for this mutation in the presence of maculopathy , even without rod- cone dystrophy.

Our case presents a severe phenotype as proven by ophthalmic examination, EDTs, FAF and SD-OCT when compared to her siblings. We believe that the more severe phenotype may be due to her additional OTX2 new mutation in association to the familial RPGR genetic mutation. Therefore, that the OTX2 mutation in our proband may have led to digenic RP. We suggest considering OTX2 mutation when genetically testing individuals with rod-cone dystrophy.

FIGURES LEGEND

Figure 1

Colour fundus photographs (CFF) of the patient with RPGR and OTX2 mutation (A right eye and B left eye) showing peripapillary hyperpigmentation and chorioretinal atrophy along the main vascular arcades associated with typical bone spicule pigmentation. C and D are CFF of the father showing peripapillary atrophy and tilted discs with visible large choroidal vessels. E and F show typical tapetal-like reflex CFF of the middle sister.

Figure 2

Fundus Autofluorescence (FAF) of the patient with *RPRG* and *OTX2* mutation (A right eye and B left eye) showing symmetrical changes with peripapillary hypoautofluorescence extending along the vascular arcades; hyperautofluorescence was noted at the macula surrounding both the isoautofluorescence of the fovea and the perivascular hypoautofluorescence. C and D show FAF of the patient’s father with a central hyperautofluorescence symmetric area. E and F show normal FAF of the elderly of the three sisters. G and H, and I and J also show normal FAF of the middle and youngest sister respectively.

Figure 3

Spectral domain optical coherence tomography (SD-OCT) of the macula of the patient with *RPGR* and *OTX2* mutation showing fovea plana and complete loss of the outer retina bilaterally including the ellipsoid zone, the left eye (B) also presented an epiretinal membrane.

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