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LETTERS

De novo mutation in the *BIGH3/TGFB1* gene causing granular corneal dystrophy

Introduction

Allelic mutations in the *BIGH3/TGFB1* gene are responsible for a clinically heterogeneous group of corneal dystrophies inherited in an autosomal-dominant manner. At least seven corneal dystrophies are caused by such mutations and strong genotype-phenotype correlations have allowed classification at the genetic level.¹⁻⁴ Two mutation hotspots exist at codons 124 and 555, and over 95% of granular corneal dystrophy (CDGGI; OMIM no. 121900) cases have been associated with a Arg555Trp mutation.⁵ Patients typically present in the first decade with discrete, grey-white, "crumb-like" granules in the anterior corneal stroma, with sparing of the peripheral cornea and of the stroma between the opacities. We report a nuclear family with a mutation in *BIGH3/TGFB1*, causing granular corneal dystrophy. The daughter presented with granular corneal dystrophy caused by a Arg555Trp mutation in exon 12 of *BIGH3/TGFB1*. Her father also presented with granular corneal dystrophy (but with few corneal opacities) but had no detectable mutation. This family represents the first *de novo* instance of a mutation in *BIGH3/TGFB1* causing granular corneal dystrophy.

Case reports

The proband, an 18-year-old female, presented to the optician at age 18 with mild photophobia and minimally reduced vision. She had no history of recurrent erosions. On examination she had corrected visual acuities of 6/9 R and L and her corneas demonstrated classical granular corneal dystrophy, with numerous discrete midstromal and anterior stromal whitish opacities that did not extend to the limbus (not shown). Her general health was good. The provisional diagnosis was of classical granular corneal dystrophy. There was no family history of granular corneal dystrophy.

Both parents were examined. The proband's mother had a normal corneal examination. The father was asymptomatic with corrected visual acuities of 6/5 R and L. However, on examination he had a small number (<5) of small, midstromal, semi-transparent opacities in both eyes (fig 1). These were not visually significant. He had no significant ocular history of trauma or infection, and had not previously sought specialist ophthalmic advice.

To confirm the diagnosis, DNA was extracted from peripheral leucocytes of the proband. Exon 12 of *BIGH3/TGFB1* was amplified by polymerase chain reaction and directly sequenced and compared with the published NCBI sequence using the Blast2seq tool.⁶ A single heterozygous C to T base pair transition at position 1710 (c.1710C>T, fig 2a) was found that gives rise to a p.Arg555Trp change at the protein level. This mis-sense mutation has previously, and repeatedly, been reported as

the major cause of classical granular corneal dystrophy.

After counselling, both parents gave consent for DNA to be extracted from peripheral leucocytes for both mutation analysis and paternity testing. Analysis of *BIGH3/TGFB1* exon 12 sequence from both parents revealed no mutation and maternity and paternity were confirmed (data not shown). The possibility of mosaicism in the father was evaluated by direct sequencing of subcloned polymerase chain reaction products. All products cloned (n = 23) were found to be wild-type exon 12 sequences. The patient declined any further analysis from other tissues.

Comment

Granular corneal dystrophy is an autosomal dominant disorder characterised by discrete white corneal opacities within the anterior stroma. Onset is within the first decade of life and the deterioration in vision is associated with a progressive opacification of the cornea. The condition is caused by a variety of mutations in the *BIGH3/TGFB1* gene, with the p.Arg555Trp mis-sense alteration being by far the most common. In all previously reported

cases the mutation has been familial and has been inherited in an autosomal dominant fashion.

Here we describe an instance of granular corneal dystrophy in a nuclear family comprising of the proband and her two parents. The proband was confirmed to have classical granular corneal dystrophy caused by a p.Arg555Trp mis-sense mutation in *BIGH3/TGFB1*. Sequencing of both parents, who were asymptomatic, did not reveal the existence of this mutation. Paternity and maternity were confirmed. Importantly, examination of the father demonstrated bilateral semi-opaque midstromal corneal opacities of low frequency. The corneal phenotype and the lack of the c.1710C>T mutation in white blood cell genomic DNA suggests that the father is mosaic for the *BIGH3/TGFB1* mutation observed in the proband. For a mosaic individual, the novel genetic change may be present in some, but not all, of his or her cells. In this case we propose that the c.1710C>T mutation is carried within a proportion of the father's germ cells as well as his corneal cells and possibly at low levels in other tissues. It is of interest that the small number of opacities observed in the father's corneas was present

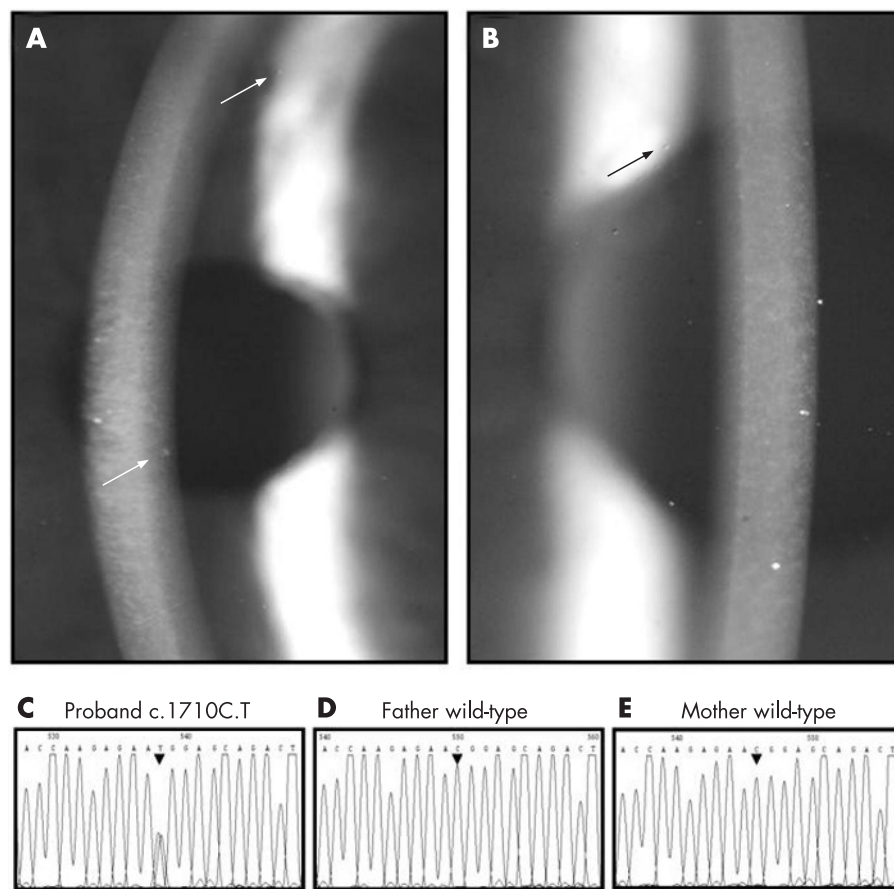


Figure 1 Slit lamp photographs of the left (A) and right (B) eyes of the proband's father. Small white opacities were observed in the anterior cornea at low frequency (arrows). Electropherograms of exon 12 of the *BIGH3* gene. (C) The proband has a heterozygous C to T transition at nucleotide position 1710 (arrow). Sequence obtained from the proband's father (D) and mother (E) was wild type.

within the stroma and that there was no clinical evidence of epithelial disturbance or of disruption at the level of Bowman's layer. This is similar to primary granular dystrophy and lends support to the suggestion that the primary disease may be of stromal (keratocyte) origin.

In the event of mosaicism a genetic change may not be observed in DNA from all tissue types and in this case the c.1710C>T mutation was absent in the affected father's blood leucocytes. As sequencing would normally only identify a mutation if it was present at a level of 10–20% of the total DNA, further sequencing analysis of multiple amplicon clones was performed and confirmed that mutation is unlikely to be present in the father's leucocytes down to a level of 5% of the normal allele. Germline mosaicism would result in vertical transmission of the mutated gene, with offspring displaying 100% penetrance in the case of an autosomal dominant disorder. Furthermore, the risk for the daughter of passing the mutant allele to her children would be 50%, as for any other individual affected by an autosomal dominant condition.

To date, all described cases of granular corneal dystrophy type I have been familial. The family in this study represents the first reported example of *de novo* granular corneal dystrophy type I – importantly, Tanhehco *et al.* have also recently reported the occurrence of two patients with a *de novo* prezygotic Arg124Leu mutation causing granular corneal dystrophy type III (CDB1; OMIM no. 608470).⁷ While the daughter may be considered as the first *de novo* case of true granular corneal dystrophy we propose that the mild phenotype of the father suggests that it is actually he who represents the first case of an individual carrying a *de novo* postzygotic mutation in the *BIGH3/TGFB1* gene.

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Multifocal electroretinography in dengue fever-associated maculopathy

Dengue fever is a viral disease transmitted by *Aedes* mosquitoes and is endemic in the tropics.¹ The severity of dengue fever varies from mild non-specific febrile illness to potentially fatal dengue haemorrhagic fever causing thrombocytopenia and shock. Patients with dengue fever may develop various ophthalmic manifestations causing visual loss, including macular oedema, macular haemorrhage, retinal vasculitis, "cotton-wool" spots and optic disc swelling.^{2–8} We report the use of multifocal electroretinography (mfERG) in the assessment of a patient with dengue fever-associated maculopathy in whom there were no clinical or angiographic abnormalities.

Case report

A 16-year-old girl with serologically confirmed dengue fever presented with left relative scotoma and reduced vision 7 days after the onset of fever. Her visual acuity was 20/20 and 20/40 in the right and left eye, respectively. Anterior segment examination was unremarkable. Fundus examination showed

no abnormality and particularly no retinal haemorrhage. Optical coherence tomography demonstrated normal foveal depression and retinal thickness. Fluorescein and indocyanine green angiographies showed no abnormal leakage (fig 1). Owing to the lack of clinical evidence of maculopathy, mfERG was performed to evaluate the macular function. mfERG demonstrated reductions in both N1 and P1 response amplitudes at the central and nasal macula, corresponding to the scotoma on perimetry (fig 2). The patient was managed conservatively without treatment. After 1 year, her visual acuity remained at 20/40 with absence of fundus abnormality. Repeat mfERG recording showed persistent response abnormalities.

Comment

Ocular manifestations in dengue fever have been reported in several case series, and the commonest fundus findings are retinal haemorrhage and macular oedema.^{2–8} The onset of visual symptoms usually coincides with the resolution of fever and the lowest point of thrombocytopenia.^{6–8} In our patient and in previous reports, the interval between fever onset and ophthalmic symptoms was around 7 days.^{2–8} It has been postulated that this time interval corresponds to the time of antibody formation, deposition of immune complexes or production of autoantibodies.⁶

Visual disturbances in dengue fever-associated maculopathy were suggested to be because of retinal haemorrhages, or retinal and choroidal vasculopathy.^{6–9} In our patient, visual loss developed in the absence of fundus abnormality including retinal haemorrhages or oedema, and without any angiographic abnormality. With the use of mfERG, retinal dysfunction in the central and nasal macula corresponding to the scotoma was detected. As both the N1 and P1 responses were reduced, the findings suggested that dengue fever-associated maculopathy might be due to damage to the photoreceptors or bipolar cells. After 1 year, the mfERG abnormalities were found to be persisting in the absence of any visible fundus changes.

Our mfERG and clinical findings support the hypothesis that formation of autoantibodies against the retina could cause visual loss in dengue fever-associated maculopathy, and that the functional changes could be irreversible. A similar clinical picture and mfERG findings

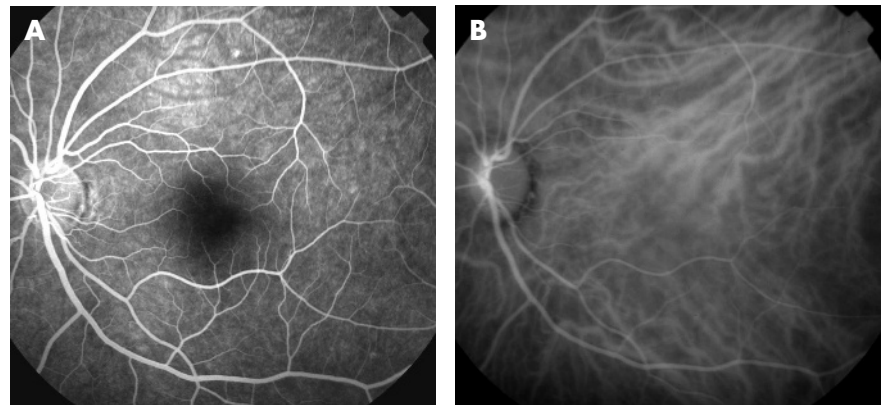


Figure 1 (A) Mid-phase fluorescein angiography and (B) mid-phase indocyanine green angiography of the left eye showing normal retinal and choroidal vasculature and the absence of abnormal hyperfluorescence, hypofluorescence or blocked fluorescence at the macula.