KIF11 mutations are a common cause of autosomal dominant familial exudative vitreoretinopathy

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ABSTRACT

Background/aims To identify KIF11 mutations in patients with familial exudative vitreoretinopathy (FEVR) and to describe the associated phenotypes.

Methods Mutation analysis in a cohort of patients in a single institute was conducted. Bioinformatics was performed for whole exome sequencing, and the variants were confirmed by Sanger sequencing. Clinical data and DNA samples were collected from 814 unrelated Chinese probands, including 34 with FEVR, at the Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Centre, Guangzhou, China.

Results Four novel heterozygous truncation mutations in KIF11, including c.131_132dupAT (p.P45Ifs*92), c.2230C>T (p.Q744*), c.2863C>T (p.Q955*) and c.2952_2955delGCAG (p.G985fs*6), were detected in four of 34 probands with FEVR. Combined with our previously identified mutations in FEVR cases (n=14), KIF11 mutations were identified in 8.3% (4/48) of all probands with FEVR. Ocular phenotypes documented in patients with KIF11 mutations showed a significant great variability of FEVR from the avascular zone in the peripheral retina to bilateral complete retinal detachment. Analysis of available family members in family QT1314 and QT937 showed segregation of KIF11 mutations with the phenotype of FEVR as expected. The family QT964 with two affected siblings and unaffected parents demonstrated a peculiar somatic mosaicism in the mother who had a low copy number variant (about 7% in her leucocyte DNA).

Conclusions Identification of mutations in 8.3% patients suggests KIF11 mutations as a common cause of FEVR. Patients with KIF11 mutations showed typical, but variable, signs of FEVR with or without microcephaly, lymphoedema and mental retardation.

INTRODUCTION

Familial exudative vitreoretinopathy (FEVR, MIM 133780), a rare hereditary disease causing blindness, is characterised by developmental anomalies of the peripheral retinal vasculature. Aberrant vascularisation results in various fundus changes, including non-perfusion in the peripheral retina, straightening of the temporal arcade, retinal neovascularisation, vitreoretinal traction, retrolenticular fibrotic mass, retinal fold and tractional retinal detachment. Variable clinical manifestations are presented in patients with FEVR, ranging from asymptomatic peripheral vascular anomalies to congenital blindness due to complete retinal detachment. FEVR can be inherited as autosomal dominant, autosomal recessive, and X-linked trait, in which the autosomal dominant form is the most common.

Mutations in six genes have been reported to be responsible for FEVR, including FZD4, LRP5, TSPAN12, NDR, ATOH7 and ZNF408. Mutations in these genes have been identified in about 50% cases of FEVR, suggesting a genetic basis for the rest half cases remains to be identified. Mutations in KIF11 were reported to be associated with microcephaly with or without choriotetinopathy, lymphoedema or mental retardation (MLCRD, MIM152950), in which, unclassified chorioretinal dyspla was identified in about half of patients. Recently, KIF11 mutations were first identified in patients with FEVR by Robitaille et al. The purpose of this study was to estimate the contribution of KIF11 mutations in our patient cohort of FEVR and the associated phenotypes.

In this study, potential pathogenic variants in KIF11 were selected from whole exome sequencing on 814 Chinese probands with different forms of eye diseases, including 34 with FEVR. Then, the variants were confirmed by Sanger sequencing and further evaluated in the available family members. Four novel heterozygous truncation mutations were identified, and the associated phenotypes were documented.
Whole exome sequencing
Genomic DNA from the 814 probands was initially analysed by whole exome sequencing, which was performed by Marogen (http://www.marogen.com). Briefly, genomic DNA was captured using the Illumina TruSeq Exome Enrichment Kit (62 M) and exome-enriched DNA fragments were sequenced on the Illumina HiSeq2000. The average sequencing depth was 125-fold. The high quality reads were mapped to the human genome reference sequence hg19 using BWA (http://bio-bwa.sourceforge.net) and variants were detected through SAMtools (http://samtools.sourceforge.net).

Variants analysis
Initially, all variants in KIF11 were collected from the data based on whole exome sequencing of 814 probands. Multi-bioinformatics analysis was performed to filter the potential pathogenic variants as follows: (1) excluding variants in non-coding regions and synonymous variants in exonic regions without affecting splicing site predicted by the Berkeley Drosophila Genome Project (http://www.fruitfly.org/seq_tools/splice.html); (2) excluding variants with minor allele frequency >0.01 by Exome Aggregation Consortium (http://exac.broadinstitute.org/gene/ENSG00000138160); (3) excluding variants predicted to be benign by both PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant From Tolerant (Table 1).

Sanger sequencing
Candidate pathogenic variants in KIF11 were validated by Sanger sequencing. The targeted fragments containing variants were amplified by a touchdown PCR as previously described. The primers were designed using the online tool Primer3 (http://primer3.ut.ee/) (Table 1). The amplicons were analysed using an Applied Biosystems (ABI) 3130 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) with BigDye Terminator cycle sequencing kit V.3.1. Variants were identified as causative,22 and the potential recombinants containing the mutant allele as well as those with the normal allele were counted, respectively. The mutant allele frequency was calculated as the ratio of the number of the recombinants containing the mutant allele to that of the total positive ones.

RESULTS
Mutations detected
Four novel heterozygous truncation variants in KIF11 were detected through whole exome sequencing in four of 34 probands with FEVR: c.131_132dupAT (p.P45fs*92), c.2230C>T (p.Q744*), c.2863C>T (p.Q955*) and c.2952_2955delGCAG (p.G985fs*6) (Table 1). The detection rate of KIF11 mutations in patients with FEVR was 8.3% (4/48) and 12.9% (4/31) of the probands without the known FEVR gene mutations. These four variants were then confirmed by Sanger sequencing. None of these four mutations were presented in the other 780 inhouse controls. Of the four mutations, two were nonsense and two were frameshift mutations. Of the four families with KIF11 mutations, three families had a familial history of FEVR, and one was an isolated case (Figure 1). Segregation analysis in family QT1314 and QT937 suggested that mutations in KIF11 segregated with the phenotypes of FEVR. Two missense variants, c.994A>G (p.I332V) and c.2747A>G (p.D916G), were detected, but considered to be less likely causes of FEVR (see online supplementary Table S1) for the following reasons: (1) the two missense variants presented in four probands with different ocular diseases; p.i332V in one with FEVR and one with cone dystrophy, p.D916G in one with congenital stationary night blindness in whom Nycatalopin c.121delG had been identified as causative,23 and one with glaucoma; (2) segregation analysis showed that the two variants in KIF11 were present in unaffected as well as affected individuals (see online supplementary figure S1).

Table 1 Primers used in Sanger sequencing

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Forward primer (5’-3’)</th>
<th>Reverse primer (5’-3’)</th>
<th>Amplicon (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIF11-E2-94366076</td>
<td>tcctacacaccaaatgcttgct</td>
<td>tcagccacataaagcct</td>
<td>387</td>
<td>65–58</td>
</tr>
<tr>
<td>KIF11-E17-94399620</td>
<td>tcaccaaaaagtctccttcct</td>
<td>agaagaacacactagctaag</td>
<td>469</td>
<td>65–58</td>
</tr>
<tr>
<td>KIF11-E20-94409684</td>
<td>acaggtagcaagaagcct</td>
<td>cggttgtgagtgcggtggtggtg</td>
<td>400</td>
<td>65–58</td>
</tr>
<tr>
<td>KIF11-E21-94410186</td>
<td>caaccttaccctgccctcat</td>
<td>gttgtcctgtcctaccaga</td>
<td>531</td>
<td>65–58</td>
</tr>
</tbody>
</table>
Clinical information of the variant carriers was present in the online supplementary table S2 and figure S2.

**Phenotypic characteristics**

The seven patients from four families with KIF11 mutations showed typical signs of FEVR. Clinical data for patients with KIF11 mutations are summarised in table 3. All probands with KIF11 mutations had poor vision in early childhood. Visual acuity of individuals with mutations varied from normal, without any symptoms, to blind, with no response to light. Fundus changes varied significantly in different individuals, ranging from avascular zone of the peripheral retina to severe ocular changes, including temporal dragging of the optic disc, falciform retinal folds, retinal detachment and/or retrolenticular fibrotic masses in affected patients (figure 2). Microcephaly (3SD below the mean) was identified in four patients with KIF11 mutations, and lymphoedema in none. Mental retardation was not present in the two adult patients, QT1314I:2 and QT937I:1, but was present in QT761I:2 (identified by the parents when the patient was 5 years old) while the examination was not available for the remaining four patients.

**Mosaicism detected**

In family QT964, the KIF11 c.2230C>T mutation was present in both siblings with FEVR, but not in the clinically asymptomatic parents with visual acuities of 20/20 and normal fundus examinations. Genotyping and haplotype analysis showed that the mutation KIF11 c.2230C>T might be inherited from the mother (figure 3A). Genetic testing of the mother showed a small variant peak for the mutant allele c.2230C>T (figure 3B), which might further indicate the existence of mosaicism with a low mutant allele frequency. After cloning sequencing, the mutation in 72 resultant clones, c.2230C>T was detected in about 7% (5/72) of the clones isolated from the mother’s leucocyte DNA (figure 3C).

In family QT761, cloning followed by sequencing of 32 clones was performed, but the c.2952_2955delGCAG mutant allele was not detected in any of the clones from both parents’ leucocyte DNA, which suggested a de novo mutation in the proband, but not mosaicism in the parents.

**DISCUSSION**

In this study, four novel KIF11 mutations were detected in four probands with FEVR, but not in the 780 inhouse controls. Segregation analysis in these four families indicated a dominant role of the mutations. These data provide evidence that the KIF11 mutations are the cause of FEVR in these patients.

<table>
<thead>
<tr>
<th>Family no.</th>
<th>Exon no.</th>
<th>DNA change</th>
<th>Protein change</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT1314</td>
<td>2</td>
<td>c.131_132dupAT</td>
<td>p.P45iffs*92</td>
</tr>
<tr>
<td>QT964</td>
<td>17</td>
<td>c.2230C&gt;T</td>
<td>p.Q744*</td>
</tr>
<tr>
<td>QT937</td>
<td>20</td>
<td>c.2863C&gt;T</td>
<td>p.Q955*</td>
</tr>
<tr>
<td>QT761</td>
<td>21</td>
<td>c.2952_2955delGCAG</td>
<td>p.G985iffs*6</td>
</tr>
</tbody>
</table>

All the mutations were novel and heterozygous, and the allele frequency of them in FEVR, other ocular diseases and Exome Aggregation Consortium were 1/68, 0/1560 and not present, respectively.

**Figure 1** Pedigrees and Sequence chromatography. The columns from left to right display the family number, pedigree, sequence chromatography from patients and controls. In the pedigrees, M sign represents a variant; +, a normal allele; arrows, probands; squares, males; circles, females; blackened symbols, affected individuals. In the sequence chromatography, the variations are marked with arrows and named under the sequence.
Mutations in KIF11 have been identified in patients with MLCRD, which presented with four common features including microcephaly, mental retardation, lymphoedema and unclassified chorioretinopathy. A recent study identified mutations in KIF11 in a cohort of patients with FEVR. They found that KIF11 mutations were identified in four patients, all of whom showed typical signs of FEVR, two with microcephaly and none with mental retardation or lymphoedema. In our study, KIF11 mutations were identified in seven patients with FEVR. Systemic anomalies were also presented in seven patients, including four with microcephaly and one with mental retardation. The presence of patients without microcephaly, mental retardation or lymphoedema in our study was consistent with the findings of Jones et al. and Robitaille et al. These patients were usually diagnosed as FEVR only, without MLCRD. However, it is important to evaluate the head circumference, lymphoedema and mental development in patients with FEVR for a comprehensive diagnosis. Thus, this study confirms and extends the findings of Jones et al and Robitaille et al that patients with KIF11 mutations can show a milder phenotype and can often be clinically labelled as FEVR.

Mutations in six genes have been reported to cause FEVR. In our previous studies, the mutation frequencies of the three known causative genes were 11.5% (6/52) in LRP5, 9.6% (5/52) in FZD4 and 5.7% (3/52) in TSPAN12, respectively. In the current study, KIF11 mutations were identified in 8.3% (4/}

### Table 3 Clinical information of the patients with KIF11 mutation

<table>
<thead>
<tr>
<th>Patient no./gender</th>
<th>Age at onset/examination</th>
<th>Mutation</th>
<th>First symptom</th>
<th>Visual acuity</th>
<th>Axial length (mm)</th>
<th>Main phenotypes</th>
<th>Ultrasonography</th>
<th>OFC†</th>
<th>Lymphoedema</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT1314I:2/F</td>
<td>6 months/23 years</td>
<td>c.131_132dupAT</td>
<td>NYS</td>
<td>20/250; 20/250</td>
<td>18.99; 17.19</td>
<td>RFM, TDOD, FRF, RPC</td>
<td>NA</td>
<td>−4.1</td>
<td>−</td>
</tr>
<tr>
<td>QT1314I:1/F</td>
<td>4 months/6 months</td>
<td>c.131_132dupAT</td>
<td>NYS</td>
<td>NA, NA</td>
<td>2020; 20200</td>
<td>MC, RFM, TDOD, FRF, RPC</td>
<td>MCOD</td>
<td>−5.5</td>
<td>−</td>
</tr>
<tr>
<td>QT964II:2/F</td>
<td>2 months/5 months</td>
<td>c.2230C&gt;T</td>
<td>CO</td>
<td>NYS</td>
<td>20/500; 20/500</td>
<td>RFM, TDOD, FRF, CA, RPC</td>
<td>MCOD</td>
<td>−1.3</td>
<td>−</td>
</tr>
<tr>
<td>QT964II:1/F</td>
<td>3 months/6 months</td>
<td>c.2863C&gt;T</td>
<td>None</td>
<td>19.77; NA</td>
<td>22.37; 22.37</td>
<td>MC, CO, TDOD, FRF, RPC</td>
<td>NA</td>
<td>−0.1</td>
<td>−</td>
</tr>
<tr>
<td>QT937II:1/F</td>
<td>6 months/6 months</td>
<td>c.2952_2955delGAG</td>
<td>NRLO</td>
<td>NA, NA</td>
<td>22.37; 22.37</td>
<td>NA</td>
<td>NA</td>
<td>Low‡</td>
<td>−</td>
</tr>
</tbody>
</table>

*The visual acuity was obtained when the patients were older than 4 years.
†Head circumference was measured in cm and corrected for age and sex following the discovery of the KIF11 mutations.
‡No measurements was available, but the parents complained microcephaly in the patient.

AZ, avascular zone; CA, chorioretinal atrophy; CO, corneal opacity; FRF, falciform retinal fold; LP, light pursuit; MC, microcornea; MCOD, membrane connected with optic disc; NA, not available; NRLO, no response to light or object; NYS, nystagmus; OFC, occipitofrontal head circumference; RD, retinal detachment; RFM, retrolenticular fibrotic mass; RPC, retinal pigment change; TDOD, temporal dragging of optic disc.

Figure 2 Fundus changes of patients with KIF11 mutations. Typical signs of familial exudative vitreoretinopathy (FEVR) included temporal dragging of the optic disc (A, B, E), falciform retinal fold (A, B, E), retinal detachment (C, D) and avascular zone in the peripheral retina (F). Retinal pigment changes (A, B, E) and chorioretinal atrophy (E) were present. The patient ID number is marked at the bottom left of each picture. OD and OS represent right and left eyes, respectively.
FEVR families with children who have clinically defined de novo mutations and with a family history counterintuitive for autosomal dominant inheritance. Accurate genetic counselling is necessary to avoid the recurrence of the same genomic disorder.

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Contributors QZ conceived and designed the study. XX, SL, XJ, XG and QZ contributed in collecting samples. HH, XX, SL, XJ and QZ participated in analysing and interpreting data. HH drafted the manuscript. QZ, XX, SL, XJ and XG revised the manuscript critically for important intellectual content. All authors approved the final version.

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Competing interests None declared.

Patient consent Obtained.

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REFERENCES