Germline Mutations in CTNNB1 Associated With Syndromic FEVR or Norrie Disease

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PURPOSE. Germline and somatic mutations in CTNNB1 have been found in different types of human diseases. This follow-up study aimed to identify causative germline mutations in CTNNB1 and their associated ocular phenotypes through a comparative analysis of whole-exome sequencing data.

METHODS. Annotated sequence variations in CTNNB1 were selected from in-house data from whole-exome sequencing of genomic DNA prepared from leucocytes of 3280 unrelated probands with different forms of eye diseases. Potentially pathogenic variants in CTNNB1 were analyzed by multistep bioinformatics analyses. Clinical data from probands with pathogenic variants in CTNNB1 were collected, and potential genotype-phenotype correlations were analyzed.

RESULTS. Eleven rare variants that potentially affect the coding regions of CTNNB1 were detected in 11 of the 3280 samples, and four variants were considered to be potentially pathogenic. All four mutations, namely, c.999delC (p.Tyr333*), c.1104delT (p.His369Thrfs*2), c.1758_1742delinsACA (p.Leu580Thrfs*28), and c.1867C>T (p.Gln623?), were heterozygotes and considered to have a germline origin. Three of the four mutations are de novo mutations, and the status of the remaining mutation is unavailable. All four probands had the same class of closely related ocular diseases: one proband had FEVR, and three probands had Norrie-like retinopathy. The molecular results indicated that three probands showed systemic anomalies, as demonstrated by a follow-up survey, but relevant information for the remaining proband was unavailable.

CONCLUSIONS. The data suggest that germline truncating mutations in CTNNB1 cause autosomal dominant syndromic FEVR or Norrie disease. Patients with mutations in CTNNB1, KIF11, or NDP may have similar or overlapping phenotypes, but this phenomenon needs to be studied further.

Keywords: CTNNB1, mutation, FEVR, Norrie
### MATERIALS AND METHODS

A total of 3280 unrelated probands with different hereditary eye diseases (106 probands with FEVR and 3174 probands with other genetic eye diseases, including retinal degeneration, high myopia, glaucoma, hyperopia, and ocular dysplasia) were recruited to participate in this study. Peripheral blood samples and clinical data were collected from each proband, and genomic DNA was obtained from leucocytes isolated from the peripheral blood samples. In accordance with the tenets of the Declaration of Helsinki, written informed consent was obtained from all the participants or their guardians before the study. This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center.

Whole-exome sequencing of the genome of all 3280 probands was performed by Macrogen (http://www.macro.gen.com/en/main/index.php). In brief, genomic DNA from each proband was captured using an Agilent SureSelect Human All Exon Enrichment V5 Kit (50M; Agilent, Santa Clara, CA, USA), and the exome-enriched DNA fragments were then sequenced using the Illumina HiSeq4000 system (Illumina, San Diego, CA, USA). The average sequencing depth was at least 125-fold. Variant calling and annotation were performed using SAMtools (http://samtools.sourceforge.net/) and ANNOVAR (http://annovar.openbioinformatics.org/en/latest/), respectively.

The variants in CTNNB1 were selected and analyzed through a multistep bioinformatics analysis as follows: low-occurrence variant positions with coverage less than 10 were excluded; variants with a minor allele frequency ≥0.01, as determined based on the dbSNP database, 1000 Human Genome Project, Exome Variant Server (EVS), and Exome Aggregation Consortium (ExAC, Cambridge, MA, USA; http://exac.broadinstitute.org; accessed October 15, 2018), were excluded; the remaining mutation is unknown because of the unavailability of genomic DNA from the parents.

### RESULTS

A total of 11 rare variants that potentially affect the coding regions of CTNNB1 were detected in 11 of the 3280 samples, and these consisted of 5 missense variants, 2 splicing variants, 2 nonsense variants, and 2 frameshift variants. The five missense variants were identified in four patients with glaucoma and one patient with high myopia, whereas the two splicing variants were identified in one patient with retinitis pigmentosa and one patient with high myopia (Supplementary Table S1). The two potentially pathogenic frameshift variants (c.1104delT, p.His369Thrfs*2 and c.1738_1742delinsACA, p.Leu580Thrfs*28) were identified in two probands with FEVR or Norrie-like retinopathy, and the two potentially pathogenic nonsense variants (c.999del, p.Tyr333* and c.1867C>T, p.Gln623*) were identified in two probands with Norrie-like retinopathy (Table 1). The four mutations were classified as loss-of-function (LoF) mutations resulting in protein truncation. This type of mutation is extremely rare in CTNNB1, as determined by a review of the ExAC database (http://exac.broadinstitute.org/), were excluded; and the pathogenic variants identified through a comparative analysis of variants among different groups of diseases were filtered.

A review of the mutations and phenotypes of CTNNB1 was performed to detect potential genotype-phenotype correlations.

### TABLE 1. Four Potential Pathogenic Mutations in CTNNB1 Identified in Patients

<table>
<thead>
<tr>
<th>Hg19 Position</th>
<th>Chr</th>
<th>Hg19 Position</th>
<th>NM</th>
<th>Nucleotide Change</th>
<th>Acid Change</th>
<th>Status</th>
<th>Patient ID</th>
<th>Allele Frequency in Patients</th>
<th>Novel or Known</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr03 41268761 NM_001904.3 c.999del</td>
<td>chr03 41274854 NM_001904.3 c.1104delT</td>
<td>chr03 41277269 NM_001904.3 c.1738_1742delinsACA</td>
<td>chr03 41277903 NM_001904.3 c.1867C&gt;T</td>
<td>p.Tyr353*</td>
<td>p.His369Thrfs*2</td>
<td>p.Leu580Thrfs*28</td>
<td>p.Gln623*</td>
<td>Het</td>
<td>QT2648</td>
</tr>
<tr>
<td>chr03 41274854 NM_001904.3 c.1104delT</td>
<td>chr03 41274854 NM_001904.3 c.1104delT</td>
<td>chr03 41277269 NM_001904.3 c.1738_1742delinsACA</td>
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<td>p.Tyr353*</td>
<td>p.His369Thrfs*2</td>
<td>p.Leu580Thrfs*28</td>
<td>p.Gln623*</td>
<td>Het</td>
<td>QT1615</td>
</tr>
<tr>
<td>chr03 41277269 NM_001904.3 c.1738_1742delinsACA</td>
<td>chr03 41277903 NM_001904.3 c.1867C&gt;T</td>
<td>chr03 41277269 NM_001904.3 c.1738_1742delinsACA</td>
<td>chr03 41277903 NM_001904.3 c.1867C&gt;T</td>
<td>p.Leu580Thrfs*28</td>
<td>p.Gln623*</td>
<td>Het</td>
<td>QT409</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>chr03 41268761 NM_001904.3 c.999del</td>
<td>chr03 41274854 NM_001904.3 c.1104delT</td>
<td>chr03 41277269 NM_001904.3 c.1738_1742delinsACA</td>
<td>chr03 41277903 NM_001904.3 c.1867C&gt;T</td>
<td>p.Tyr353*</td>
<td>p.His369Thrfs*2</td>
<td>p.Leu580Thrfs*28</td>
<td>p.Gln623*</td>
<td>Het</td>
<td>QT1504</td>
</tr>
</tbody>
</table>

Hg19 position refers to human GRCh37/hg19 version from the UCSC genome. Chr, chromosome; Het, heterozygous.

### TABLE 2. Clinical Data of Four Patients With Potential Pathogenic Variants in CTNNB1

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Mutations</th>
<th>Sex</th>
<th>Age at Onset, mo</th>
<th>Best Visual Acuity</th>
<th>Axial Length, mm</th>
<th>Main Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT2648</td>
<td>c.999del</td>
<td>Female</td>
<td>3</td>
<td>NA</td>
<td>14.00</td>
<td>CAT, cataract, CO, corneal opacity; IPS, iris posterior synchia; MC, microcornea; MD, macular dystrophy; NA, not available; NRO, no response to object; OA, optic atrophy; OD, right eye; OP, occlusion of pupil; OS, left eye; PVF, poor vision fixation; RD, retinal detachment; RFM, retrolenticular fibrotic mass; TTOD, temporal dragging of optic disc.</td>
</tr>
<tr>
<td>QT1615</td>
<td>c.1104delT</td>
<td>Female</td>
<td>8</td>
<td>NA</td>
<td>19.00</td>
<td>CAT, cataract, CO, corneal opacity; IPS, iris posterior synchia; MC, microcornea; MD, macular dystrophy; NA, not available; NRO, no response to object; OA, optic atrophy; OD, right eye; OP, occlusion of pupil; OS, left eye; PVF, poor vision fixation; RD, retinal detachment; RFM, retrolenticular fibrotic mass; TTOD, temporal dragging of optic disc.</td>
</tr>
<tr>
<td>QT409</td>
<td>c.[1738_1742delinsACA]</td>
<td>Male</td>
<td>6</td>
<td>NA</td>
<td>17.50</td>
<td>CAT, cataract, CO, corneal opacity; IPS, iris posterior synchia; MC, microcornea; MD, macular dystrophy; NA, not available; NRO, no response to object; OA, optic atrophy; OD, right eye; OP, occlusion of pupil; OS, left eye; PVF, poor vision fixation; RD, retinal detachment; RFM, retrolenticular fibrotic mass; TTOD, temporal dragging of optic disc.</td>
</tr>
<tr>
<td>QT1504</td>
<td>c.[1867C&gt;T]</td>
<td>Male</td>
<td>3</td>
<td>NA</td>
<td>11.80</td>
<td>CAT, cataract, CO, corneal opacity; IPS, iris posterior synchia; MC, microcornea; MD, macular dystrophy; NA, not available; NRO, no response to object; OA, optic atrophy; OD, right eye; OP, occlusion of pupil; OS, left eye; PVF, poor vision fixation; RD, retinal detachment; RFM, retrolenticular fibrotic mass; TTOD, temporal dragging of optic disc.</td>
</tr>
</tbody>
</table>
posterior synechia of iris, or pupil occlusion (Table 2). After
the molecular results were obtained, the four families were re-
contacted. Three of the four probands showed systemic
anomalies, as determined via telephone surveys, and the
remaining proband was lost to follow-up. The proband
QT2648, who is currently 15 months of age, had scoliosis
and experienced difficulty in controlling her head. She was
also unable to speak, and a cranial computed tomography scan
taken when she was 5 months of age showed a local osteolysis
lesion on the left parietal, as determined by a review of her
medical records. Both proband QT1615 and QT1504 are
currently 4 years of age and exhibit autism and learning
disabilities. QT1615 showed microcephaly, with a head
circumference of 40 cm, when she was 9 months of age.

Since mutations in CTNNB1 were identified in patients with
intellectual disabilities in 2012, 245 mutations have been
identified in 56 families with variable diagnoses and pheno-
types (Supplementary Table S2).2–15,24–27 The mutation types
and locations in the 56 families with or without eye
abnormalities are shown in Figure 2.

DISCUSSION

In this study, four truncating mutations in CTNNB1 were
identified in four patients with syndromic FEVR or Norrie
disease but not in patients with other eye diseases. These
results provide additional evidence for an association of
truncating mutations in CTNNB1 with FEVR or Norrie-like retinopathy.

Nonsense and frameshift mutations in CTNNB1 are
associated with FEVR because of several points. First, the
same type of mutations in CTNNB1 were identified in four
unrelated patients with the same class of diseases, namely,
FEVR or Norrie-like retinopathy. Second, neither nonsense nor
frameshift mutations in CTNNB1 were detected in more than
3000 in-house control individuals in our laboratory or more
than 60,000 individuals in the ExAC database. Third, three of
the four mutations are de novo, and fourth, all four mutations
in CTNNB1 identified in the current study are LoF mutations,
which is consistent with the previous finding that most (92.7%,
38/41) mutations in the gene are LoF mutations (Supplemen-
tary Table S2). Moreover, this type of mutation results in the
haploinsufficiency of CTNNB1 by nonsense-mediated mRNA
decay and a conditional knockout mouse model of ctnnb1
established in previous studies showed specific vascular
mutations in CTNNB1 and NDP may cause dominant syndromic FEVR or Norrie disease. Furthermore, phenotypes can be caused by mutations in NDP, which can cause Norrie disease, and occasionally FEVR. Therefore, similar retinal vascular masses secondary to similar vascular dysgenesis of the retina accompanied by mental disorders. Mutations in NDP were identified in patients with syndromic FEVR and Norrie-like retinopathy, and all of them were examined before 1 year of age due to severe visual defects. The follow-up study after the molecular results were obtained showed that three of the four patients exhibited features of patients with FEVR at the age of 3 years. In the current study, four patients with CTNNB1 mutations exhibited FEVR or Norrie-like retinopathy, and all of them were examined before 1 year of age due to severe visual defects. The follow-up study after the molecular results were obtained showed that three of the four patients exhibited features of systemic anomalies, including autism, learning disability, motor delay, and scoliosis. The other patient was not available (QT409) but displayed features of Norrie disease. In addition to this study, two previous studies identified two mutations in patients with FEVR or retinal detachment accompanied by intellectual disability, developmental delay, and microcephaly. Therefore, variable manifestations of FEVR might be part of the phenotypes resulting from CTNNB1 mutations. This trend is similar to that found for mutations in KIF11, which have been identified in patients with syndromic and nonsyndromic FEVR. Some patients with mutations in KIF11 are initially diagnosed with FEVR but found to exhibit mental retardation or microcephaly when reexamined after 17 years of age, potentially because intellectual disability is easily ignored in infants. Moreover, Norrie disease is characterized by fibrovascular masses secondary to similar vascular dysgenesis of the retina accompanied by mental disorders. Mutations in NDP can cause Norrie disease, and occasionally FEVR. Therefore, similar phenotypes can be caused by mutations in CTNNB1, KIF11, and NDP.

In conclusion, we identified four heterogeneous truncating mutations in CTNNB1 in patients with syndromic FEVR or Norrie disease. Our results provide additional evidence for an association between CTNNB1 haploinsufficiency and autosomal dominant syndromic FEVR or Norrie disease. Furthermore, mutations in CTNNB1, KIF11, or NDP might cause similar or overlapping phenotypes, but the mechanism needs to be studied further.

Acknowledgments

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