Novel Recessive Cone-Rod Dystrophy Caused by \textit{POC1B} Mutation

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\textbf{IMPORTANCE} A new form of cone-rod dystrophy (CORD) is described and the gene responsible for the disease is identified.

\textbf{OBJECTIVE} To clinically evaluate 4 patients and 5 control relatives, perform disease gene mapping, and identify the gene defect responsible for CORD.

\textbf{DESIGN, SETTING, AND PARTICIPANTS} Prospective observational case series of 13 members of a consanguineous family and 113 unrelated control individuals.

\textbf{INTERVENTIONS} Clinical investigations included eye examination with color fundus and autofluorescent imaging, spectral-domain optical coherence tomography, and electrophysiologic measurements. Linkage mapping was performed using single-nucleotide polymorphism genotype data. Candidate genes were analyzed for mutations via Sanger sequencing.

\textbf{MAIN OUTCOMES AND MEASURES} Clinical diagnosis of CORD, disease gene mapping, and mutation identification.

\textbf{RESULTS} The onset of CORD occurred in early childhood. The clinical phenotype was typical CORD with photophobia, decreased central vision, and dyschromatopsia. In all patients, a disrupted inner segment/outer segment line and the external limiting membrane were noted as a single blurry line at the central fovea, and the cone outer segment tip line was absent. In the midperipheral retina, the rod inner segment/outer segment line was disrupted and blurry, and the rod outer segment tip line was absent. Cone response was nonrecordable in all patients, whereas rod response was nonrecordable in the eldest patient and subnormal in the others. The Arden Index was abnormal in the youngest patient and flat in the others. The disease gene mapped to a less than 2-megabase recessive locus at 12q21.33 with a logarithm of odds score of 3.92. At the locus, we identified a homozygous missense \textit{POC1B} p.R106P mutation that was predicted as damaging by online tools.

\textbf{CONCLUSIONS AND RELEVANCE} \textit{POC1B} is a novel gene for a new disease typical of CORD except that patients did not report night blindness. The clinical course was slowly progressive. Screening for \textit{POC1B} mutation could benefit families afflicted with CORD.
One-rod dystrophy (CORD) is a rare disease with onset generally in the first decade of life. It is characterized by progressive degeneration of the cone and rod cells, manifesting as an early decrease in central visual acuity, photophobia, color vision defects, and nystagmus, with later development of night blindness (nyctalopia). Clinical heterogeneity of CORD is well known. The differential diagnosis of CORD with cone dystrophy and retinitis pigmentosa (RP) is crucial from a clinical perspective. Photophobia, abnormal color vision, and diminished central vision are known as a clinical triad in CORD. The main symptoms in RP are nyctalopia and progressive peripheral vision loss.

In some patients with retinal dystrophy, the symptoms and clinical manifestations do not allow a definite clinical diagnosis, and electrophysiologic testing usually is helpful. Electroretinography (ERG) shows a markedly decreased photopic (cone) response and a mildly reduced scotopic (rod) response in most patients with CORD. The electrooculogram (EOG) results may be normal in the early stages of CORD, whereas a decrease in the Arden Index, which quantifies slow oscillations, may follow as the disease progresses. In cone dystrophy, photopic ERG is reduced and scotopic ERG is normal, whereas in RP, both the rod and cone ERG responses are decreased.

The Arden Index and fast oscillations of the EOG reflect different metabolic changes in the retinal pigment epithelium (RPE). In most cases of retinal degeneration, the Arden Index is reduced and coincides with reduced responses in ERG. However, in early RP, the Arden Index is normal, but the ERG and fast oscillations are abnormal.

In the present study, we report the clinical and genetic findings in a consanguineous family with CORD. A full clinical evaluation was performed on 4 patients, and 5 of their healthy relatives served as controls. Linkage mapping followed by mutation screening identified a homozygous mutation in a centriolar gene not previously associated with a disease.

**Methods**

**Participants**

Thirteen members of a consanguineous family were investigated (Figure 1). Four members of the family had CORD. Written informed consent was obtained from or for all participants. The Boğaziçi University Institutional Review Board for Research with Human Participants approved the study protocol. The participants did not receive financial compensation.

**Clinical Investigations**

Detailed ophthalmologic examinations included visual acuity, biomicroscopy, intraocular pressure, and fundus examination after pupil dilation by mydriatics. Fundus color and autofluorescence pictures were then taken (Kowa VK-2; Kowa Company Ltd) and visual field examinations were performed (Kowa AP-7000 Automatic Perimeter; Kowa Company Ltd) using center 1 threshold 30 and peripheral 30-60 tests. Spectral-domain optical coherence tomography (SD-OCT) sections of the macula and midperipheral retina were obtained (RTVue, Model RT100; Optovue Inc). Central foveal thickness was manually measured as the distance between the vitreoretinal interface and the posterior edge of the RPE at umbo. Electroretinography and EOG (EP-1000 Pro; Tomey GmbH) were recorded and interpreted according to the guidelines provided by the International Society for Clinical and Electrophysiology of Vision. The Arden Index and fast oscillations were evaluated as described in the literature. Color vision was evaluated with the Ishihara color blindness test. Statistical analysis was performed using SPSS, version 15.0 (SPSS Inc).

**Molecular Genetic Studies**

Samples of DNA from 10 individuals were genotyped (Human 370-Quad BeadChip; Illumina), which included 370 000 single-nucleotide polymorphism markers. Parental consanguinity prompted us to assume a recessive inheritance model. Assuming full penetrance and a disease frequency of 0.0001, we calculated multipoint logarithm of odds (LOD) scores using GeneHunter software, version 2.1r5 (http://www.mybiosoftware.com/population-genetics/4674), on EasyLinkage, version 5.08 (http://www.mybiosoftware.com/population-genetics/4674), including all markers and using marker sets of 100. Because the original large pedigree exceeded the computational limits of the program, a simplified pedigree was used (eFigure 1 in the Supplement).

Genome scans for the 3 individuals who were later included in the study were performed (Human 610-Quad BeadChip; Illumina), which included 610 000 single-nucleotide polymorphism markers. Markers common to both of the chips were selected, and multipoint LOD scores were calculated with SimWalk, version 2.91 (http://www.mybiosoftware.com/population-genetics/1630), using markers at 0.07 centimorgan spacing and in sets of 100. The actual pedigree...
was used (Figure 1) except that individuals 401, 404, and 506 were not included to hasten the process. Even then, the calculations were lengthy and so were performed only for the 4 chromosomes that yielded the highest LOD scores in the initial linkage analysis. Haplotype segregation was investigated via HaploPainter, version 029.5 (http://haplopainter.sourceforge.net/), to assess identity by descent, that is, both of the parental chromosomal segments have possibly descended from the same common ancestor.

Mutational Analysis
Coding sequences of 6 of the genes at the 2 shared homozygosity regions were analyzed for mutations in a patient using Sanger sequencing. Primer sequences are available on request.

The presence of a homozygous deletion was inferred from the single-nucleotide polymorphism genotype data; 2 consecutive single-nucleotide polymorphisms were recorded as no-call in the patients but not in the other family members. To verify the deletion, amplification of a 303–basepair (bp) region within the deduced minimal deletion region was attempted by polymerase chain reaction in the patients and the controls. To narrow the maximal deletion region delineated by the flanking read markers, amplification of arbitrarily selected regions within the maximal deletion region but outside the minimal deletion region was attempted in patients and unrelated control samples. The products were visualized on agarose gels.

All family members and 113 unrelated control individuals from the population were screened for the mutation identified using high-resolution melting curve analysis (LightCycler 480; Roche).

Results
Clinical Findings
The patients and 5 control relatives (405, 501, 502, 506, and 507) underwent a complete ocular examination. According to the family history, none of the patients was blind at birth, but all experienced progressive vision loss later. The clinical manifestations and electrophysiologic findings in the patients are summarized in Table 1 and Table 2, respectively. A photopic response was not detectable, whereas a scotopic response was relatively preserved in the 3 younger patients; no ERG response was obtained in the eldest patient (305) (Table 2). An EOG disclosed a flat Arden Index in 3 patients and an abnormal Arden Index in the youngest patient. In addition, fast oscillations were very high in all patients (Table 2). Thus, the main clinical signs of the disease were decreased: central vision, extreme photophobia, and dyschromatopsia (Table 1).

Color fundus and autofluorescence pictures of the patients and a control relative are presented in Figure 2A, B, and C. We noted moderate bilateral peripheral chorioretinal atrophy, and autofluorescent imaging showed decreased hypoautofluorescence at the perifoveal region in patient 305; asteroid hyalosis of the left eye was the additional ocular finding in this patient. In patient 503, bilateral mild peripheral chorioretinal atrophy and optic disc drusen of the left eye were observed (Figure 2A and B). Results of the fundus examinations and autofluorescence images were normal in patients 504 and 505 (Figure 2C). Visual field examination that was performed in patients 504 and 505 disclosed marked sensitivity reduction in the center 1 threshold 30 test and scotoma in the peripheral 30-60 test (data not shown). Color testing showed that all pa-
Patients could differentiate only the 38th color plate. The control participants had no remarkable clinical features.

We were unable to distinguish the external limiting membrane (ELM) and inner segment (IS)/outer segment (OS) lines at the central fovea; a single blurry line was observed in this region. The cone outer segment tip (COST) line, also known as the Verhoeff membrane, was clearly absent in all patients. In contrast, the COST line was easily distinguishable in all control participants (Figure 2C). Spectral-domain optical coherence tomography of the midperipheral retina disclosed disruption of the rod IS/OS, and the absence of the rod OS tip line resulted in a single blurry line in contrast to the 2 distinct lines.

Figure 2. Color Fundus, Autofluorescence (AF), and Spectral-Domain Optical Coherence Tomography (SD-OCT) Results

A. Color montage fundus photograph of the left eye of patient 503. Peripheral mild chorioretinal atrophy was evident. B. Autofluorescence montage fundus photograph of the same eye. The AF image was normal except for marked hyperfluorescence due to optic disc drusen (arrow) at the inferior margin of the optic disc. C. Clinical results of a control participant and all patients. From left to right: color picture, AF imaging, and SD-OCT results of the 2-mm central macular region. In patient 305, moderate midperipheral chorioretinal atrophy and decreased hypofluorescence during AF imaging at the perifoveal region were noted. The fundus examinations and AF images were normal in patients 504 and 505. The SD-OCT scan shows the external limiting membrane and the inner segment (IS)/outer segment (OS) lines as a single blurry line and that the cone outer segment tip (COST) line is absent in all patients. D. SD-OCT results of the midperipheral retina of a control participant and patients 305 and 505. In the patients, disruption of the IS/OS line resulted in a single blurry line and the rod outer segment tip line (ROST) was absent, whereas the innermost external limiting membrane (ELM) was relatively preserved. RPE indicates retinal pigment epithelium.
at this level in the control participants (Figure 2D). Central foveal thickness was decreased in patients, with a mean (SD) of 174.25 (5.56) μm (95% CI, 165.40-183.10) in 4 patients and 214.0 (3.67) μm (95% CI, 209.44-218.56) in control participants. Patients with these ocular findings received a diagnosis of CORD.

**Genetic Findings**
Initial linkage analysis using the genome scan data of 10 family members yielded the same maximal multipoint LOD score of 2.65 at 4 loci (eFigure 2A in the Supplement). Haplotype analysis showed that all patients, but none of the other participants, were homozygous for the same haplotype only at 12q21.33, which was possibly identical by descent. A second linkage analysis that included the data for the 3 subsequent participants confirmed the result, yielding a significant (>3.0) maximal LOD score (3.92) only at 12q21.33 (eFigure 2B in the Supplement). The maximal region of shared homozygosity in the patients at the locus was approximately 1.14 megabases (Mb), flanked by markers rs12311684(89 435 177 bp) and rs934891(90 571 042 bp). Haplotype analysis around the gene locus revealed a second region with shared homozygosity in the patients, with a maximal length of 784 517 bp and a LOD score of 3.40. This region was delineated by rs2408366(91 192 135 bp) and rs2130402(91 976 653 bp) and was 621 093 bp telomeric to the first one. The haplotypes are presented in the eTable in the Supplement. We concluded that the disease gene was in 1 of those 2 homozygous regions (almost 2 Mb together and separated by a heterozygous region of approximately 621 kilobases). Of the 11 genes in the regions, 4 were pseudogenes (MRPS5P4 [NCBI Entrez Gene NG_002898.2], CENPCT1P1 [NCBI Entrez Gene NG_003044.4], MRPL2P1 [NCBI Entrez Gene NG_002838.3], and LOC100287505 [NCBI Entrez Gene NG_030127.1]) and 1 was a noncoding RNA gene (LOC338758 [NCBI Entrez Gene NR_028138.1]). The remaining 6 genes were protein coding: DUSP6 (OMIM 602748), POCIB (OMIM 614750), GALNT4 (OMIM 603565), and ATP2B1 (OMIM 108731) in the first, larger region, and LUM (OMIM 600616) and DCN (OMIM 125255) in the smaller region; the coding regions of these genes were analyzed by Sanger sequencing in a patient, and a novel sequence variant POCLB c.317G>C (p.R106P) was identified (eFigure 3 in the Supplement). The variant segregated with the eye disease in the family and was not found in the 113 control participants from the population. It is not reported in the Database of Single-Nucleotide Polymorphisms (http://www.ncbi.nlm.nih.gov/snp/) or the National Heart, Lung, and Blood Institute Exome Sequencing Project (http://evs.gs.washington.edu/EVS/) databases. The amino acid substitution was predicted to be damaging to protein function by the PolyPhen-2 online tool (http://genetics.bwh.harvard.edu/pph2) and disease causing by MutationTaster (http://www.mutationtaster.org/). The other 3 variants that we found at the locus were common polymorphisms with frequencies greater than 0.17.

We also identified a novel homozygous deletion at the disease locus. The genome scan results showed that adjacent markers rs12817868(90 488 249 bp) and rs10858953(90 490 259 bp) were read in all participants except the patients, indicating a possible homozygous deletion. The flanking read markers were rs1105501(90 482 636 bp) and rs10777224(90 508 309 bp), suggesting that the deletion was maximally 26 kilobases. We verified the deletion and attempted to identify the break points, but we could narrow the maximal deletion region only to between 90 486 683 bp and 90 492 571 bp because further amplification at this highly adenine-thymine–rich region was not possible. We concluded that the deletion was maximally 5888 bp and minimally 3280 bp.

**Discussion**
In this study, we report a new type of CORD caused by recessive mutation in POCIB, a novel CORD gene. The symptoms of all of our patients included the same triad of decreased visual acuity, severe photophobia, and color vision disturbances typical for CORD. None of the patients reported night blindness, although rod degeneration was apparent in all. Furthermore, our patients stated that they were more comfortable during nighttime compared with daytime and that their vision was better at night. We hypothesize that the extreme photophobia in the patients masks nyctalopia. We did not test any other CORD families for POCIB mutations.

Examination using SD-OCT enables the clinician to analyze the foveal microstructure. In control participants, as expected, 4 separate hyperreflective bands were clearly observed in the outer retina of foveola (Figure 2C). Starting from the innermost layer, these bands are the ELM (zonular adherence between cone IS and Müller cell process),8 the IS/OS line (ellipsoid region of the cone inner segments),9 the COST line (envelopment of the cone OS by the apical processes of RPE, Verhoeff membrane),10 and the RPE. Spectral-domain optical coherence tomography findings correlated well with the clinical findings in our study (Figure 2C and D). At the central and midperipheral retina, a disruption of the IS/OS line was observed, which is a clear indication that not only the cones but also the rods were affected. Results of SD-OCT of the midperipheral retina, scotopic ERG, and visual field examination also showed that rods were affected. We were unable to discern the ELM and IS/OS lines in the patients; instead, a blurry line at the central fovea was observed. The IS/OS line appears to be intact, but its intensity is low in both cone dystrophy and achromatopsia.11 Lima et al12 reported that the ELM and IS/OS lines were intact outside the foveal area in CORD. However, in our patients, we noticed intact ELM but a disrupted rod OS tip line midperipherally. In addition, we noted a moderate decrease of the central foveal thickness by 95% CI in all of our patients. By SD-OCT, although we found signs of rod degeneration in the midperipheral retinal region in our patients, the structural alterations in the central foveal region, including the absence of the COST line and the disappearance of the ELM and IS/OS lines as separate entities, were more marked. Thus, the ERG results were in agreement with the SD-OCT results, showing that cone degeneration was more advanced than rod degeneration.

Cone dystrophies exhibit wide clinical variability.1 The appearance of the fundus could be entirely normal in cone dystrophy as well as in CORD. In patients with stationary cone dystrophy, rod function tends to be normal. However, our patients with CORD exhibited abnormal rod function revealed by the subnormal scotopic ERG. We found disruption of the IS/OS line in

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the midperipheral retinal region, a sign of rod degeneration. In 2 of our patients (504 and 505), both the fundus examination and autofluorescence were entirely normal, but the other 2 patients (305 and 503) exhibited mild to moderate peripheral chorioretinal atrophy. Mild atrophy of the peripheral chorioretinal region, generally a common clinical finding in CORD, was found in only 2 of our patients (305 and 503). Bone spicule pigmentation is a frequent sign of RP as well as some forms of CORD, but none of our patients exhibited such pigmentation. Also, our patients did not have optic disc pallor. We concluded that the clinical phenotype of POC1B mutation is more like CORD than any of the other diseases discussed in the present report.

We mapped the disease gene to a greater than 2-Mb region with interrupted shared homozygosity in the patients. Within the gene locus, we first identified a novel deletion of less than 6 kilobases. No deletion is reported in this region (Database of Genomic Variants; http://projects.tcag.ca/variation). The sequences in the maximal deleted region do not contain part of a gene, so we investigated whether they contained any expressed sequences or predicted gene regulatory elements. We found only 2 computationally predicted transcription factor binding regions but no evidence indicative of a gene regulatory function, such as histone modifications, cytosine-guanine islands and DNase hypersensitivity clusters, or regulation of the expression of distant sequences (eFigure 4 in the Supplement), and concluded that the deletion most likely did not underlie the disease. We then analyzed all coding regions of the genes that were not pseudogenes or hypothetical. The only novel/rare variant that we found was POC1B p.R106P. Substitution of the basic amino acid arginine by nonpolar cyclic proline is expected to have a drastic effect on the structure of the protein. The substitution was predicted by 2 online tools to be damaging to the protein function. In addition, the mutation was not found in the 113 population samples tested, showing with greater than 80% power that it was not a normal sequence variant in the Turkish population. The entire 478 amino acid sequence of the larger POC1B protein isoform is fully conserved between humans and chimpanzees except for the single residue at position 402. Residue R106 is within a stretch of 278 amino acids that are conserved in full among humans, chimpanzees, and rhesus monkeys, a stretch of 20 amino acids that are fully conserved across mammals, and a stretch of 4 amino acids that are conserved in all organisms investigated, including fruit fly and zebrafish (eFigure 5 in the Supplement) (HomoloGene; http://www.ncbi.nlm.nih.gov/homologene). The 7 tryptophan-aspartic acid repeats (amino acids 1–298) in the protein are crucial for the localization of POC1B to basal bodies, and POC1B localizes to basal bodies of primary cilia in human RPE-1 cells. We propose that alteration of the conserved R106 would interfere with proper localization to basal bodies, which in turn would impair cilia formation and phagocytosis in RPE. Thus, the POC1B mutation identified is compatible with CORD. We therefore concluded that the POC1B mutation was responsible for the disease in our patients.

A centriolar protein, POC1B plays a key role in centriole duplication and lengthening and is required for primary cilia formation in human RPE cells. Two other proteins localized to the primary cilium are responsible for recessive retinal dystrophies, C2ORF71 for RP54 and C8orf37 for CORD16 and RP64. All 3 diseases have the common features of bone spicule pigmentation, attenuated blood vessels, and optic disc pallor. Our patients had none of those features.

Conclusions
We have identified POC1B as a new CORD gene and assessed the disease as severe and slowly progressive compared with most other CORDs. The clinical findings for the oldest patient (61 years) provided an opportunity to evaluate the disease as slowly progressive. Our results widen the clinical spectrum of CORD diseases and add to the genes associated with ciliogenesis in RPE. We suggest that screening for POC1B mutation in patients with CORD of an unknown cause, and especially with photophobia, could benefit families with this severe eye disease.
Cystic retinal tuft (CRT) is a developmental vitreoretinal abnormality found in 5% of autopsy eyes. Clinical examination reveals a focal, elevated gliotic lesion in the peripheral retina associated with vitreous traction (Figure, A and B). Histopathological analysis demonstrates a dome-shaped area with internal microcysts, glial cell proliferation, outer retinal degeneration, and photoreceptor loss. Optical coherence tomography reveals a similar configuration with separation of the retina from the retinal pigment epithelium (Figure, C and D). The characteristic intralesional cystic changes on histopathological analysis are not apparent with optical coherence tomography. To our knowledge, optical coherence tomography of CRT has not previously been reported and imaging may be precluded by its peripheral location. The firm vitreous adhesion to a CRT may predispose to retinal tear during posterior vitreous detachment. However, the incidence of retinal detachment from CRT is low and estimated at 0.3%. Thus, prophylactic therapy of asymptomatic CRT is typically not indicated.