**REPORT**

**Isolated and Syndromic Retinal Dystrophy Caused by Biallelic Mutations in \textit{RCBTB1}, a Gene Implicated in Ubiquitination**

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Inherited retinal dystrophies (iRDs) are a major cause of blindness worldwide. They compose a group of genetic eye disorders with a broad phenotypic spectrum and variable age of onset and are caused by progressive degeneration of rod and cone photoreceptors and/or the retinal pigment epithelium (RPE).1 Most iRDs are genetically heterogeneous; mutations have been identified in over 250 genes thus far (RetNet), allowing a molecular diagnosis in heterogeneous; mutations have been identified in over 250 genes thus far (RetNet), allowing a molecular diagnosis in

Inherited retinal dystrophies (iRDs) are a group of genetically and clinically heterogeneous conditions resulting from mutations in over 250 genes. Here, homozygosity mapping and whole-exome sequencing (WES) in a consanguineous family revealed a homozygous missense mutation, c.973C>T (p.His325Tyr), in \textit{RCBTB1}. In affected individuals, it was found to segregate with retinitis pigmentosa (RP), goiter, primary ovarian insufficiency, and mild intellectual disability. Subsequent analysis of WES data in different cohorts uncovered four additional homozygous missense mutations in five unrelated families in whom iRD segregates with or without syndromic features. Ocular phenotypes ranged from typical RP starting in the second decade to chorioretinal dystrophy with a later age of onset. The five missense mutations affect highly conserved residues either in the sixth repeat of the RCC1 domain or in the BTB1 domain. A founder haplotype was identified for mutation c.919G>A (p.Val307Met), occurring in two families of Mediterranean origin. We showed ubiquitous mRNA expression of \textit{RCBTB1} and demonstrated predominant RCBTB1 localization in human inner retina. RCBTB1 was very recently shown to be involved in ubiquitination, more specifically as a CUL3 substrate adaptor. Therefore, the effect on different components of the CUL3 and NFE2L2 (NRF2) pathway was assessed in affected individuals’ lymphocytes, revealing decreased mRNA expression of \textit{RCBTB1} and several NFE2L2 target genes. In conclusion, our study puts forward mutations in \textit{RCBTB1} as a cause of autosomal-recessive non-syndromic and syndromic iRD. Finally, our data support a role for impaired ubiquitination in the pathogenetic mechanism of \textit{RCBTB1} mutations.

Inherited retinal dystrophies (iRDs) are a major cause of blindness worldwide. They compose a group of genetic eye disorders with a broad phenotypic spectrum and variable age of onset and are caused by progressive degeneration of rod and cone photoreceptors and/or the retinal pigment epithelium (RPE).1

In recent years, however, an increasing number of defects have been found in ubiquitously expressed genes playing roles not only in retinal pathways but also in more general pathways, e.g., \textit{DHDDS} (dehydrodolichyl diphasosphate synthase subunit [MIM: 608172]), \textit{HGSNAT} (heparan-alpha-glucosaminide N-acetyltransferase [MIM: 610453]), \textit{MFSO} (major facilitator superfamily domain containing 8 [MIM: 611124]), and \textit{MVK} (mevalonate kinase [MIM: 251170]) (RetNet). The isolated retinal phenotypes can often be explained by hypomorphic mutations resulting in partial loss of function, whereas syndromic phenotypes are caused by more severe mutations.1–5

The initial aim of this study was to unravel the genetic etiology in a Belgian consanguineous family of Turkish origin (F1) in whom two autosomal-recessive traits segregate in two branches (Figure 1). In the first branch, three females present with retinitis pigmentosa (RP [MIM: 268000], the most common iRD), goiter (MIM: 138800), primary ovarian insufficiency (POI [MIM: 311360]), and mild intellectual disability. In the second branch, two individuals display postaxial polydactyly (MIM: 174200), a feature not present in any of the individuals with RP.

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A summary of the retinal manifestations can be found in Figure 2 and Table 1, and the other clinical features are provided in Table 1 and the Supplemental Note (case report S1). This study was approved by the ethics committee of Ghent University Hospital, adhered to the tenets of the Declaration of Helsinki, and obtained informed consent from all participants. Peripheral blood was collected from affected individuals, parents, and unaffected relatives if available. Genomic DNA was extracted from blood leukocytes according to standard procedures.

Identity-by-descent (IBD) mapping in three affected individuals (IV:3, V:1, and V:2) and one unaffected individual (V:3) revealed a single genomic region (hg38 chr13: 41,246,578–52,963,036) that is homozygous in the affected individuals and heterozygous in the healthy sibling (Affymetrix GeneChip Human Mapping 250K). Subsequent whole-exome sequencing (WES; TruSeq Exome Enrichment, HiSeq 2000, Illumina) in two affected individuals (IV:3 and V:1) identified in RCBTB1 (RCC1 and BTB domain containing protein 1 [MIM: 607867]) the homozygous missense variant c.973C>T (p.His325Tyr) (GenBank: NM_018191.3), which is predicted to affect protein function (Figure S1 and Table S1). Segregation of this variant with the disease in the family was confirmed by Sanger sequencing of RCBTB1 (Figure 1). The variant was found to be absent in 142 control individuals, 68 of whom are of Turkish origin. This change is known as rs200826424 in dbSNP and has an overall allele frequency of 0.0091% in the Exome Aggregation Consortium (ExAC) Browser (no homozygotes were observed).

In order to identify additional iRD-affected families with mutations in RCBTB1, we performed targeted next-generation sequencing on the coding region of RCBTB1 as previously described (Table S2) in a Belgian cohort of 281 probands with autosomal-recessive or sporadic iRD. This did not reveal any mutations. Inspection of WES data (Table S3) in ~450 unsolved iRD cases from four cohorts from the European Retinal Disease Consortium revealed homozygous mutations in the probands of five additional families; these individuals display isolated or syndromic iRD with thyroid involvement or sensorineural hearing loss (Table 1).

In all families, the RCBTB1 mutations are the most likely cause of the common retinal phenotype identified by WES (Table S4). All mutations are missense changes, were identified in a homozygous state in the affected individuals, and segregate with disease in the family (Figure 1). RCBTB1 is located in the largest (F1 and F4) or third largest (F3) homozygous region in families in whom IBD mapping was performed (Figure S2). As summarized in Table S1, all mutations have very low minor allele frequencies or are absent in the ExAC Browser, and all are predicted to be deleterious. In family F5, two RCBTB1 variants were identified in cis, and both are present in a homozygous state in the
proband and in a heterozygous state in her unaffected daughter. It is still unclear which of these variants is causal. Both variants have comparable in silico predictions on protein function (Table S1). The c.1151A>G (p.His384Arg) variant affects a highly conserved residue, and protein modeling suggests a disruptive effect, whereas the c.1202C>T (p.Ser401Leu) variant affects a less conserved, surface-exposed residue for which protein modeling is inconclusive (see below).

The c.919G>A (p.Val307Met) mutation was found in two families originating from Italy (F2) and Greece (F3). Segregation analysis with microsatellite markers and SNPs revealed a 3 Mb common haplotype, which suggests a Mediterranean founder mutation (Figure S3).

RCBTB1 has a regulator of chromosome condensation 1 (RCC1)-like domain (RLD) and two broad complex, tramtrack, and bric-a-brac (BTB) domains (UniProt: Q8NDN9).7 Three (F1–F4) and two (F5 and F6) of the mutations are located in the sixth repeat of the RLD (RCC6) and in the first BTB domain (BTB1), respectively (Figure 3). The affected and surrounding amino acids are highly conserved throughout evolution (Figure S4).

The BTB domain is a protein-protein-interaction motif with a high degree of sequence variability. Sequence comparison based on structure superposition of different protein families revealed only 15 significantly conserved residues out of 95 amino acids composing the core BTB, and 12 of them are buried in the monomer core. In contrast, highly variable residues are located on the exposed interaction surface and probably contribute to interaction behavior.15,16 Interestingly, the RCBTB1
The phenotypes associated with RCBTB1 mutations vary from a more severe iRD (i.e., RP) and shared extra-ocular features (goiter, POI, and mild ID) in three F1 individuals to progressive iRD with or without extra-ocular features in seven individuals from five families (F2–F6). The clinical onset of iRD in these families is between 30 and 50 years of age, mostly with decreasing visual acuity and an absence of complaints about the peripheral visual field. Fundus pictures show reticular dystrophy in the retinal periphery and rounded spots of chorioretinal macular atrophy, which enlarge with age. Electroretinography is characterized by moderate alterations of all responses (which worsen with age), indicating loss of both rods and cones. Abbreviations are as follows: hom, homozygous; ID, intellectual disability; POI, primary ovarian insufficiency; iRD, inherited retinal dystrophy; and RP, retinitis pigmentosa.

We built homology models for both RCBTB1 domains. The models predict a deleterious effect for mutations c.919G>A (p.His325Tyr) (F5) and c.1164G>T (p.Leu388Phe) (F6) affect 2 of the 12 highly conserved amino acids. As for the RLD domain, the residues Val307 and Trp310 are both hydrophobic amino acids highly conserved in human RLD superfamily proteins.\(^\text{17}\)

Table 1. Overview of RCBTB1 Mutations and the Associated Phenotypes Identified in This Study

| Family | Origin | Mutation (Zygosity) | Individual | Age of Onset (Years) | Characteristics | Extra-ocular Phenotypic Manifestations |
|--------|--------|---------------------|------------|----------------------|----------------|----------------------------------------|
| F1     | Turkey | c.973C>T (p.His325Tyr) (hom) | V:1        | 17                   | severe iRD compatible with RP | goiter, POI, and mild ID |
|        |        |                      | V:2        | 14                   | severe iRD compatible with RP | goiter, POI, mild ID, recurrent otitis media, psoriasis, and allergy to house dust mites |
|        |        |                      | IV:3       | 18                   | severe iRD compatible with RP | goiter, POI, and mild ID |
| F2     | Italy  | c.919G>A (p.Val307Met) (hom) | II:4       | 40                   | progressive pattern-like reticular dystrophy | none reported |
|        |        |                      | II:5       | 55                   | progressive pattern-like reticular dystrophy | none reported |
| F3     | Greece | c.919G>A (p.Val307Met) (hom) | II:1       | 50                   | central chorioretinal atrophy and peripheral reticular dystrophy | thyroid nodules, cold intolerance, and dyslipidemia; son with son with Hashimoto thyroiditis |
| F4     | Greece | c.930G>T (p.Trp310Cys) (hom) | II:5       | 45                   | central chorioretinal atrophy and peripheral reticular dystrophy | sensorineural hearing loss (adult onset) and spinal ganglioglioma |
|        |        |                      | III:2      | 30                   | central chorioretinal atrophy and peripheral reticular dystrophy | sensorineural hearing loss (adult onset); mother with reported Hashimoto thyroiditis |
| F5     | Algeria| c.1151A>G (p.His384Arg) (hom) and c.1202C>T (p.Ser401Leu) (hom) | II:6       | 48                   | progressive pattern-like reticular dystrophy | lung fibrosis |
| F6     | China  | c.1164G>T (p.Leu388Phe) (hom) | II:2       | 33                   | retinal dystrophy starting with bilateral vision loss; fundus with bilateral irregular pigmentation mainly in the mid-periphery | none reported |

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with three aliphatic sidechains of blade five. p.Trp310Cys therefore introduces a big void between both blades and is probably highly destabilizing. Val307 is part of a hydrophobic core between blades six and seven. The more bulky methionine side chain introduced by p.Val307Met is clashing with residues of blade seven. His325 cannot be modeled accurately because alignments with different methods and against different templates give diverging outcomes for the exact position of this residue. Most likely, His325 is surface exposed at the end of the fourth strand of blade six. p.His384Arg (c.1151A>T) introduces drastic steric clashes of the phenyl ring with surrounding hydrophobic residues. Ser401 is a surface-exposed residue that is either close to or at the edge of the BTB-CUL3 interface, depending on the template that was used. It is unclear whether p.Ser401Leu (c.1202C>T) can disrupt the interaction with CUL3. Homology models were built on the basis of different structure templates with YASARA Structure.9,10 Additional models were built in MODELER and YASARA Structure with alignments based on HHpred and Phyre2.8–12 On the basis of the initial models, the alignments were edited for model improvement, as judged by the DOPE score in MODELER, visual inspection, and Verify_3D 3D profile analysis of the models.9,10 The models are based on template structures of RLD (PDB: 4O2W) and the BTB domain complex (PDB: 4J8Z and 4AP2). The effects of the variants were analyzed in YASARA Structure.9,10Figures were generated with UCSF Chimera.14

Figure 3. Location and Structural Modeling of Identified RCBTB1 Missense Variants

(A) Schematic diagram of RCBTB1 shows the location of the missense variants within two distinct domains: three (F1–F4) in the sixth repeat of the RCC1-like domain (RLD), i.e., RCC6, and two (F5 and F6) in the first BTB domain (BTB1).

(B) A homology model for the β-propeller structure of the RLD is shown in rainbow colors, evolving from blue (N-terminal) to red (C-terminal). The RCBTB1 RLD contains seven repeats that form a seven-bladed β-propeller, in which each blade consists of a four-stranded antiparallel β sheet. The p.Val307Met (c.919G>A), p.Trp310Cys (c.930G>T), and p.His325Tyr (c.973C>T) variants are all found in the sixth blade. Val307 and Trp310 are part of the third strand of blade 6. Trp310 is a conserved aromatic. The bulky indole group of Trp310 is buried in the hydrophobic core between blades five and six and makes extensive Van der Waals contacts between POI and goiter without autoimmunity has not been described before in a known clinical entity.

For the F3 proband, who suffers from thyroid nodules, cold intolerance, and dyslipidemia, no endocrine data are available. In F4, the mother of the proband (II:6) was diagnosed with Hashimoto thyroiditis (MIM: 140300) on the basis of clinical appearance and laboratory findings. Ultrasound showed a multinodal goiter (increased total thyroid volume with multinodular appearance without neoplastic characteristics). TSH and hormones T3 and T4 were within normal limits, but antibodies against TSH and thyroglobulin were elevated.

Because the combination of iRD and thyroid disease is rare, WES data of F1 were also analyzed for the presence of variants in genes important for thyroid function and/or in which mutations are known to cause thyroid disease, as well as genes located in the shared IBD region and predicted to be related to goiter on the basis of gene-prioritization tools (Table S5 and Figure S5). However, we did not identify variants that could explain the thyroid phenotype (Table S6). Despite this extensive variant analysis, we cannot completely rule out the possibility that mutations in other genes cause the non-ocular phenotypes such as thyroid involvement, especially linked mutations in autogamous regions in the case of consanguineous origin.

So far, little is known about the function of RCBTB1. RCBTB1 was initially identified as a candidate gene for chronic lymphocytic leukemia (MIM: 151400) and was shown to activate the pathway for DNA damage and repair.7,20,21 In addition, overabundant RCBTB1 induces cellular hypertrophy in cultured rat vascular smooth muscle and renal proximal tubular cells as an angiotensin II type 1 receptor-associated protein, and a synonymous SNP in RCBTB1 modifies the effect of smoking on carotid intima-media thickness.12,23 In a final stage of this study, haploinsufficiency of RCBTB1 was shown in two families in whom mutations segregate with Coats disease (MIM: 300216) or familial exudative vitreoretinopathy (FEVR [MIM: 133780]). Functional analysis suggested a role for RCBTB1 in retinal angiogenesis through Norrin-induced


Figure 4. Expression Analysis of RCBT1 mRNA

(A) Expression analysis was performed according to the manufacturer’s instructions with an in-house-designed custom array (SurePrint G3 Human Gene Expression array version 2, AMADID 041648, Agilent Technologies) covering all protein-coding genes and 22,980 long non-coding RNA transcripts (LNCipedia version 2.1). Data normalization was performed with the VSN package in R. All values were log2 transformed. Samples included total RNA from whole brain, colon, heart, kidney, liver, lung, breast, and adrenal gland (Stratagene Europe; all adult tissues); cerebellum, brain stem, striatum, frontal cortex, occipital cortex, and parietal cortex (Agilent; adult tissues); and fetal whole brain (Agilent).

(B) qPCR-based expression analysis of mRNA from RCBT1 and two positive control genes strongly expressed in the retina and retinal pigment epithelium (RPE) was performed as previously described on commercial human cDNA from retina (BioChain) and RPE (3H Biomedical). High retinal and limited RPE expression was observed. Error bars represent the SE of the relative quantities.

β-catenin signaling. A clinical overlap with RP was excluded in one of the probands given the absence of night blindness, a typical fundus aspect of FEVR without bony spicules or narrow vessels, and a preserved electroretinogram in one eye. In the families included here, no FEVR signs could be observed, sustaining the hypothesis that distinct molecular consequences of RCBT1 mutations and zygosity cause different clinical entities.

Because of the syndromic phenotypes observed in this study, we explored the expression pattern of RCBT1 human mRNA by analyzing in-house whole-transcriptome expression array data, which showed ubiquitous expression (SurePrint G3 Human Gene Expression array version 2, AMADID 041648, Agilent Technologies) (Figure 4A). Thyroid RCBT1 expression was observed in several experiments centralized in the EMBL-EBI Expression Atlas. The gene was found to be moderately expressed in the cochlea, saccule, utricle, and ampulla of the adult human inner ear. Next, we performed targeted analysis of the expression of RCBT1 and Rcbtb1 mRNA in different human and murine tissues, respectively. RCBT1 mRNA showed relatively high and limited expression in the human retina and RPE, respectively, and Rcbtb1 mRNA showed strong expression in the murine retina, RPE, and ovary (Figure 4B and Figure S6). On the basis of these expression results, staining of RCBT1 was performed on murine and human retinal sections (Figure 5). In the murine retina, RCBT1 was found mainly in the inner retina with strong signals reaching up to the outer plexiform layer (Figures 5A and 5B). In human sections, immunostaining was present in the nerve fiber layer and to a lesser extent in the inner and outer plexiform layers (Figures 5C and 5D). The staining signal in the photoreceptor layer is very likely due to autofluorescence of outer segments, as described before.

The RCC1-like domain is present in several ciliary proteins, whose encoding genes [RPGR (retinitis pigmentosa GTPase regulator (MIM: 312610)), NEK8 (NIMA related kinase 8 (MIM: 609799)), and recently NEK9 (NIMA related kinase 9 (MIM: 609798))] are implicated in Mendelian disease. For RPGR and NEK8, this domain is involved in targeting the protein to the photoreceptor connecting cilium and centrosome, respectively. Hence, co-staining of RCBT1 with acetylated α-tubulin was performed in the retina. However, no clear co-staining was observed (Figure S7).

RCBT1 has previously been shown to be involved in ubiquitination, a post-translational modification with a wide variety of functions, among which is the recognition of proteins for proteasome degradation. In this process, ubiquitin is first activated by an activating enzyme (E1) and then carried by a conjugating enzyme (E2) to a substrate through interaction with a ubiquitin ligase (E3) (Figure S8). RCBT1 was identified as a putative substrate adaptor for cullin 3 (CUL3). CUL3 is the major component of the CULLIN3-RING ubiquitin ligases (CRL3), an emerging class of E3 enzymes regulating a wide range of cellular and developmental processes (Figure S8). Substrate recognition is highly specific and mediated by substrate adaptors such as RCBT1, which recruit substrates to the CRL3 complex. In addition, RCBT1 was shown to interact with UBE2E3, an E2 enzyme that is highly present in the retina and is important for modulating the balance between RPE cell proliferation and differentiation. Recent evidence has shown that UBE2E3 regulates the localization and activity of the stress-response transcription factor NFE2L2 (nuclear factor, erythroid 2 like 2, often called NRF2) in concert with members of the CRL3 complex (Figure S8). The retina is known to be extremely sensitive to oxidative stress. In this way,
NFE2L2 is crucial for protecting and preserving retinal health.37–40 The stress response mediated by CRL3 and NFE2L2 is also important for other organs, such as the thyroid (affected in families F1, F3, and F4) and the ovaries (affected in family F1). The thyroid in particular requires a stringent regulation of the production and removal of reactive oxygen species in the context of normal homeogenesis and thyroid gland growth.41–42 Interestingly, a germline loss-of-function mutation in KEAP1 (kelch like ECH associated protein 1 [MIM: 606016]), encoding a CUL3 substrate adaptor that negatively regulates NFE2L2, has been associated with multinodular goiter.43 In ovarian cells, NFE2L2 is an essential sensor and regulator of chemical homeostasis. NFE2L2-null mice display accelerated ovarian failure after treatment with an ovarian toxicant, and the lack of NFE2L2 results in accelerated ovarian aging.44,45

Here, we have demonstrated co-expression of CUL3 (cullin 3 [MIM: 603136]) and RCBTB1 in the human retina and RPE and co-expression of Cul3 and Rcbtb1 in the murine retina, RPE, and ovary (Figure S6). In addition, Cul3 was found in the human retina (faint) and the murine retina, ovary, and thyroid (Figure S9). In order to assess the molecular consequences of RCBTB1 mutations on the CRL3 complex and the NFE2L2 pathway, we analyzed the mRNA expression of CUL3 and RBX1 (ring-box 1 [MIM: 603814]) (encoding two components of the CUL3 complex), UBE2E3 (ubiquitin conjugating enzyme E2 E3 [MIM: 604151]; encoding the protein interacting with RCBTB1), NFE2L2 (nuclear factor, erythroid 2 like 2 [MIM: 600492]), and a selection of 21 NFE2L2 target genes (Table S7).46,47 Because RCBTB1 is ubiquitously expressed, this analysis was performed on total RNA extracted from peripheral-blood mononuclear cells from two affected individuals from F1 (V:1 and V:2) and six healthy control individuals. Interestingly, we observed significantly lower expression in affected individuals than in control individuals for CUL3, NFE2L2, and three NFE2L2 target genes: RXRA (retinoid X receptor alpha [MIM: 180245]), IDH1 (isocitrate dehydrogenase [NADP(+)] 1, cytosolic [MIM: 147700]), and SLC25A25 (solute carrier family 25 member 25 [MIM: 608745]) (Figure 6 and Table S8). The decreased expression of CUL3 and NFE2L2 can be explained by autoregulatory feedback loops. NFE2L2 is known to positively regulate the expression of CUL3 in order to control its own degradation.48 In addition, NFE2L2 is able to autoregulate its own expression.49 Apart from CUL3 and NFE2L2, some of the NFE2L2 target genes are interesting with respect to the systems affected in the families with RCBTB1 mutations. For example, retinoid X receptor alpha (RXRalpha), encoded by RXRA, is known to be present in the rod inner segment layer, and activation of RXRs prevents photoreceptor oxidative stress-induced apoptosis.50,51 In addition, RXRs form heterodimers with thyroid hormone receptor and can regulate response elements.52 IDH1 is highly expressed in the retina,53 and IDH1 mutations occur in thyroid cancer.54 The downregulation of only a limited number of the selected NFE2L2 target genes could be related to the source of the material and/or the absence of oxidative stress at the moment of RNA extraction.

In addition to regulating NFE2L2, RCBTB1 might exert other functions as well. Ubiquitination plays an important role in retinal development, modulation of the visual cycle, and removal of aberrant or misfolded proteins.55 Pathological accumulation and aggregation of proteins escaping or saturating proteasome degradation is a known iRD disease mechanism.56,57 This hypothesis requires further studies, however, because RCBTB1 substrates are yet to be identified.
So far, only a few genes in which mutations cause iRD are known to play a role in ubiquitination. Mutations in \textit{KLHL7} (kelch like family member 7 [MIM: 611119]), encoding a CUL3 substrate adaptor, cause autosomal-dominant RP by attenuating ubiquitin ligase activity.\textsuperscript{58,59} A second example is \textit{TOPORS} (TOP1 binding arginine/serine rich protein [MIM: 609507]), mutations in which underlie autosomal-dominant RP as well. TOPORS was initially characterized as both a ubiquitin and a SUMO-1 E3 ligase.\textsuperscript{60,61} Interestingly, TOPORS is also a cilia-centrosomal protein implicated in ciliary protein trafficking.\textsuperscript{62} This is in line with recent studies linking several ubiquitination components with ciliogenesis.\textsuperscript{63,64} Mutations in ubiquitously expressed genes with a role in ciliary, lysosomal, or metabolic pathways, for instance, are increasingly described in both isolated and syndromic iRD (RetNet). Mutations in such genes cover a broad spectrum ranging from hypomorphic to null alleles. Depending on the combination of alleles, phenotypes can vary from mild (isolated iRD) to severe (syndromic iRD). In the case of \textit{RCBTB1}, we hypothesize that the identified missense mutations affect specific functions of the protein and/or distinct protein-protein interactions and thereby impair one or multiple organ systems.

Understanding the pathogenetic mechanism of iRD mutations in genes acting in ubiquitination and downstream \textit{NFE2L2} regulation is important in view of therapeutic developments. Local AAV-mediated overexpression of \textit{NFE2L2} was recently put forward as a strategy for prolonging cone survival in three RP models caused by mutations in two affected individuals (V:1 and V:2 from F1) than in six healthy control individuals (respective p values are 0.001, 0.005, 0.001, 0.026, and 0.002). For \textit{EPHX1}, the observed decrease was not significant (p value of 0.076). qPCR expression analysis was performed as previously described.\textsuperscript{65} Error bars represent the SE of the relative quantities.

In conclusion, we have identified \textit{RCBTB1} mutations as a cause of autosomal-recessive iRD with or without extra-ocular manifestations in the thyroid, ovary, and inner ear. This study has linked autosomal-recessive iRD with impaired ubiquitination and \textit{NFE2L2} regulation, an emerging pathway that regulates oxidative stress in the retina and is amenable to gene therapy.

\section*{Accession Numbers}

The accession numbers for the variants reported in this article are ClinVar: SCV000292417, SCV000292418, SCV000292419, SCV000292420, SCV000292421, and SCV000292422.

\section*{Supplemental Data}

Supplemental Data include a Supplemental Note, nine figures, and eight tables and can be found with this article online at \url{http://dx.doi.org/10.1016/j.ajhg.2016.06.017}.

\section*{Conflicts of Interest}

F.C. is a co-founder of pxlence.

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