Mutations in *C2ORF71* Cause Autosomal-Recessive Retinitis Pigmentosa

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With a worldwide prevalence of 1 in 4,000, retinitis pigmentosa (RP) is the most common form of hereditary retinal degeneration. More than 30 genes and loci have been implicated in nonsyndromic autosomal-recessive (ar) RP. Genome-wide homozygosity mapping was conducted in one Dutch and one Israeli family affected by arRP. The families were found to share a 5.9 Mb homozygous region on chromosome 2p23.1-p23.3. A missense variant in one of the genes residing in this interval, *C2ORF71,* has recently been reported to be associated with RP. *C2ORF71*, encoding a putative protein of 1,288 amino acids, was found to be specifically expressed in human retina. Furthermore, RT-PCR analysis revealed that in the mouse eye, *C2orf71* is expressed as early as embryonic day 14. Mutation analysis detected a 1 bp deletion (c.946 del; p.Asn237MetfsX5) segregating with RP in the Dutch family, whereas a nonsense mutation (c.556C > T; p.Gln186X) was identified in the Israeli family. Microsatellite-marker analysis in additional Israeli families revealed cosegregation of a *C2ORF71*-linked haplotype in one other family, in which a 13 bp deletion (c.2756_2768 del; p.Lys919ThrfsX) was identified. Clinically, patients with mutations in *C2ORF71* show signs of typical RP; these signs include poor night vision and peripheral field loss, typical retinal bone-spicule-type pigment deposits, pale appearance of the optic disk, and markedly reduced or completely extinguished electroretinograms. In conclusion, truncating mutations in *C2ORF71* were identified in three unrelated families, thereby confirming the involvement of this gene in the etiology of arRP.

With a worldwide prevalence of 1 in 4,000, retinitis pigmentosa (RP [MIM #268000]) is the most common form of hereditary retinal degeneration.[1] RP reflects a heterogeneous group of retinal dystrophies characterized by night blindness followed by visual-field loss and often resulting in severe visual impairment. The disease is heterogeneous, both clinically and genetically. Nonsyndromic RP can be inherited as an autosomal-recessive, autosomal-dominant, or X-linked trait.[1] Digenic patterns of inheritance have also occasionally been described.[2] More than 40 genes and loci have been implicated in nonsyndromic RP, and of these at least 32 are associated with an autosomal-recessive mode of inheritance (RetNet—Retinal Information Network). However, the contribution of each one of these genes to the overall prevalence of RP is relatively small, and for the majority, pathogenic mutations have been reported in only a few families worldwide. It is estimated that the genes underlying 50% of RP cases are still unknown.

Homozygosity mapping is an efficient tool for the identification of the underlying genetic defects in recessive traits, not only in consanguineous families but also in non-consanguineous families originating from countries, such as The Netherlands, in which little migration has occurred over the last centuries.[3–5] In an effort to identify additional genes causative for retinal degeneration, we performed homozygosity mapping in a number of Dutch and Israeli families affected by arRP by using the Affymetrix GeneChip Human Mapping 5.0 and the Affymetrix GeneChip Human Mapping 250K SNP arrays, respectively (Affymetrix, Santa Clara, CA, USA). Homozygous regions were calculated with Partek Genomic Suite software (Partek Inc., St. Louis, MO, USA). The study was approved by the institutional review boards at Bnai Zion and Ha'Emek Medical Centers in Israel and at the Radboud University Nijmegen Medical Centre in The Netherlands. Written informed consent was obtained from all participants or their parents.

In four affected siblings from a Dutch family (W97-111), only one shared homozygous segment was identified. This was a region of 6.8 Mb on chromosome 2p23.1-p24.1, between SNPs rs10495742 and rs10514769. In a consanguineous Muslim Arab Israeli family (TB52), a shared homozygous region of 8.7 Mb on chromosome 2p22.3-p23.3 was identified; this region was flanked by SNPs rs12470303 and rs218195. Microsatellite-marker analysis in both families, including nonaffected siblings and parents, confirmed that the region segregated with arRP (Figures 1A and 1B). To determine whether arRP was linked to this regio in additional families, we genotyped microsatellite markers within the interval in four additional consanguineous Muslim Arab Israeli families. In one of these families (TB44), all four affected individuals, but

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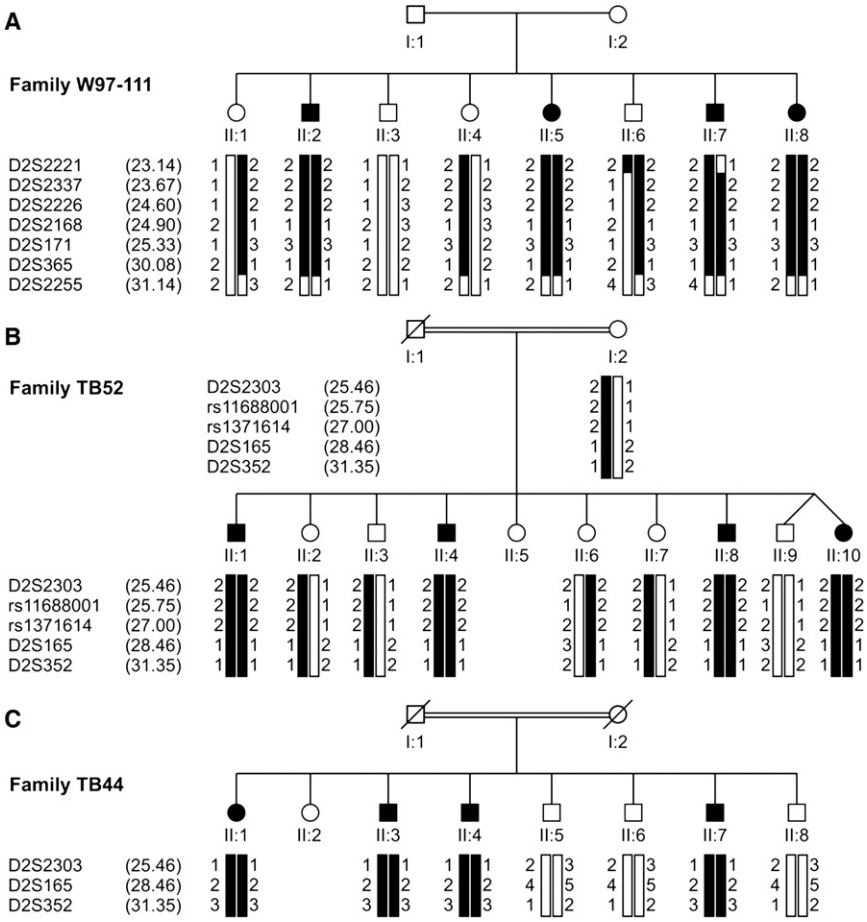

Figure 1. Chromosome 2 Haplotypes in Families W97-111, TB52, and TB44
Shown are three families affected by arRP. One family is of Dutch origin (W97-111 [A]), and two are of Israeli origin (TB52 [B] and TB44 [C]). Microsatellite marker and SNP analyses performed on each of the families demonstrate cosegregation of a 2p22.3-p23.3-linked haplotype with RP. Double lines indicate consanguineous unions. Filled symbols represent affected individuals, and clear symbols represent unaffected individuals. Mutation-bearing haplotypes are marked by black bars. The position of the markers, based on the human genome browser working draft hg18, is indicated between parentheses.

not their unaffected siblings, were homozygous for these markers (Figure 1C), indicating that the genetic defect underlying arRP in this family might also be located within the linkage interval on chromosome 2.

Together, the linkage intervals in the three families resulted in a combined region on chromosome 2 predicted to harbor a previously unrecognized gene causative for arRP (Figure 2A). This region of 5.9 Mb is flanked by markers rs12470303 (recombinant distal marker in TB52) and rs10514769 (recombinant proximal marker in W97-111). One of the genes residing in the combined region is *C2ORF71* (GenBank accession number NM_001029883). This predicted gene harbors two exons (Figure 2A) and encodes a putative protein of 1,288 amino acids. A missense variant in this gene (p.Ile201Phe) has recently been reported to be associated with RP in a single patient from Spain (L.M. Baye et al., 2009, The American Society of Human Genetics, abstract). Sequence analysis of the two exons and intron-exon boundaries of *C2ORF71* was performed with the Big Dye terminator cycle sequencing kit (PE Applied Biosystems, Foster City, CA, USA). Primers were designed with Primer3 software,[6] and primer sequences are listed in Table S1.

A homozygous 1 bp deletion (c.946 del; p.Asn316-MetfsX5) that is predicted to cause a frameshift and premature termination of the protein was detected in family W97-111 (Figure 2B). In family TB52, a homozygous C-to-T transition (c.556C > T; p.Gln186X) that generates a premature stop codon was identified (Figure 2B). In family TB44, a homozygous 13 bp deletion (c.2756_2768 del; p.Lys919ThrfsX) that is predicted to result in a frameshift that immediately generates a stop codon was identified (Figure 2B). In each family the identified mutations completely cosegregated with the disease; unaffected siblings or parents were either heterozygous carriers or homozygous wild-type. None of the three mutations was detected among chromosomes from ethnically matched control individuals (242 chromosomes for c.946 del, 286 chromosomes for c.556C > T, and 308 chromosomes for c.2756_2768 del).

In a search for the genetic causes of various retinal dystrophies, we have applied high-resolution SNP genotyping combined with homozygosity mapping in hundreds of isolated patients with RP, Leber congenital amaurosis (LCA), and cone-rod dystrophy (CRD) over the last 2 years.[7] In one additional RP patient, one LCA patient, and two patients with CRD, homozygous regions that harbor *C2ORF71* were identified. Mutation analysis in the RP patient revealed one additional variant, a homozygous missense change that is predicted to substitute a leucine residue for a proline (c.2600C > T; p.Pro867Leu). The same variant was detected in the heterozygous state in one out of 90 ethnically matched control individuals. In addition, the proline residue that is substituted is not conserved throughout mammalian evolution; for instance, in mice a leucine residue (to which the proline residue is mutated) is present at this position. These data suggest that the c.2600C > T; p.Pro867-Leu change might be a polymorphism.

Clinically, the affected individuals from the families described here all show typical signs of RP. Such signs include poor night vision and the presence of bone-spicule-type pigment deposits in the peripheral retina.

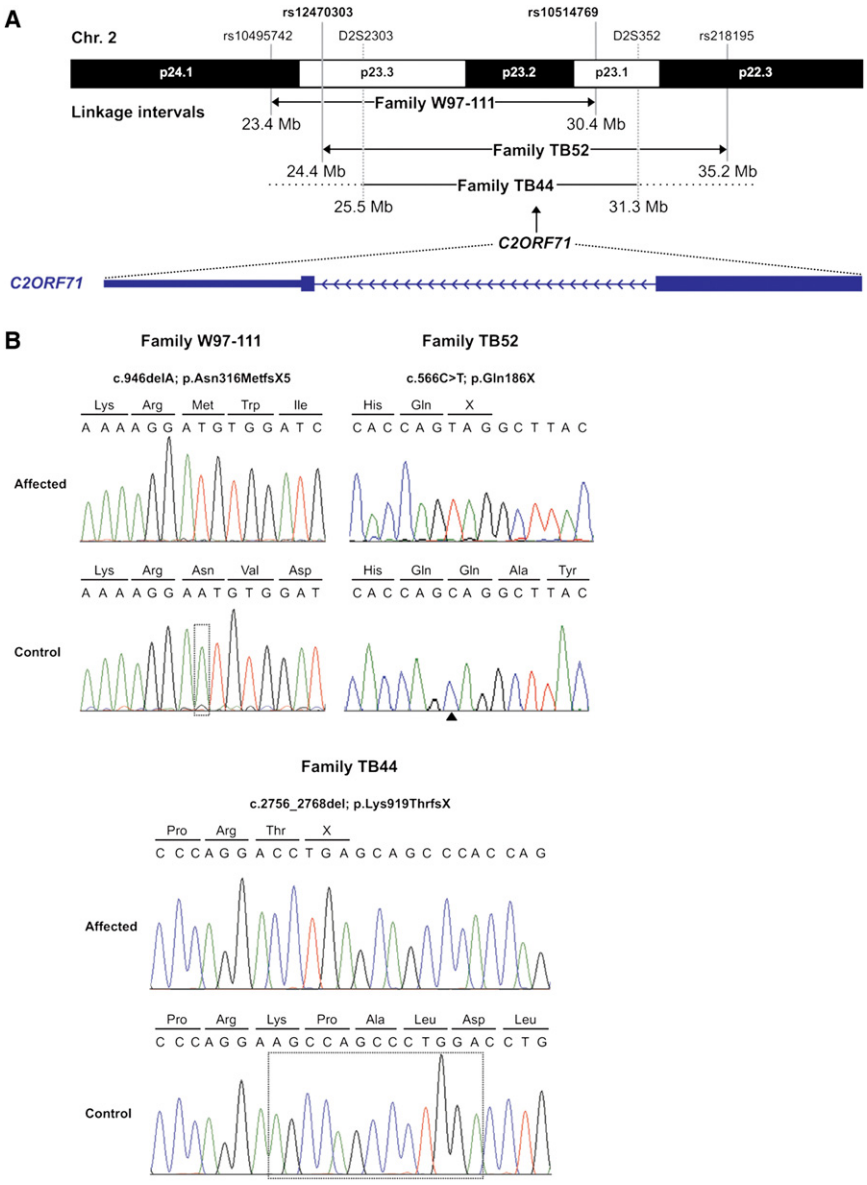

Figure 2. Linkage Intervals and Sequence Analysis of *C2ORF71*

(A) Upper panel: part of chromosome 2p showing the linkage intervals and the corresponding flanking microsatellite markers or SNPs for arRP families W97-111 and TB52. The flanking markers that determine the shared interval are indicated in bold. For family TB44, flanking markers were not determined, so the linkage interval might continue beyond the indicated markers, as is shown with the dotted line. *C2ORF71* resides within the region that is shared by the three families. Lower panel: genomic structure of *C2ORF71*. Two exons are indicated by blue bars. The noncoding part of exon 2 is indicated with a smaller bar.

(B) Sequence chromatograms for the three mutations in *C2ORF71*. Mutant sequences are indicated above the wild-type counterparts. In family W97-111, a 1 bp deletion (indicated in a dotted box in the wild-type sequence) that results in a frame shift and premature termination of the protein was detected. In family TB52, a nonsense mutation (indicated with an arrowhead) was detected, whereas in family TB44, a 13 bp deletion (also indicated in a dashed box in the corresponding wild-type sequence) was identified. This change also causes a shift in the reading frame and results in premature termination of the C2ORF71 protein. For all chromatograms, the corresponding amino acids are indicated above the sequence traces. The nucleotide positions of *C2ORF71* are according to GenBank accession number NM_001029883.

The four affected Dutch siblings from family W97-111 are part of a large pedigree in which affected individuals have an autosomal-dominant granular corneal dystrophy, retinal degeneration, and albinism. The clinical characteristics of this family were described almost three decades ago.[7] The four affected siblings were the only family members that manifested retinal degeneration. The photoreceptor dystrophy in these siblings resembles RP, or rod-cone dystrophy, in most aspects. All patients complained of night blindness and demonstrated characteristic ophthalmoscopic abnormalities such as peripheral bone spicules and attenuated retinal vessels (Figures 3A and 3B). There is, however, also evidence of early degeneration of the cone photoreceptor system. This is exemplified by the macular abnormalities in all four patients and, more specifically, by the electroretinogram (ERG) recordings in patient II-2 and II-7 (Table 1). In both patients, the ERG recordings indicate that although both photoreceptor functions are severely damaged, the cone function appears to be slightly more affected than the rod function. In light of the night blindness, the relatively good visual acuity, and the mild color vision disturbances (especially in II-7), as well as the ringscotoma (II-7) on the visual field, we still favor the diagnosis RP, albeit somewhat atypical. A decade later, the ERG in both patients was nonrecordable. The progression of the photoreceptor dystrophy was also reflected in the decline of the visual acuity; in all patients vision deteriorated to finger counting and light perception over the next two decades.

In family TB52, affected individuals show typical signs of arRP: poor night vision and peripheral-field loss are noticed in the third decade of life. Both scotopic and photopic ERGs were markedly reduced or completely extinct (Table 1). Visual evoked potentials, when recordable, were also reduced with normal latency (data not shown). Funduscopic findings included typical peripheral bone-spicule-type pigment deposits, attenuation of retinal blood vessels, severe retinal atrophy, and pale appearance of the optic disk (Table 1 and Figure 3C). In addition, most

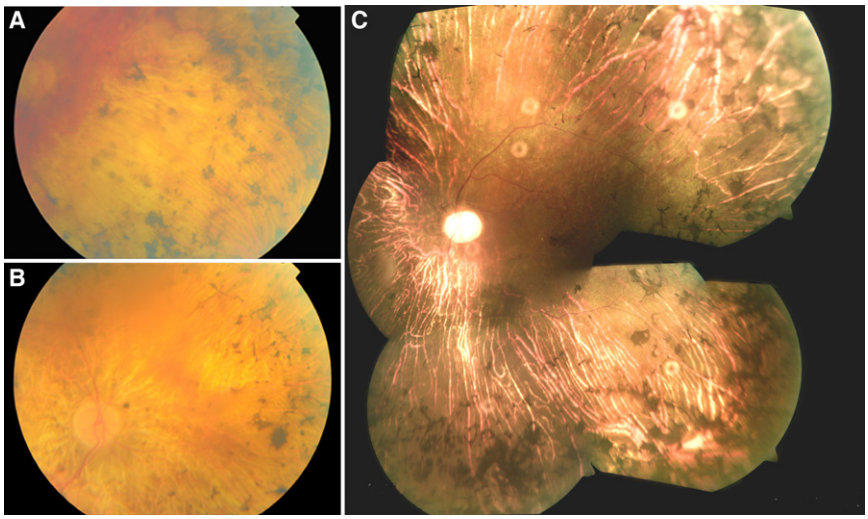

Figure 3. Fundus Photographs of Affected Individuals from Families W97-111 and TB52

(A and B) Fundus photographs of patients II-7 (age 67) and II-8 (age 66) from family W97-111. In both patients, visual acuity has deteriorated to no light perception. The fundus photographs are somewhat hazy as a result of severe cataracts. (A) Peripheral fundus of II-7, showing widespread atrophy, severe attenuation of retinal vessels, and irregular pigmentations. (B) Posterior pole of II-8 showing central atrophy as well as midperipheral atrophy. The optic nerve head has a waxy aspect, the retinal vessels are severely attenuated, and bone-spicule pigmentations are present.

(C) Fundus photographs of individual II-4 (age 50) from family TB52, showing peripheral bone-spicule-type pigment deposits, attenuation of retinal blood vessels, severe retinal atrophy (with demonstration of choroidal vessels), and pale appearance of the optic disk.

patients from family TB52 had been myopic since childhood. Patients from family TB44 also showed the typical signs of RP, including peripheral-field loss, extinct ERGs, and bone-spiculed pigmentation in the peripheral retina (Table 1).

Little is known about the physiological role of the protein encoded by *C2ORF71*. The predicted C2ORF71 protein contains 1288 amino acids that do not harbor any known functional domain. Very recently, others have identified a homozygous missense *C2ORF71* variant segregating with arRP in a small family of Spanish origin. The authors selected the gene from a 21.9 Mb homozygous linkage interval after comparing retinal-expression-array data from wild-type mice with those from mice lacking photoreceptor cells. The homozygous p.Ile201Phe variant that was identified in the Spanish family was expressed at a significantly lower level than the wild-type protein upon overexpression in ARPE-19 cells, indicating that this amino acid substitution reduces C2ORF71 protein stability. Using morpholinos to knock down the expression of the zebrafish ortholog of *C2ORF71*, the authors were able to show that morpholino-injected embryos exhibited defects in vision, illustrating an important role for this protein in photoreceptor function in vivo (L.M. Baye et al., 2009, The American Society of Human Genetics, abstract). To determine the spatial expression of *C2ORF71*, we synthesized cDNA by using RNA that was extracted from several adult human tissues. Using a forward primer located in exon 1 and a reverse primer located in exon 2, we performed RT-PCR analysis and found a high expression of *C2ORF71* in retina, whereas in the other tissues examined, we detected no or hardly any expression (Figure 4A). To examine the ocular expression of *C2orf71* at different developmental time points, we performed RT-PCR analysis of total RNA from the mouse eye. *C2orf71* was found to be expressed in the developing mouse eye as early as embryonic day 14 (E14) (Figure 4B). This finding indicates that, in addition to playing a role in the mature retina, C2ORF71 might be important for retinal development.

The mutations that were identified in this study all result in premature termination of the C2ORF71 protein or induce nonsense-mediated degradation of the *C2ORF71* mRNA.[8] Either way, the three mutations can be considered true loss-of-function alleles. Clinically, patients with mutations in *C2ORF71* show the typical hallmarks of RP. As such, the data described here clearly confirm the involvement of C2ORF71 in photoreceptor function and contribute to the etiology of this gene in causing arRP. Additional studies will be required to elucidate the exact role of this protein in retinal function and disease.

Supplemental Data

Supplemental Data include one table and can be found with this article online at http://www.cell.com/AJHG.

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Table 1. Clinical Characteristics of Patients with Mutations in *C20RF71*

Family / Patient ID[a]	Gender[b]	Age (yr)[c]	Visual Acuity[d]		FFERG[e]		Ophthalmoscopic Findings	Additional Findings
			OD	OS	LA	DA		
W97-111								
II-2	M	37	20/60	20/30	SA[f]	SA	Oval, sharply defined macular atrophy. Dust-like pigmentations in the peripheral retina.	Cornea dystrophy, albinism.
II-5	F	32	20/60	20/60	NR	NR	Oval, sharply defined macular atrophy. Large pigment clumps and bone spicules in the periphery.	Cornea dystrophy. Visual field: pericentral annular defects.
II-7	M	30	20/25	20/16	SA	NR	Subtle alterations of the macular RPE. Peripheral retina: bone spicules and attenuated vessels.	Corneal dystrophy. Visual field: ringscotomas (at age 41)
II-8	F	31	20/50	20/40	NR	NR	Oval atrophic lesions at the macula. Attenuated retinal vessels and bone-spicule pigmentations in the periphery.	Subcapsular posterior cataract.
TB52								
II-1	M	39	FC at 50 cm	FC at 100 cm	NR	NR	Peripheral bone-spicule-type pigment deposits, attenuation of retinal blood vessels, severe retinal atrophy, and pale appearance of the optic disk.	Myopia; astigmatism.
II-4	M	37	20/200	20/400	NR	NR	Peripheral bone-spicule-type pigment deposits, attenuation of retinal blood vessels, severe retinal atrophy, and pale appearance of the optic disk.	Myopia; astigmatism.
II-8	M	25	20/80	20/40	NR	NR		Myopia (R −8.5, L – 6); astigmatism.
TB44								
II-3	M	47	ND		NR	NR		
II-7	M	59	ND		NR	NR		

Abbreviations are as follows: SA, severely abnormal; NR, nonrecordable; ND, not determined; RPE, retinal pigment epithelium.
[a] Individual numbers correspond to the pedigrees depicted in Figure 1.
[b] M: male. F: female.
[c] The age listed here represents the age at which the patients were first ophthalmologically examined.
[d] Best corrected visual acuity. OD: right eye, OS; left eye, FC: finger counting.
[e] For the full-field electroretinogram (FFERG), light adaptation (LA: cone ERG) was performed with a single flash, a normal amplitude of 80–180 μV, and a normal latency of 30–35 ms. For dark adaptation (DA: rod ERG), a normal a-wave was considered to be at 100–200 μV, and a normal b-wave was considered to be at 400–550 μV.
[f] The cone function was slightly more abnormal.

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Web Resources

The URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nih.gov/Genbank/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim
Retnet- Retinal Information Network, http://www.sph.uth.tmc.edu/Retnet/
The American Society of Human Genetics, http://www.ashg.org
UCSC human genome database build hg18, March 2006, http://www.genome.ucsc.edu

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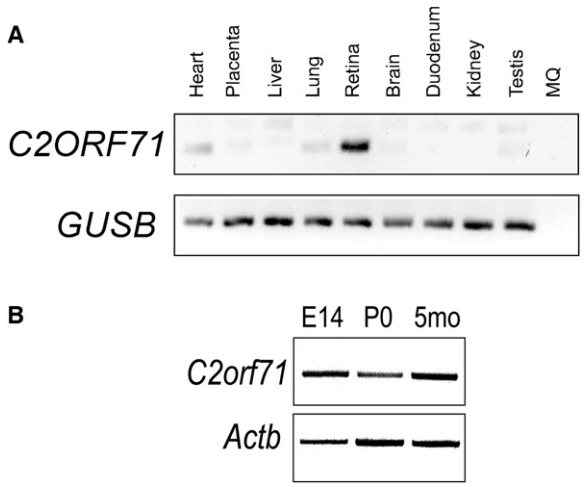

Figure 4. Expression Analysis of *C2ORF71*

(A) Expression analysis of *C2ORF71* in different adult human tissues. RT-PCR using exon-spanning primers revealed high expression of *C2ORF71* in human retina, whereas little or no expression was detected in all other tissues examined (upper panel). The expression of *GUSB*, encoding glucuronidase beta, was analyzed as a positive control (lower panel).

(B) RT-PCR analysis of *C2orf71* in the murine eye at different developmental time points: embryonic day 14 (E14), newborn (P0), and 5 months (5 mo). The analysis indicates high and relatively equal expression at all tested time points (upper panel). The *Actb* gene (β-actin) served as a positive control (lower panel).

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