Structural and Functional Abnormalities of Retinal Ribbon Synapses due to Cacna2d4 Mutation

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PURPOSE. In a spontaneous mutant substrain of C57BL/10 mice, severely affected retinal ribbon-type synapses have been described. The retinopathy was accompanied by a substantial loss in the activities of the second-order neurons. Rod photoreceptor responses were maintained with reduced amplitude, whereas cone activities were absent. This study was conducted to identify the genetic defect underlying this hitherto unknown autosomal recessive cone–rod dysfunction.

METHODS. Genome-wide linkage analysis and screening of positional candidate genes were used to identify the causative mutation. Tissue-specific transcriptional activity of the defective gene was determined by Northern blot analysis and RT-PCR approaches. The number of cone photoreceptors was estimated by immunohistochemistry.

RESULTS. The mutation was localized to a 275-kb region of chromosome 6. Within this candidate interval, a homozygous frameshift mutation (c.2367insC) was identified in the Cacna2d4 gene of affected animals. This gene codes for an L-type calcium channel auxiliary subunit of the α2δ type. The mutation introduces a premature stop codon that truncates one third of the predicted Cacna2d4 protein. A severe reduction in Cacna2d4 transcript levels observed in mutant retinas probably results in the lack of Cacna2d4 protein. The mutation leads to significant loss of rods, whereas the number of cone cells remains unaffected until 6 weeks of age.

CONCLUSIONS. The Cacna2d4 mutation underlies a novel channelpathy leading to cone–rod dysfunction in the visual system of mice and provides a new candidate gene for human retinal disorders including night blindness, retinitis pigmentosa, and cone–rod dystrophies. (Invest Ophthalmol Vis Sci. 2006;47:3523–3530) DOI:10.1167/iovs.06-0271

Clinical symptoms of hereditary retinal diseases range from moderate night blindness to severe visual impairment and complete blindness in various forms of retinal degenerations. The elucidation of the underlying genetic defects helps to understand retinal function and pathogenic mechanisms. In a previous study, an autosomal recessive cone–rod dysfunction was discovered in a substrain of C57BL/10 mice.1 The phenotype is characterized by a disturbed signal transmission of photoreceptor cells to adjacent neurons. On dark- and light-adapted electroretinography, a profound loss of the b-wave, which requires synaptic transduction, has been detected in affected animals. The scotopic a-wave, which mainly reflects membrane potentials of rods, has a consistently reduced amplitude. Under photopic conditions however, cone-specific activity is absent. The substantial loss of postphotoreceptor activities is associated with a severely compromised morphological pattern of retinal ribbon-type synapses. The causative mutation of this disorder remains unknown.

Ribbon-shaped synapses are known to involve the high-voltage-gated L-type calcium channels preferentially in their signaling pathways.2 These calcium channels are clustered at presynaptic membranes below ribbon organelles that guide synaptic vesicles to active zones for calcium-mediated fusion.3 L-type calcium channels are heteromultimers composed of four independently encoded proteins, the pore-forming α1 subunit, which triggers calcium flow across the membrane, and the auxiliary subunits α2δ, β, and γ.4 Mutations affecting the functionality in two of these calcium channel subunits lead to failure of retinal processing. Allelic variants of the retina-specific α1F subunit (CACNA1F) were found to be responsible for X-linked incomplete congenital stationary night blindness type 2 (CSNB2).5 Mutations either result in complete loss of function or modify CACNA1F kinetics.6,7 Certain variants of CACNA1F were associated with clinical symptoms of cone–rod dystrophy.7,8 An abnormal morphology of the photoreceptors ribbon synapses and seriously diminished signal transmission from photoreceptor terminals to the second-order neurons were detected in mice lacking the β2 auxiliary subunit.9 The recently reported mouse model for human CACNA1F-mediated CSNB2 revealed a cone–rod dysfunction due to a targeted deletion of the Cacna1F gene.10 Of note, striking similarities were found between the phenotypic features of ribbon synapses in Cacna1f-mutant and affected C57BL/10 mice.

We applied genetic mapping and screening of positional candidate genes to elucidate the causative mutation in affected C57BL/10 mice. By genome-wide linkage analysis, we localized the causal mutation on chromosome 6 and identified the defect in the gene encoding the fourth L-type calcium channel α2δ auxiliary subunit, Cacna2d4. This gene may be responsible for proper assembly and auxiliary modulation of biophysical properties of L-type calcium channels, similar to the previously described human CACNA2D4 orthologue.11 The mutation found in Cacna2d4 underlies a novel channelpathy leading to cone–rod disease in the visual system of mice. Our results provide insights into a new pathophysiology of retinal ribbon-type synapses and a new candidate gene for human retinal
disorders including night blindness, retinitis pigmentosa, and cone-rod dystrophies.

**MATERIALS AND METHODS**

**Animal Breeding**

Mouse strains were obtained from regular providers of laboratory animals. The research was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. To perform linkage, C57BL/10 mutant animals were crossed with animals of the AJ inbred mouse strain to generate heterozygosity in polymorphic markers. The heterozygous hybrids (F1) were subsequently mated for the second generation (F2). To refine linkage analysis, successive breeding strategy was repeated with new parental animals. Affected F2 mice were selected for mapping.

**Phenotypic Characterization**

In an earlier study, extended electrophysiological examinations of the phenotype were performed in animals up to the age of 14 months. For screening purposes, the animals were characterized by scotopic ERG at the age of 3 to 6 weeks. Briefly, dark-adapted, anesthetized mice were placed in a Ganzfeld bowl exposing white-light flashes to the dilated eye of the mouse (flash energies ranging from $10^{-4}$ to 0.5 log cd s/m$^2$). A monocular contact lens electrode served as the recording electrode. Light-induced retinal activities were visualized in ERG recordings. Representative negative scotopic ERG recordings of affected animals exhibited reduction of the a-wave amplitude and a distinct attenuation of the b-wave. Cone ERGs were nonrecordable in affected animals. Affected F2 mice were selected for mapping.

**Retinal Morphology**

To exclude modifying effects of the AJ genetic background, we looked for alterations in the retina of affected B10AF2 hybrids by light microscopy. Paraﬃn sections of 10 mutant and 10 wild-type eyes were stained with hematoxylin-eosin dye complex and evaluated with a microscope station (Axioplan 2; Carl Zeiss Meditec, Inc., Feldbach, Switzerland). For this, eyes were enucleated from mice anesthetized and subsequently killed with CO$_2$ and ﬁxed in Serra’s (60% ethanol, 30% formic acid, 10% acetic acid) over night. After dehydration in an isopropanol series (70%, 80%, 90%, 100%, 100%), eyes were embedded in paraffin blocks and sectioned into 5-μm slides.

**Genotyping and Linkage Analysis**

DNA was extracted from tail biopsy samples by standard methods. A whole-genome screen was performed with 75 informative microsatellite markers. Products of PCR assays with fluorescently labeled primers were analyzed by automated capillary genotyping (MegaBACE 1000, GE Healthcare). To perform linkage, C57BL/10 mutant animals were crossed with unaffected B10AF2 animals for ﬂanking markers. The heterozygous hybrids (F1) were subsequently mated for the second generation (F2). To refine linkage analysis, successive breeding strategy was repeated with new parental animals. Affected F2 mice were selected for mapping.

**RT-PCR and Real-Time PCR**

Gene transcription and the exonic composition of Caca2d4 were characterized by direct sequencing of the open reading frame (ORF) or whole mRNA. Mainly, RT-PCR was directed from whole-eye cDNA of two mutants and two wild types to prescreen exon splicing and deletion or insertion defects, applying primers designed to amplify ORF/mRNA-spanning fragments of $-550$ bp. As no mutation was found in all investigated candidate genes, we proceeded with sequence analysis of the entire interval, defined by fine mapping. We performed primer design through the region to amplify overlapping fragments of 500 to 650 bp corresponding to the genomic reference sequence of C57BL/6j mice (http://www.informatics.jax.org/marker). Sequences including identified SNPs and primers are available on request. Unaffected B10AF2 animals were genotyped for flanking markers of the final interval.

**Mutational Analysis**

Candidate genes were selected on functional relevance and analyzed by direct sequencing of the open reading frame (ORF) or whole mRNA. Mainly, RT-PCR was directed from whole-eye cDNA of two mutants and two wild types to prescreen exon splicing and deletion or insertion defects, applying primers designed to amplify ORF/mRNA-spanning fragments of $-550$ bp. As no mutation was found in all investigated candidate genes, we proceeded with sequence analysis of the entire interval, defined by fine mapping. We performed primer design through the region to amplify overlapping fragments of 500 to 650 bp corresponding to the genomic reference sequence of C57BL/6j mice (http://www.informatics.jax.org/marker). Sequences including identified SNPs and primers are available on request. Unaffected B10AF2 animals were genotyped for flanking markers of the final interval.

**RNA Isolation and cDNA Synthesis**

After tissue homogenization with a glass pestle, total RNA extraction was performed (RNeasy Mini Kit; Qiagen, Basel, Switzerland). Through reverse transcription, 1.5 μg total RNA was transcribed into cDNA by random hexanucleotide priming (hexamer primers pd(N)$_6$; GE Healthcare, Otelfingen, Switzerland; and Superscript III; Invitrogen, Basel, Switzerland). cDNA synthesis efﬁciency and DNA contamination were examined by standardized Gapdh PCR including negative control experiments for each sample with no reverse transcriptase used during synthesis. Absent Gapdh product in the negative control experiments excluded DNA contamination.

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**Retinal Morphology**

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on a detection system (7900HT Prism; ABI). A melting curve was analyzed to verify single-product amplification. In addition, the specificity of the amplicons was confirmed by sequencing. Transcript levels were normalized to 18S rRNA (TaqMan Ribosomal RNA Control Reagent; ABI) and quantified to the mean level in homozygous wild types according to the ΔCt-method.

**Northern Blot Analysis**

For hybridization, a 550-bp cDNA fragment encoding the Cacna2d4-specific 3′end (exons 33–38; for: 5′-tgttaggtgaagctctgg, rev: 5′-ggagcataccccacagcagtga) was used as a probe. Labeling with [α-32P]dCTP (10 μCi/μl; Hartmann Analytic, Braunschweig, Germany) was performed according to the manufacturer’s instructions (Prime-it II Random Primer Labeling Kit; Stratagene, Amsterdam, The Netherlands). Denatured DNA probe was transferred to a hybridization tube (ULTRahyb hybridization buffer; Ambion, Cambridge, UK), Mouse Cot-DNA and a Northern blot preincubated for 50 minutes at 42°C. We hybridized a Poly A+ RNA commercial murine northern blot (Multi Choice; OriGene, Rockville, MD). After incubation for 15 hours at 42°C, the blot was washed twice with 2× SSC and 0.1% SDS for 5 minutes at 42°C and twice with 0.1× SSC and 0.1% SDS for 15 minutes at 65°C. Finally, the blot was exposed to film (BioMax; Eastman Kodak, Rochester, NY) at −80°C overnight. As the loading control, mouse β-actin probe (OriGene) hybridization was included.

**Cell Quantification in the Retina**

Immunohistochemistry was performed on 8-μm whole-eye cryosections of six affected and six wild-type littermates, as described elsewhere.13 Ga cone transducin primary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used to detect the cones.14 As a secondary antibody, Cy3 labeled anti-rabbit IgG was applied (Dianova, Hamburg, Germany). Subsequently, tissue sections were embedded in mounting medium that contained DAPI (Vetashield; Vector Laboratories, Burlingame, CA), to visualize cell nuclei. Images were generated at 63× magnification by the fluorescence microscopy station (Axioplan2; Carl Zeiss Meditech) from three sections per animal, representing retinas including the optic nerve. Cone marker-positive cells were quantified in two 200 × 50-μm² subfields of the peripheral and two of the central outer nuclear layer per each image. In addition, the number of cells in the central outer and inner nuclear layers was counted in a 163 × 18-μm² subfield (n = 4) in DAPI-stained retinal cryosections for each animal.

**Statistics**

Statistical relevance for ERG measurements, real-time PCR and cell number quantification was analyzed with the Mann-Whitney test (SPSS ver. 13 for Windows; SPSS Inc., Chicago, IL) and accepted if P < 0.05. Graphic data interpretations included the calculation of the SE visualized in confidence intervals.

**RESULTS**

**Genome-Wide Linkage Analysis**

To identify the mutation, we crossed the C57BL/10 mutant genome to the genetic background of the phylogenetically distant AJ mouse strain generating substantial heterozygosity in the alleles of genetic markers. Heterozygous (C57BL/10 x AJ)F1 mice revealed no phenotypic alterations. Subsequently, (C57BL/10 x AJ)F1 mice were intercrossed. In total, two independent C57BL/10 x AJ breedings were performed. In the B10AF2 generation, segregation into affected and unaffected phenotypes in a ratio of 25:75% was found in accordance with the originally suggested autosomal recessive transmission of the mutation. Among a total number of 580 F2 mice, 142 animals (24.48%) showed the characteristic negative ERG in scotopic conditions (Fig. 1).

Structural and functional alterations in affected B10AF2 animals were similar to the symptoms of the inbred line C57BL/10 (Fig. 2).1 Affected B10AF2 offspring displayed significant reduction of the scotopic a-wave and profound loss of the b-wave. In affected B10AF2 mice, the a-wave amplitude was attenuated to 80.3% (Fig. 2A). The severe reduction of the b-wave amplitude was reflected by the a/b-waves, calculated as the difference between the a- and b-wave maxima. The a/b-wave revealed a decrease to 25% in affected B10AF2 animals.
Corresponding to the defective signal transmission, the loss of the b-wave was associated with a significantly attenuated photoreceptor synaptic layer (outer plexiform layer, OPL; Fig. 2B). Homozygous and heterozygous B10AF2 unaffected mice revealed no differences on scotopic electroretinography and morphology. Accordingly, the genetic background of the AJ strain did not apparently modify the retinal phenotype.

For genetic localization of the mutation, 64 affected mice from the first B10AF2 breeding were included in a genome-wide linkage analysis with an initial marker-spacing of 20 cM. A homozygosity region was detected on chromosome 6 between the markers D6Mit389 and D6Mit218, comprising an interval of 15.6 Mb (Figs. 3A, 3B). Linkage to chromosome 6 was evident through a distance of >30 cM, as revealed by allelic frequencies resulting in significant $\chi^2$ values (Supple-
an insertion of a cytosine nucleotide (C).

The first was found in a compound tetranucleotide repeat in intron 6 of Cacna2d4 and showed an insertion of an additional C, strongly suggesting a polymorphic nature of the tetranucleotide repeat. The second sequence alteration was detected in a recently characterized variant of 14 GAAAs, strongly suggesting a poly-G repeat. To investigate this putative repeat expansion, we sequenced the orthologous repeat in the AJ mouse strain and showed an insertion of an additional C in a 12-unit GAAA replicon. However, RT-PCR revealed a single product that included exon 25 (Fig. 5B). The mutation was verified in mutant animals by direct sequencing of RT-PCR products. No additional fragments were detected in unaffected animals, exclusively homozygous (C/C) allelic combinations were detected (n = 319), affected n = 78) and reduced the interval to 275 kb (Fig. 3B).

Identification of Sequence Variants in Cacna2d4

In parallel experiments, we excluded sequence variations in 33 candidate genes from the initially identified 15.6-Mb region (Supplementary Table S3, http://www.iovs.org/cgi/content/full/47/8/3523/DC1). We detected no further linkage elsewhere in the genome of the B10a hybrid strain (Supplementary Fig. S1, http://www.iovs.org/cgi/content/full/47/8/3523/DC1). The linkage interval was refined by genotyping of additional SNPs and microsatellite markers to an interval of 7.83 Mb flanked by SNPs in Cacna1c and Akap3 genes. For further fine mapping, we proceeded by generating and genotyping a second B10AF2 hybrid population (total n = 319, affected n = 78) and reduced the interval to 275 kb (Fig. 3B).

The identified insertion (c.2367insC) induces a frameshift that substitutes 14 amino acids and subsequently results in a premature stop in the corresponding protein sequence (Fig. 4B). The mutation removes 327 amino acid residues from the carboxyl terminus of the Cacna2d4 subunit representing 29.5% of the protein.

Qualitative and Quantitative Analysis of Cacna2d4 mRNA

We confirmed the predicted exonic structure and analyzed the expression of Cacna2d4 in wild-type and mutant retinas by RT-PCR (Fig. 5A). RT-PCR of fragment 7 (exons 14–29) revealed a single product that included exon 25 (Fig. 5B). The mutation was verified in mutant animals by direct sequencing of RT-PCR products. No additional fragments were detected in amplicon 7, which may correspond to alternatively spliced Cacna2d4 transcripts lacking exon 25 (Fig. 5B).

Real-time PCR experiments revealed a significant reduction of Cacna2d4 mRNA levels in eyes and retinas of affected and heterozygous animals (Figs. 6A, 6B). The relative amount of Cacna2d4 transcripts in eyes of homozygous mutant mice was diminished to 45% in comparison to wild-type animals. In the retina, a decline to 31% was observed. In the retina of heterozygous animals, a significant decrease to 73% was detected (Figs. 6A, 6B). In eyes of heterozygous mice, statistical analysis indicated significant reduction to 82% in comparison to wild-type control animals.

We also examined Cacna2d4 expression in 13 additional mouse tissues. On a Poly A+ Northern blot including 12 tissues, we detected bands in the size range of 1.5 to 5 kb, most likely corresponding to alternative splice variants of Cacna2d4 as suggested from EST databases (GenBank accession no.: XM_132795 with 2.2 kb, Ak030723 with 2.6 kb, Ak044427 with 3.23 kb, XM_132794 with 4.8 kb, and AK137847 with 3.1 kb; Fig. 6C). Strongest signals occurred at the size of ~4.6 and ~2.0 kb in most tissues. In brain, muscle and spleen, remarkably weaker signals were detected compared with other organs. Nevertheless, transcription of Cacna2d4 was evident in all tissues analyzed, indicating a ubiquitous expression pattern.

Broad Cacna2d4 expression was confirmed by RT-PCR of fragment 9 (exons 33–38) in 12 tissues (Fig. 6D). Additional
bands detected in amplicon 9 occurred in the range of 300 to 400 bp in kidney, muscle, spleen, and stomach and may indicate alternative splicing at the 3’ end of Cacna2d4.

**Examination of Cone Numbers in Affected Mice**

In previous studies, attenuated scotopic photoreceptor responses in mutants (scotopic a-wave) have been interpreted to reflect reduced rod photoreceptor number. The complete absence of cone-specific activities remained unexplained. To elucidate whether a preferential loss of cone photoreceptors occurs in mutant animals, immunohistochemical staining of peripheral retina of 6-week-old mutant animals did not significantly differ from that of wild-type mice (Fig. 7A). However, a ~15% reduction in the number of cells in the outer nuclear layer was detected, indicating an early degeneration of rod photoreceptors (Fig. 7B).

**DISCUSSION**

In the present study, we report genetic mapping of the respective trait and the identification of the disease-causing mutation in the voltage-gated L-type calcium channel auxiliary subunit Cacna2d4. This gene is ubiquitously expressed and shows significantly reduced transcript amounts in the retina of affected and heterozygous animals. The disease is caused by a homozygous single nucleotide insertion in exon 25 of Cacna2d4 which results in a frameshift that truncates one third of the predicted amino acid sequence. This defect represents a novel channelopathy that disturbs synaptic signal processing in the retina and leads to functional failure of cones and early onset degeneration of rods.

The causative nature of the identified mutation is supported by several lines of evidence. Linkage analysis revealed a single locus limited to only 275 kb on chromosome 6. No further candidate regions were detected in the mouse genome on linkage analysis. The mutation perfectly segregates with the disease phenotype. Homozygosity for this mutation was detected only in affected animals (n = 172). In unaffected mice (n > 500), homozgyosity for wild-type alleles or heterozygosity were observed. No sequence variations were found in any other gene from the linkage interval. Moreover, the expression of the respective Cacna2d4 mRNA was confirmed by RT-PCR analyses in the mouse retina and the mutation was verified in affected animals by direct sequencing of the relevant RT-PCR of fragment 7 comprising exon 33 to 38 in 12 mouse tissues: lane 1: muscle; lane 2: brain; lane 3: heart; lane 4: liver; lane 5: lung; lane 6: muscle; lane 7: skin; lane 8: spleen; lane 10: stomach; lane 11: testis; lane 12: thymus. Tissue-specific alternative splice variants were detected only in affected animals (Fig. 6B). In unaffected mice (n > 500), homozygosity for wild-type alleles or heterozygosity were observed. No sequence variations were found in any other gene from the linkage interval. Moreover, the expression of the respective Cacna2d4 mRNA was confirmed by RT-PCR analyses in the mouse retina and the mutation was verified in affected animals by direct sequencing of the relevant RT-PCR of fragment 7 comprising exon 33 to 38 in 12 mouse tissues: lane 1: muscle; lane 2: brain; lane 3: heart; lane 4: liver; lane 5: lung; lane 6: muscle; lane 7: skin; lane 8: spleen; lane 9: stomach; lane 10: testis; lane 11: olfactory bulb; lane 12: eye. Bands occurring at the size of ~100 to 200 bp are PCR primer dimers. Bands of 300 to 400 bp in kidney, muscle, spleen, and stomach may indicate alternatively spliced isoforms of Cacna2d4.
acid level and contains functionally important domains. Each Cacna2d4 displays 93% similarity in both species on the amino residues with its mouse orthologue. The truncated part of the protein alignments (part of data shown in Fig. 4C). The main structure in many species, as demonstrated by comparative protein alignments (part of data shown in Fig. 4C). The human CACNA2D4 protein shares 79% identical amino acid residues with its mouse orthologue. The truncated part of Cacna2d4 displays 93% similarity in both species on the amino acid level and contains functionally important domains. Each α2β subunit identified so far consists of a single-gene product cleaved posttranslationally into α2 and β peptides linked by disulfide bridges. The δ peptides possess a conserved single transmembrane segment at the carboxyl terminus for cell surface attachment. In human CACNA2D4, a homologous highly hydrophobic region encoded by the last two exons (37 and 38) potentially acts as the transmembrane domain. This motif is removed in mutant mice by the premature stop codon (Fig. 5A). Furthermore, the proteolytical cleavage site between the α2 and β peptides is encoded by a conserved alanine residue at position 971 in exon 34. The premature translational stop occurs at amino acid position 802 (exon 26) and thus eliminates the entire δ subunit. Hence, membrane integration of Cacna2d4 is probably abolished as a major consequence. An additional 170 amino acid residues (802–971) are truncated from the C terminus of the α2 peptide (exons 26–34). This deletion comprises one of the two Cache domains formed by amino acid residues 869–879. These domains, highly conserved in all α2 subunits, are supposed to be involved in interaction with calcium channel complexes. Finally, at least 11 cysteine residues, conserved in all murine and human α2δ subunits, are removed by the truncation.

Loss of Cacna2d4 function may have severe effects on the retinal physiology in C57BL/10 mutant animals. The α2δ subunits are determinants of auxiliary stimulation of calcium channel complexes. Beyond this, they aid in augmentation of α1 subunits on the plasma membrane increasing the density of functional channels on the cell surface and thus accelerating calcium current amplitudes. In cell lines, human CACNA2D4 enhances the α1C/β3-mediated calcium influx threefold. The precisely modulated calcium signaling and the overall integrity of α1 subunits into the presynaptic membranes may be severely affected in C57BL/10 mutant mice and therefore result in compromised neurotransmission of the retinal ribbon synapses. Loss of function of the retina-specific α1F subunit in the recently described Cacna1f mouse mutant revealed absence of synaptic signaling and was associated with substantial degeneration of photoreceptor ribbon terminals. Transgenic mice deficient for the β2 auxiliary subunit demonstrate loss of synaptic processing due to lack of trafficking and proper assembly of α1F complexes in photoreceptor synaptic membranes similar to our mutants, loss of ribbon-type synapses was detected.

Two additional L-type α1 subunits, Cacna1c and Cacna1d, are involved in neurotransmission and synaptic plasticity of retinal ribbon synapses. Strong expression of N- and P/Q-type calcium channels at plasma membranes of retinal neurons was also observed. Because α2δ subunits have been identified as components of all voltage-gated calcium channel complexes, these channels may be subject to Cacna2d4 modulation. Likewise, other retinal channels may underlie Cacna2d4-mediated stimulation. Preliminary results in immunofluorescence microscopy of retinal sections stained with antibodies detecting α1 subunits of voltage-gated calcium channels (anti-pan Cave1 antibody (Alomine Laboratories, Munich, Germany), anti-α1C and anti-α1F (Santa Cruz Biotechnology, Santa Cruz, CA; data not shown) indicated reduced signals in the ONL of mutant animals. This may be a consequence of the reduced thickness of the OPL or may imply a decreased density of α1 subunits at the synaptic terminals.

The phenotype in mutant mice is restricted to retina, as no further apparent abnormalities are detectable, although the expression is ubiquitous. Affected animals display normal behavior and body weight and regular breeding. In correlation to the cone–rod dysfunction of the Cacna1f mutant, these mice are probably congenitally blind. Yet, the elimination of ~15% of photoreceptor cells in mutant animals may indicate additional degenerative processes. As minor alterations in calcium concentration modulate a diversity of molecular processes, the dysregulation of the calcium homeostasis may activate further pathogenic mechanisms responsible for the rod photoreceptor cell death. The identification of the Cacna2d4 defect may have implications for corresponding retinal diseases in human patients. The deficiency of Cacna2d4 may be associated with rare human autosomal recessive incomplete CSNB. However, because a mutation in CACNA1F was also found in a family.
with a cone–rod dystrophy, the CACNA2D4-mediated channelopathy may involve both night blindness and cone–rod dystrophy. Finally, the early degeneration of rod photoreceptors in mutant mice may indicate a causative role of mutations in this gene, also in patients with retinitis pigmentosa.

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