

## TRPM1 Is Mutated in Patients with Autosomal-Recessive Complete Congenital Stationary Night Blindness

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Night vision requires signaling from rod photoreceptors to adjacent bipolar cells in the retina. Mutations in the genes *NYX* and *GRM6*, expressed in ON bipolar cells, lead to a disruption of the ON bipolar cell response. This dysfunction is present in patients with complete X-linked and autosomal-recessive congenital stationary night blindness (CSNB) and can be assessed by standard full-field electroretinography (ERG), showing severely reduced rod b-wave amplitude and slightly altered cone responses. Although many cases of complete CSNB (cCSNB) are caused by mutations in *NYX* and *GRM6*, in ~60% of the patients the gene defect remains unknown. Animal models of human diseases are a good source for candidate genes, and we noted that a cCSNB phenotype present in homozygous *Appaloosa* horses is associated with downregulation of *TRPM1*. *TRPM1*, belonging to the family of transient receptor potential channels, is expressed in ON bipolar cells and therefore qualifies as an excellent candidate. Indeed, mutation analysis of 38 patients with CSNB identified ten unrelated cCSNB patients with 14 different mutations in this gene. The mutation spectrum comprises missense, splice-site, deletion, and nonsense mutations. We propose that the cCSNB phenotype in these patients is due to the absence of functional *TRPM1* in retinal ON bipolar cells.

Congenital stationary night blindness (CSNB) is a group of genetically and clinically heterogeneous retinal disorders. The genes involved in the different forms of CSNB encode proteins, which are confined to the phototransduction cascade or are important in retinal signaling from photoreceptors to adjacent bipolar cells.<sup>1</sup> Most of the patients with mutations in these genes show a typical electrophysiological phenotype characterized by an electronegative waveform of the dark-adapted, bright-flash electroretinogram (ERG), in which the amplitude of the b-wave is smaller than that of the a-wave.<sup>2</sup> This so-called Schubert-Bornschein type of ERG response allows the discrimination of two subtypes of CSNB: incomplete (ic) (CSNB2A [MIM 300071], CSNB2B [MIM 610427]) and complete (c) (CSNB1A [MIM 310500], CSNB1B [MIM 257270]).<sup>3</sup> The incomplete type is characterized by both a reduced rod b-wave and substantially reduced cone response, due to

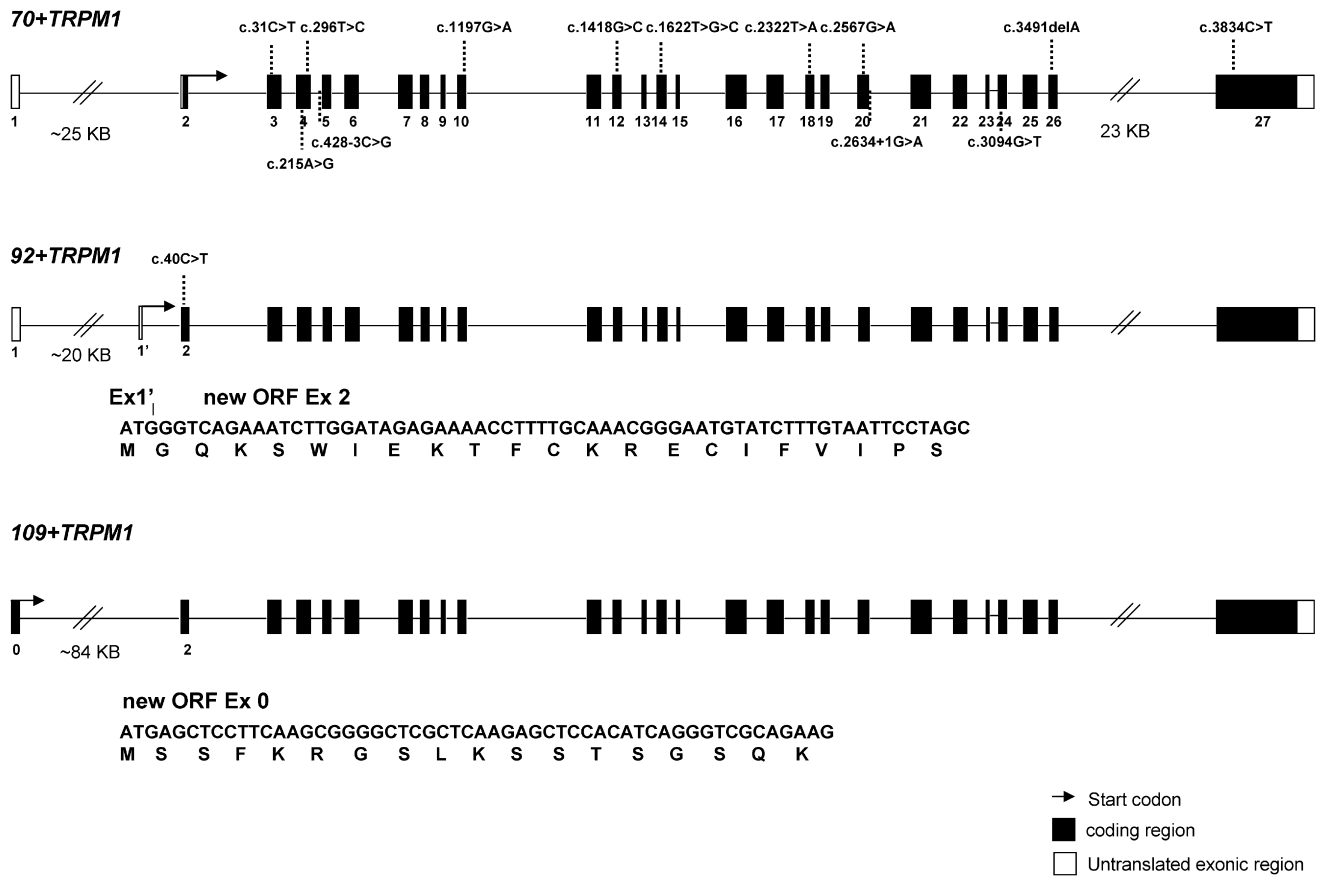
both ON and OFF bipolar cell dysfunction, whereas the complete type is associated with a drastically reduced rod b-wave response but largely normal cone b-wave amplitudes, due to ON bipolar cell dysfunction.<sup>4</sup>

In a considerable fraction of CSNB patients, mutations have been identified by direct sequencing of candidate genes or microarray analysis.<sup>5</sup> However, from our CSNB cohort, the phenotype in ~60% of the patients could not be associated with mutations in known genes, indicating that additional genes remain unidentified. Recently, a type of CSNB in *Appaloosa* horses has been described.<sup>6,7</sup> Affected animals initially showed reduced vision in dim light conditions, which subsequently progressed to reduced vision even in normal light conditions in severely affected animals. No fundus abnormalities were present, but strabismus and nystagmus were described. Electrophysiological studies revealed a “negative ERG” resembling

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**Figure 1. TRPM1 Isoforms and CSNB Mutations**

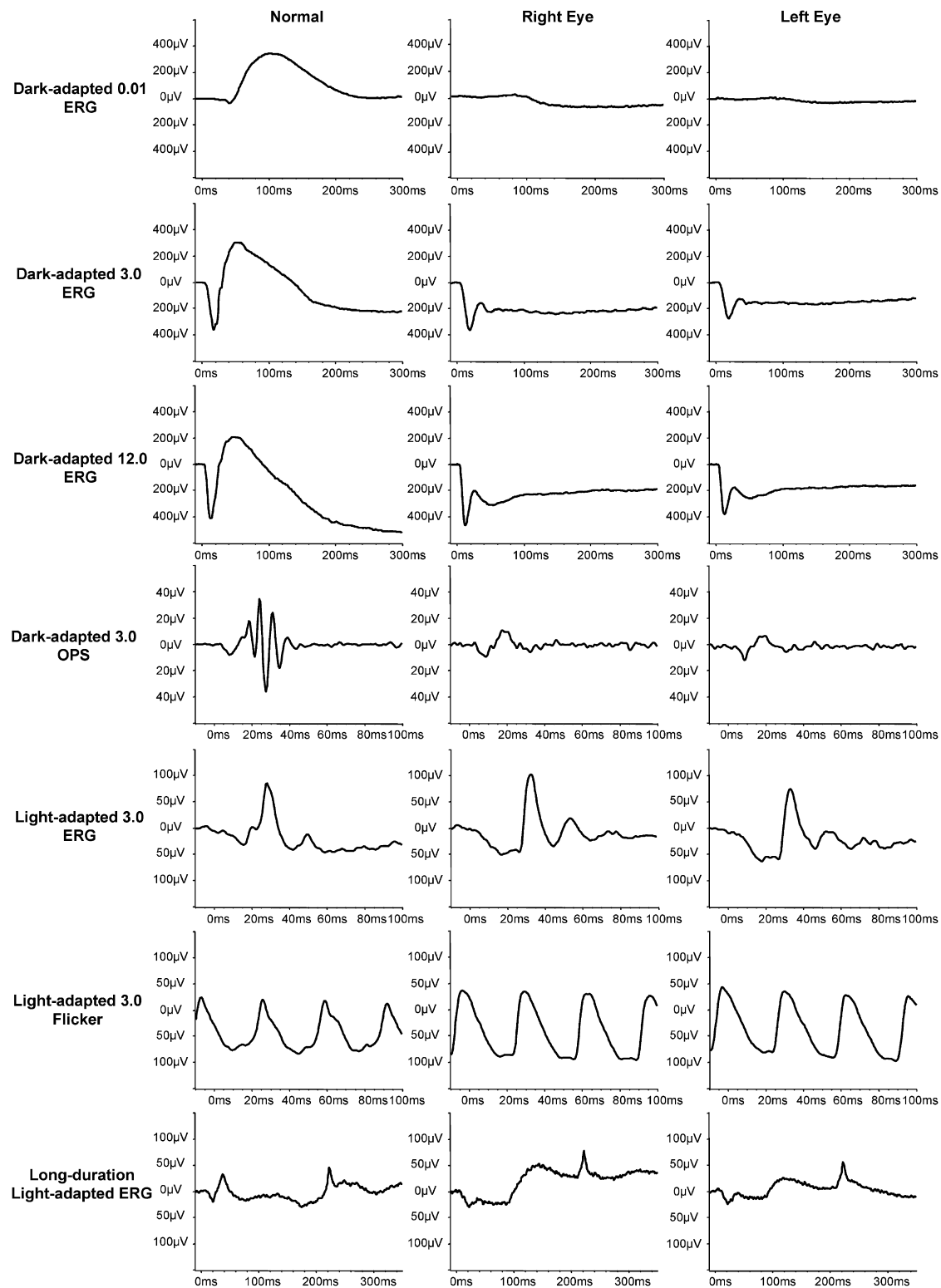
Three isoforms of *TRPM1* are presented: the *70+TRPM1* variant represents the previously published reference sequence (RefSeq NM\_002420.4), whereas the *92+TRPM1* and *109+TRPM1* isoforms were only recently identified.<sup>13</sup> In comparison to *70+TRPM1*, the *92+TRPM1* isoform contains 22 additional amino acids, and the *109+TRPM1* isoform contains 39 additional amino acids. The new open reading frame of *92+TRPM1* is made up of the complete exon 2 with the initiation codon located in a new exon (exon 1'). The new ORF of *109+TRPM1* is made up of the complete exon 2 and a new exon (exon 0). Both new ORFs continue in the isoform *70+TRPM1*, coding for 1625 and 1642 amino acids, respectively.

the human Schubert-Bornschein type of ERG response.<sup>8</sup> Furthermore, the photopic flicker responses of affected horses seemed to be similar when compared with those of unaffected horses,<sup>6</sup> suggesting a phenotype reminiscent of cCSNB. Association studies of the coat coloring in these horses revealed that this trait is directly linked with the CSNB phenotype. Gene expression analysis of genes linked to this disorder revealed that *TRPM1* (MIM 603576), also known as melastatin (*MLSN1*), was significantly downregulated in the retina and skin of affected animals. Thus, it was proposed that *TRPM1* is responsible for altering bipolar signaling as well as melanocyte function, causing both CSNB and the coat color phenotype in *Appaloosa* horses.<sup>9</sup> Studies in mice lacking *Trpm1* revealed a severely reduced b-wave in ERG recordings, similar to the Schubert-Bornschein type of ERG response.<sup>10</sup> These findings support the hypothesis that this gene is important for night vision.

*TRPM1* is a member of the transient receptor potential (TRP) channel family. These channels permit  $\text{Ca}^{2+}$  entry into hyperpolarized cells, producing intracellular responses linked to the phosphatidylinositol and protein kinase C

signal transduction pathway.<sup>11</sup> Because of the downregulation of *TRPM1* in *Appaloosa* horses with CSNB, it was suggested that this gene may play a role in neural transmission in the human retina through changing cytosolic-free  $\text{Ca}^{2+}$  levels in the retina in ON bipolar cells. The mGluR6 protein (MIM 604096) of the ON bipolar cells is coupled to  $\text{G}\alpha_o$  proteins (MIM 139311) and to *TRPM1*. *TRPM1* might be the cation channel that is downstream of the  $\text{G}\alpha_o$  protein in the ON bipolar cells.<sup>9</sup>

Altogether, the phenotype of *Appaloosa* horses, the downregulation of *TRPM1* in affected animals, and its localization downstream of mGluR6 in ON bipolar cells rendered this gene a good candidate. Thus, we screened this gene in 38 clinically diagnosed CSNB patients from different centers in Europe and the United States (Belgium: Ghent; France: Paris, Montpellier, and Lille; Germany: Berlin, Freiburg, Giessen, and Tuebingen; Switzerland: Lausanne; United States: Philadelphia, PA). Prior to this study, most patients were excluded either for known mutations, by a CSNB genotyping microarray, or for known CSNB genes and additional candidate genes, by direct sequencing.<sup>5</sup> Research procedures were conducted in accordance with



**Figure 2. Electrophysiologic Description of Patient CIC00238 with cCSNB, as an Example**

Full-field ERGs show typical ON bipolar pathway dysfunction: there are no detectable responses for the dark-adapted 0.01 ERG; dark-adapted 3.00 and 12.0 ERGs show an a-wave with a normal amplitude and implicit time but a severely reduced b-wave, leading to an electronegative waveform. Dark-adapted oscillatory potentials (OPs) are not detectable. Light-adapted 3.0 ERGs show normal amplitudes but implicit time shift for both the a-wave and the b-wave. The a-wave has a broadened trough, and there is a sharply rising b-wave with no OPs. Light-adapted 3.0 flicker ERGs show normal amplitudes but a broadened trough and a mildly delayed implicit time. These photopic ERG appearances are characteristic of selective dysfunction of the ON bipolar pathway with OFF bipolar pathway preservation.<sup>31</sup> This is further confirmed with long-duration stimulations, which reveal a normal a-wave but a severely reduced ON-response b-wave and a preserved OFF-response d-wave.

**Table 1. Patients with Likely Pathogenic TRPM1 Mutations**

Index Patient, Location, Family Members	Ethnicity	Exon	Nucleotide Exchange	Allele State	Protein Effect	Control Alleles (Mut/WT)	Phenotype Index
<b>CIC00238:</b> Paris, France	Portuguese-French	12	c.1418G>C	hom?	p.Arg473Pro	0/286	cCSNB, myopia, nystagmus, strabismus
unaff. father CIC03424		12	c.1418G>C	het	p.Arg473Pro		
unaff. mother CIC03423		-	no	-	-		
unaff. sister CIC03421		-	no	-	-		
unaff. sister CIC03422		-	no	-	-		
aff. sister CIC03452		12	c.1418G>C	hom?	p.Arg473Pro		cCSNB, myopia, nystagmus, strabismus
<b>4497<sup>a</sup>, II-1:</b> Tuebingen, Germany	German	3	c.31C>T	het	p.Gln11X	0/352	cCSNB, nystagmus, myopia
		4	c.296T>C	het	p.Leu99Pro	0/224	
unaff. father 4608, I-1		4	c.296T>C	het	p.Leu99Pro		
unaff. mother 4610, I-2		3	c.31C>T	het	p.Gln11X		
unaff. sister 4600, II-2		-	no	-	-		
unaff. sister 4712, II-4		3	c.31C>T	het	p.Gln11X		
unaff. brother 4740, II-3		3	c.31C>T	het	p.Gln11X		
<b>691:</b> Tuebingen, Germany	Turkish	20	c.2567G>A	hom?	p.Trp856X	0/366	cCSNB, myopia, nystagmus, strabismus
<b>8214:</b> Tuebingen, Germany	German	10	c.1197G>A	het	c.Pro399Pro/splice defect?	0/350	cCSNB, myopia, strabismus
		26	c.3491delA	het	p.Gln1164ArgfsX31	0/266	
<b>CIC00612:</b> Paris, France	French	4	c.215A>G	het	p.Tyr72Cys	0/210	cCSNB, myopia, nystagmus, strabismus
		24	c.3094G>T	het	p.Glu1032X	0/370	
unaff. mother: CIC03359		24	c.3094G>T	het	p.Glu1032X		
unaff. brother: CIC03360		-	no	-	-		
<b>23625<sup>a</sup>, II-3:</b> Lausanne, Switzerland	Italian	4	c.215A>G	het	p.Tyr72Cys	0/210	cCSNB, myopia
		int4	c.428-3C>G	het	splice defect	0/298	
unaff. father 23628, I-1		4	c.215A>G	het	p.Tyr72Cys		
unaff. mother 23728, I-2		int4	c.428-3C>G	het	splice defect		
unaff. brother CIC03365, II-1		-	no	-	-		
unaff. brother CIC03364, II-2		4	c.215A>G	het	p.Tyr72Cys		
<b>758.01:</b> Giessen, Germany	German	int20	c.2634+1G>A	het	splice defect	0/366	cCSNB
		27	c.3834C>T	het	p.Asn1278Asn	0/304	
<b>D0704708<sup>a</sup>, II-3:</b> Ghent, Belgium <sup>b</sup>	Flemish-Belgian	2	c.1-27C>T (70+TRPM1) or c.40C>T (92+TRPM1)	hom	5' UTR expression defect or p.Arg14Trp	0/348	cCSNB, strabismus, hypermetropia
unaff. father CIC03386, I-1		2	"	het	5' UTR expression defect or p.Arg14Trp		
unaff. mother D0704709, I-2		2	"	het	5' UTR expression defect or p.Arg14Trp		
unaff. sister CIC03389, II-1		2	"	het	5' UTR expression defect or p.Arg14Trp		
		2	"	het	5' UTR expression defect or p.Arg14Trp		

(Continued on next page)

**Table 1. Continued**

Index Patient, Location, Family Members	Ethnicity	Exon	Nucleotide Exchange	Allele State	Protein Effect	Control Alleles (Mut/WT)	Phenotype Index
unaff. sister CIC03390, II-2		2	"	het	5' UTR expression defect or p.Arg14Trp		
unaff. brother CIC03391, II-3		-	no	-			
<b>14101</b> : Philadelphia, PA, USA	Austrian-Russian-Ashkenazi Jewish	18	c.2322T>A	het	p.Tyr774X	0/380	cCSNB, myopia
		?	?	-	?		
unaff. father 19037		?	?	-	?		
unaff. mother 19038		18	c.2322T>A	het	p.Tyr774X		
<b>10731</b> : Berlin, Germany	German	14	c.1622T>A	het	p.Met541Lys	0/214	cCSNB, myopia
		?	?	-	?		

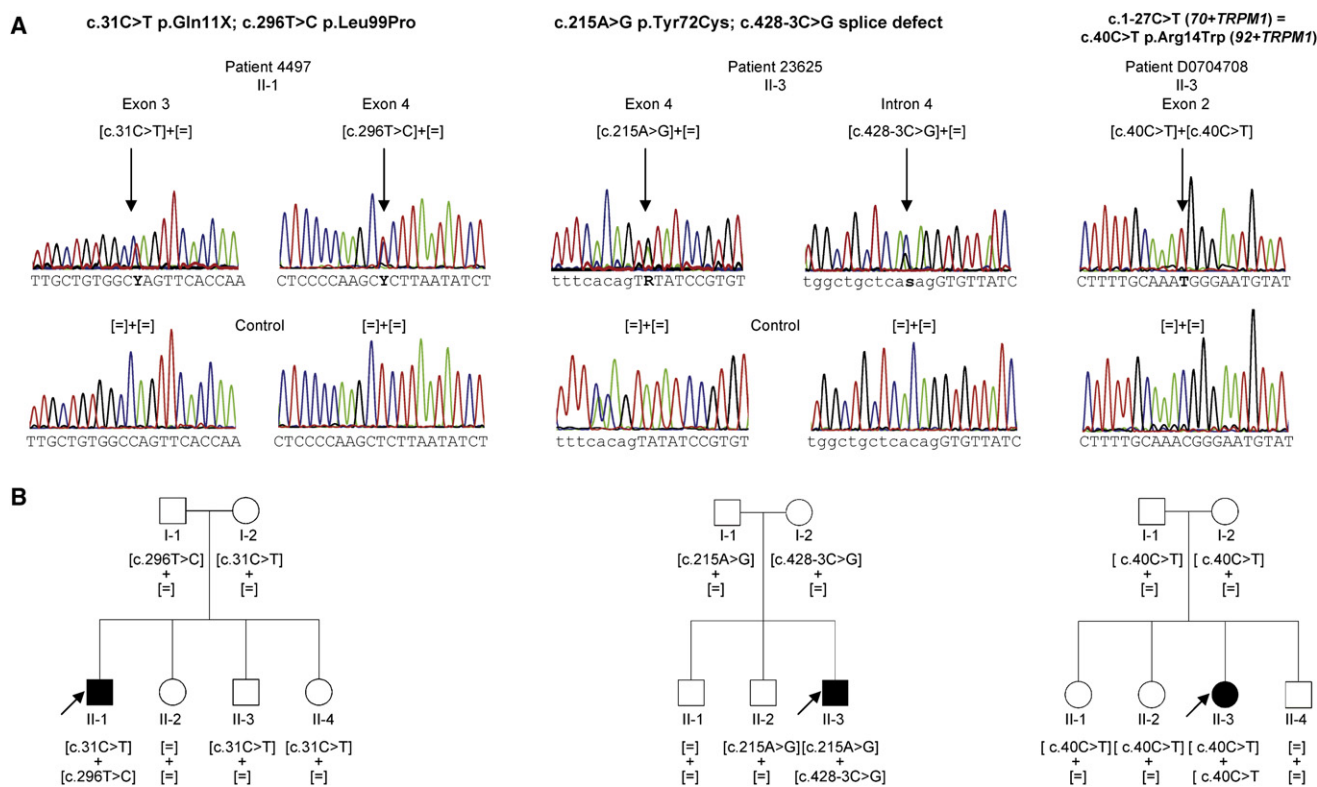
Index patients are presented in bold. Abbreviations are as follows: Mut, mutated; het, heterozygous; hom, homozygous; unaff., unaffected; aff., affected. CSNB mutations are annotated according to the recommendation of the Human Genome Variation Society, with nucleotide position +1 corresponding to the A of the translation-initiation codon ATG in the cDNA nomenclature RefSeq NM\_002420.4, 70+*TRPM1*. For Exon 0 and Exon 1', the respective A of the new translation initiation codon ATG was used.

<sup>a</sup> See Figures 3A and 3B.

<sup>b</sup> 27533 Diagnostic: Zurich, Switzerland.

institutional guidelines and the Declaration of Helsinki. Prior to genetic testing, informed consent was obtained from all patients and their family members. Ophthalmic examination included best corrected visual acuity, slit

lamp examination, funduscopy, Goldmann kinetic perimetry, full-field ERG incorporating the ISCEV (International Society for Clinical Electrophysiology of Vision) standards,<sup>12</sup> fundus autofluorescence, and optical coherence



**Figure 3. TRPM1 Mutations and Cosegregation Analysis in Families with CSNB**  
 (A) Electropherograms of three index patients provided as an example, showing *TRPM1* mutations, which are highlighted by an arrow. Exonic sequence is shown in capital letters. Intronic sequence is shown in lowercase letters.  
 (B) Corresponding pedigrees of selected cCSNB patients with *TRPM1* mutations and cosegregation in available family members. Filled symbols represent affected individuals, and unfilled symbols represent unaffected persons. Squares indicate males, and circles indicate females. Arrows reflect the index patients.

**Table 2. Likely Non-Disease-Causing *TRPM1* Variants Identified in Patients with CSNB**

Exon	Nucleotide Exchange	Allele State	Protein Effect	Control Alleles (Mut/WT)	Conclusion
0	c.16C>T	het or hom	p.Arg6Trp	frequent in patients and controls	new, but T occurs also in Platypus; thus, SNP
10	c.1195C>A	het	p.Pro399Thr	8/350	SNP
26	c.3483G>C	het	p.Gln1161His	2/266	SNP
27	c.4123G>T	het	p.Glu1375X	20/320	SNP
	c.4264C>T	het or hom	p.Arg1422Trp	frequent in patients and controls	new, but 2/334 alleles showed exchange; thus, SNP

Abbreviations are as follows: Mut, mutated; het, heterozygous; hom, homozygous. CSNB mutations are annotated according to the recommendation of the Human Genome Variation Society, with nucleotide position +1 corresponding to the A of the translation-initiation codon ATG in the cDNA nomenclature RefSeq NM\_002420.4, 70+*TRPM1*. For Exon 0 and Exon 1', the respective A of the new translation initiation codon ATG was used.

tomography (OCT) (extent of investigation depending on the referring center). Thirty fragments covering 27 exons of *TRPM1* (RefSeq NM\_002420.4, variant 70+*TRPM1*<sup>13</sup>), two fragments corresponding to two recently identified exons (exon 1' [variant 92+*TRPM1*<sup>13</sup>] and exon 0 [variant 109+*TRPM1*<sup>13</sup>]) of this gene (Figure 1), and the flanking intronic regions were directly sequenced from the PCR-amplified products (primers are listed in Table S1, available online) with the use of a sequencing mix (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems, Courtabœuf, France) and analyzed on an automated 48-capillary sequencer (ABI 3730 Genetic Analyzer, Applied Biosystems). The results were interpreted by a software application (SeqScape, Applied Biosystems).

Analysis in *TRPM1* revealed causative mutations in ten cCSNB patients (Figure 2: patient CIC00238 shown as an example of cCSNB) with a total of 14 different mutations (Figure 1 and Table 1). These comprise nonsense mutations, a deletion leading to a predicted premature stop codon, splice-site mutations, silent mutations, and missense muta-

tions. None of these changes were found among control chromosomes (210–380 chromosomes). In those patients from whom family members could be investigated, the cCSNB phenotype cosegregated with the mutations and the genotypes were indicative for autosomal-recessive inheritance (Figures 3A and 3B: three patient examples). Five index patients (4497, 8214, CIC00612, 23625, and 758) showed compound heterozygous mutations (Table 1). Patients CIC00612 and 23625 both revealed a heterozygous p.Tyr72Cys substitution. From the origins of these patients, no close familial relationship was obvious. In three index patients (CIC00238, 691, and D0704708), an apparently homozygous mutation was found (Table 1). Homozygosity was proven for index patient D0704708 (Figure 3). Cosegregation analysis from family members of index patient CIC00238 revealed that another affected sister was apparently homozygous for the mutation, whereas the father was heterozygous. Two unaffected sisters and, interestingly, the mother did not show the mutation (Table 1). These findings indicated that the patient is most likely heterozygous for the missense mutation inherited from the father and would have a deletion in *TRPM1* or a mutation in another gene, which would have been inherited from the mother. Four investigated SNPs (rs4779818 in intron 1, rs4779816 in exon 2, rs2241493 in exon 3, and rs2288242 in exon 18) were apparently homozygous in the patient and the parents. Therefore, the putative deletion could not be defined. Analyses of additional SNPs in genomic regions of *TRPM1* or screening of candidate genes may enable us to localize the second mutation and will be investigated in the future. The parents of patient 691 were not available for genetic testing, and thus homozygosity could not be proven. For two patients, 14101 and 10731, only one heterozygous mutation was identified (Table 1). Again, the second mutation may be a large heterozygous deletion and thus not detectable by PCR-based sequencing. In addition, a mutation located in a second gene may disable signaling important for nocturnal vision. Three investigated SNPs (rs4779816 in exon 2, rs2241493 in exon 3, and rs3782599 in exon 4) were apparently homozygous in the patient and the parents, and thus the putative deletion could not be defined. Mutation analysis in patient

**Table 3. Benign *TRPM1* Variants Identified in CSNB Patients**

Exon	Nucleotide Exchange	Protein Effect	SNP ID
2	c.2T>C	p.Met1Thr	rs4779816
3	c.95G>A	p.Ser32Asn	rs2241493
11	c.1239G>A	p.Thr413Thr	rs1035705
16	c.1813G>A	p.Val605Met	rs17815774
18	c.2307T>C	p.Tyr769Tyr	rs12913672
	c.2340T>C	p.Asn780Asn	rs2288242
19	c.2475C>T	p.Asn825Asn	rs12911350
27	c.3686A>C	p.Asn1229Thr	rs17227996
	c.4135C>A	p.Pro1379Thr	rs61734298
	c.4139G>A	p.Val1395Ile	rs3784588
	c.4494T>A	p.His1483Gln	rs12898290

CSNB mutations are annotated according to the recommendation of the Human Genome Variation Society, with nucleotide position +1 corresponding to the A of the translation-initiation codon ATG in the cDNA nomenclature RefSeq NM\_002420.4, 70+*TRPM1*.



	72 C	99 P	473 P	541 K
Human	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Rhesus	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Tarsier	SNKAMYIRVSY	LLEPKLLISVH	-----	VLEYLMGGAYR
Mouse	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Dog	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Elephant	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	-----
Opossum	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Platypus	SNKAMYIRVSD	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Chicken	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Lizard	SNKSMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
X tropicalis	SNKAMYIRVSY	LLEPKLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR
Stickleback	VNKAMYIRVAN	LLEPTLLISVH	ALVLD <sup>DR</sup> VDFVK	VLEYLMGGAYR

**Figure 4. Evolutionary Conservation of the Altered Amino Acid Residues in Other Orthologs**

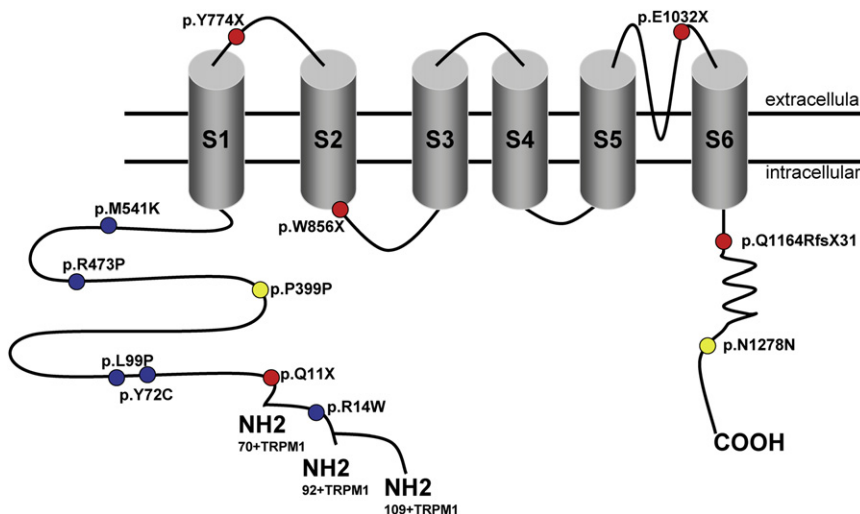
Multiple amino acid sequence alignments show evolutionary conservation of mutated residues (depicted in green). Amino acid substitutions are highlighted in red. The position of the respective amino acids is shown in black numbers.

14101 on other known or candidate genes (*NYX*, AJ278865 [MIM 300278]; *GRM6*, NM\_000843; *CABP4*, NM\_145200 [MIM 608965]; *CACNA2D4*, NM\_172364 [MIM 608171]; *BHLHB4*, BK000274 [MIM 609331]; *CACN2B*, NM\_000724 [MIM 600003]; *GNA01*, NM\_020988 and NM\_138736; and *TBC1D2*, NM\_018421 [MIM 609871]) did not reveal any mutation. Previous mutation analyses in the simplex case 10731 in known and candidate genes (*NYX*, *CACNA1F*, AJ006216 [MIM 300110], *GRM6*, *CABP4*, *CACNA2D4*, *BHLHB4*, *CACN2B*, *GNA01*, and *TBC1D2*) did not reveal any mutation. Thus, for both patients, the second mutation may be found in other regions of *TRPM1*, such as regulatory sequences or unidentified exons, or may represent a deletion in an as-yet-uninvestigated region of *TRPM1*. Alternatively, the second mutation may be found in a novel CSNB gene.

One other patient (13830) with icCSNB was compound heterozygous for two missense changes: c.1195C>A, causing a p.Pro399Thr substitution in exon 10, and c.3483G>C, leading to a p.Gln1161His substitution in exon 26, respectively (Table 2). However, the c.1195C>A change was found in eight of 350 control chromosomes and the c.3483G>C in two of 266. Thus, both variants are most likely non-disease-causing variants. This is also consistent with the fact that *TRPM1* mutations in our study specifically lead to cCSNB and not to icCSNB. Another variant (c.4123G>T) in exon 27, leading to

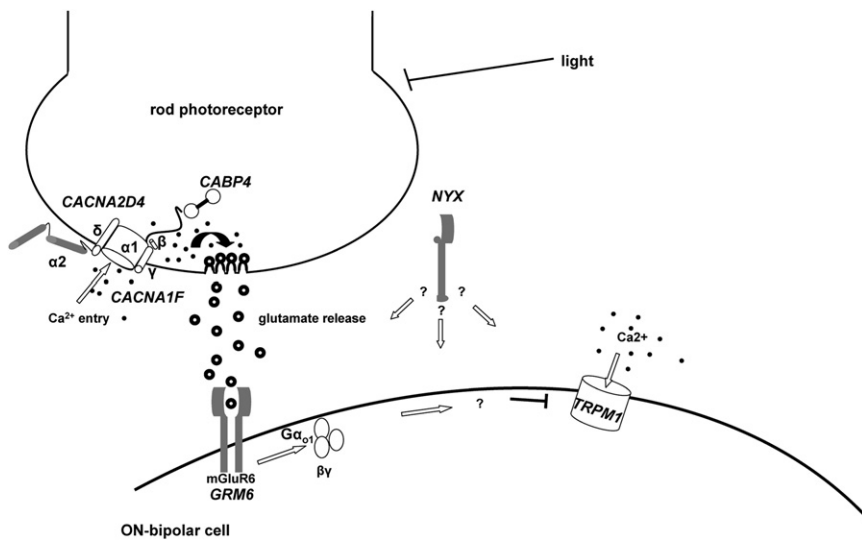
a p.Glu1375X, was detected in three patients but turned out to be a SNP (rs378489), which was detected in 20 of 320 control chromosomes. Other presumably non-disease-causing variants were detected and are summarized in Table 3.

The most likely pathogenic mutations identified herein were predicted to localize at different sites of the TRP channel. Five missense mutations, which were found in evolutionarily conserved residues (Figure 4), one silent mutation, and one nonsense mutation were predicted to localize in the N-terminal intracellular part of TRPM1 (Figure 5), the function of which is not yet understood.<sup>11</sup> All missense mutations were predicted by homology-based programs (SIFT and Polyphen, data not shown) to be pathogenic. Another silent mutation was identified in the C terminus of TRPM1. For all of these, in addition to the splice-site mutations, splicing could be influenced because different splicing proteins were predicted to bind to the mutated variants in comparison to the control (ESEfinder, data not shown). In addition, mislocalization of the mutated proteins or channel-gating defects could be the underlying pathogenic mechanisms leading to cCSNB. In total, five different mutations, predicted to lead to premature-termination codons in different locations of the protein, were identified. We assume that the corresponding mutant mRNAs of these alleles would probably be subjected to nonsense-mediated decay or produce a



**Figure 5. Localization of TRPM1 Mutations with Respect to Predicted Channel Domains**

The specific domains for the TRPM1 channel were estimated by the use of different publications and prediction programs<sup>14,32</sup> (UniProtKB-Swiss-Prot).



**Figure 6. Schematic Drawing of Proteins Involved in Signal Transmission from Photoreceptors to Adjacent Bipolar Cells, the Disruption of Which Leads to CSNB**

Arrows indicate the course of the signal transmission. In darkness,  $\text{Ca}^{2+}$  ions enter the rod photoreceptors, which results in glutamate release from the photoreceptors. Activated glutamate receptor activates  $G\alpha_{o1}$  (arrow), which then closes the TRPM1 channel by an unknown mechanism, indicated by a question mark, and thus ON bipolar cells are hyperpolarized. The exact role of NYX, encoding nyctalopin in this signal transduction cascade, remains to be solved in the future (indicated by a question mark).

short nonfunctional form of TRPM1. Previous studies showed that a shorter, alternatively spliced N-terminal form of TRPM1 devoid of any putative transmembrane segments (amino acids ~1–500) can directly interact and suppress the activity of the full-length form by preventing its translocation to the plasma membrane and thus inhibiting  $\text{Ca}^{2+}$  entry into the cell.<sup>14</sup> It was suggested that under normal conditions, this mechanism regulates the exact amount of molecules necessary for proper channel function.

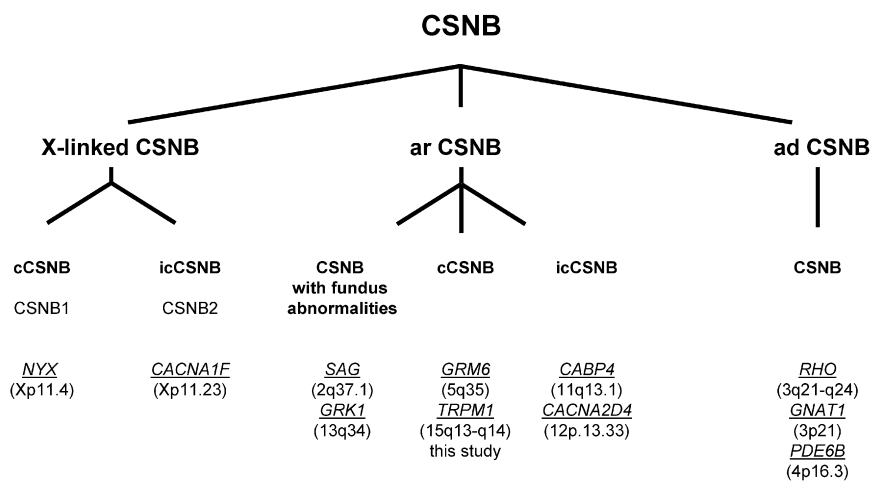
Currently, there are two genes implicated in complete CSNB: NYX and GRM6.<sup>15–18</sup> They code for the proteins nyctalopin and mGluR6, respectively, which localize postsynaptically to the photoreceptors in the retina in ON bipolar cells.<sup>19</sup> Whereas the function of nyctalopin is not yet understood, mGluR6 was shown to be important for the glutamate uptake released from the photoreceptors (Figure 6). The most obvious phenotypical feature of patients with cCSNB is a defect of the ON response, resulting in an electronegative combined rod-cone ERG, based on a severely reduced b-wave.<sup>2</sup> In the dark, glutamate is released from photoreceptors, binds to mGluR6, and activates the  $G\alpha_{o1}$  subunit of a heterotrimeric G protein. This in turn leads by an unidentified mechanism to the closure of an as-yet-unknown cation channel (Figure 6).<sup>1,20–25</sup> Upon light exposure, photoreceptor glutamate release decreases and the ON response is initiated with the shutting down of the G protein cascade. Subsequently, the cation channel opens, leading to ON bipolar cell depolarization, giving rise to the b-wave. Mutations in GRM6 lead to the loss of mGluR6 at the cell surface. Modulation of glutamate released from the photoreceptors cannot be correctly sensed by the bipolar cells, resulting in the failure of depolarization and thus a severely reduced b-wave.<sup>26</sup> Recent findings in Appaloosa horses with CSNB and a specific coat patterning caused by low expression of a TRP channel, *Trpm1*, suggested that this specific channel is specifically linked to the depolarization of the

ON bipolar cells during light exposure. However, no direct sequencing of the *Trpm1* gene was performed in the horse, and thus the loss of ON bipolar cell function could rather be due to a secondary effect than to the mutated *Trpm1*. Nevertheless, mice lacking this channel showed the same ocular phenotype with a severely reduced b-wave.<sup>10</sup> Together these findings indicated that mutations in this gene could be responsible for CSNB in patients, and indeed, our study presented herein revealed 14 different mutations in TRPM1 in ten different families with autosomal-recessive cCSNB.

Patients carrying mutations in TRPM1 reveal a similar ocular phenotype. All showed cCSNB with selective dysfunction of the ON bipolar pathway and OFF bipolar pathway preservation. Most of them revealed at least one of the following additional ocular abnormalities: myopia, nystagmus, or strabismus. These clinical observations are in accordance with the phenotype observed in the night-blind Appaloosa horses also showing nystagmus and strabismus. Although Bellone et al. showed downregulation of *Trpm1* in these horses,<sup>9</sup> it will be interesting to see which mutations in *Trpm1* lead to its downregulation. Because of the fact that the ocular phenotype was similar in all patients and because of the presence of a large fraction of nonsense and splice-site mutations, we hypothesize that this form of autosomal-recessive cCSNB is due to a lack of TRPM1 mRNA or functional TRPM1 protein on the surface, rather than to functional alterations in the biophysical properties of this channel. The study of animal models carrying the identified mutations and investigation of transcript in the retina are needed for verification of this hypothesis.

To date, three genes have been associated with icCSNB (*CACNA1F*, *CABP4*, and *CACNA2D4*),<sup>27–30</sup> and with the findings now reported here, there are also three genes associated with cCSNB (*NYX*, *GRM6*, and *TRPM1*)<sup>15–18</sup> (Figure 7). With respect to the other autosomal-recessive CSNB genes identified so far, TRPM1 seems to be the most frequently mutated gene.





**Figure 7. Genes Underlying CSNB**  
Different forms of human CSNB are classified according to their mode of inheritance, phenotype, and mutated genes. Abbreviations are as follows: cCSNB, complete CSNB; icCSNB, incomplete CSNB; ar, autosomal recessive; ad, autosomal dominant. Genes are indicated in italics and underlined. Chromosomal location is given between brackets. The phenotype of patients with mutations in icCSNB is more variable and can even lead to progressive cone or cone-rod dystrophy.<sup>1</sup>

## 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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## Web Resources

The URLs for data presented herein are as follows:

ESEfinder, <http://rulai.csh2.edu/tools/ESE>

GenCards, PolyPhen (Polymorphism Phenotyping), <http://tux.embl-heidelberg.de/ramensky/>

National Center for Biotechnology Information (NCBI), <http://ncbi.nlm.nih.gov/>

Online Mendelian Inheritance in Man (OMIM), <http://ncbi.nlm.nih.gov/Omim/>

SIFT (Sorting Intolerant From Tolerant), <http://blocks.fhcrc.org/sift/SIFT.html>

University of California-Santa Cruz (UCSC) Human Genome Browser <http://genome.ucsc.edu/>

UniProtKB-Swiss-Prot, <http://www.uniprot.org>

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