# Mutation in the Auxiliary Calcium-Channel Subunit *CACNA2D4* Causes Autosomal Recessive Cone Dystrophy

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Retinal signal transmission depends on the activity of high voltage–gated L-type calcium channels in photoreceptor ribbon synapses. We recently identified a truncating frameshift mutation in the *Cacna2d4* gene in a spontaneous mouse mutant with profound loss of retinal signaling and an abnormal morphology of ribbon synapses in rods and cones. The *Cacna2d4* gene encodes an L-type calcium-channel auxiliary subunit of the $\alpha_2\delta$ type. Mutations in its human orthologue, *CACNA2D4,* were not yet known to be associated with a disease. We performed mutation analyses of 34 patients who received an initial diagnosis of night blindness, and, in two affected siblings, we detected a homozygous nucleotide substitution (c.2406C→A) in *CACNA2D4.* The mutation introduces a premature stop codon that truncates one-third of the corresponding open reading frame. Both patients share symptoms of slowly progressing cone dystrophy. These findings represent the first report of a mutation in the human *CACNA2D4* gene and define a novel gene defect that causes autosomal recessive cone dystrophy.

Voltage-gated L-type calcium channels contribute to retinal signal transmission.[1] They cluster predominantly in presynaptic membranes of photoreceptors and ON-type bipolar cells, an excitatory class of second-order neurons, and mediate calcium-dependent neurotransmitter release.[2,3] At the molecular level, these calcium channels constitute heteromultimeric protein complexes composed of a pore-forming $\alpha_1$ subunit, which triggers calcium influx across the synaptic membrane, and the auxiliary subunits $\beta$, $\gamma$, and $\alpha_2\delta$.[4] The $\alpha_1$ subunit imparts most of the conductive properties of the channel, whereas the accessory subunits modulate calcium currents and channel activation/inactivation kinetics.[5–7] The auxiliary subunits are also involved in proper assembly and membrane localization of the calcium-channel complexes.[6] In a spontaneous mouse mutant with abnormal photoreceptor ribbon synapses and cone-rod dysfunction, we identified, by positional cloning, a homozygous protein-truncating frameshift mutation in exon 25 of the *Cacna2d4* gene, which encodes the fourth voltage-gated L-type calcium-channel auxiliary subunit of the $\alpha_2\delta$ type.[8] To date, mutations in its human orthologue, *CACNA2D4* (MIM 608171), had not been associated with a disease. Because of the retinal phenotype of *Cacna2d4*-mutant mice, gene defects in *CACNA2D4* seemed a likely cause of human retinal disorders.

Among 34 patients who received the initial diagnosis of an electronegative electroretinogram (ERG) indicative of night blindness, we detected a homozygous c.2406C→A transversion in exon 25 of *CACNA2D4* in the index patient, II-1 (fig. 1). The family of this patient consists of one affected sister, eight unaffected siblings, and the unaffected father. The affected sister (II-2) also showed homozygosity for this DNA sequence variant, whereas the unaffected father (I-1) was heterozygous for the mutation (fig. 1*B*). Two unaffected siblings (II-3, aged 51 years, and II-4, aged 47 years) displayed homozygosity for the wild-type allele. The mutation was excluded from 224 control chromosomes by direct sequencing. The nucleotide substitution (c.2406C→A) introduces a premature termination codon at aa position 802 (p.Y802X) that presumably leads to nonsense-mediated decay (NMD) and/or removes 335 aa residues (29.6%) from the C-terminus of CACNA2D4.[9] The premature termination signal occurs 64 bp upstream of the boundary between exons 25 and 26, a position that is expected to be recognized by the NMD machinery.[9] In *Cacna2d4*-deficient mice, the truncating frameshift mutation, which also occurs in exon 25, showed a reduction of retinal *Cacna2d4* mRNA levels to ~30%.[8] At the protein level, the nonsense mutation likely eliminates the entire $\delta$ peptide of CACNA2D4, encoded by residues 992–1137 (fig. 2). The $\delta$ peptides of all $\alpha_2\delta$ subunits possess a transmembrane segment, at the C-terminus, for attachment to the cell surface.[12] In addition, 189 residues (19.2%) are presumed to be deleted from the C-terminus of the CACNA2D4 $\alpha_2$ peptide (residues 802–991). As a consequence of the truncation, membrane anchoring of CACNA2D4 is presumed to be abolished.

The two patients, II-1 and II-2, reported a mild decrease

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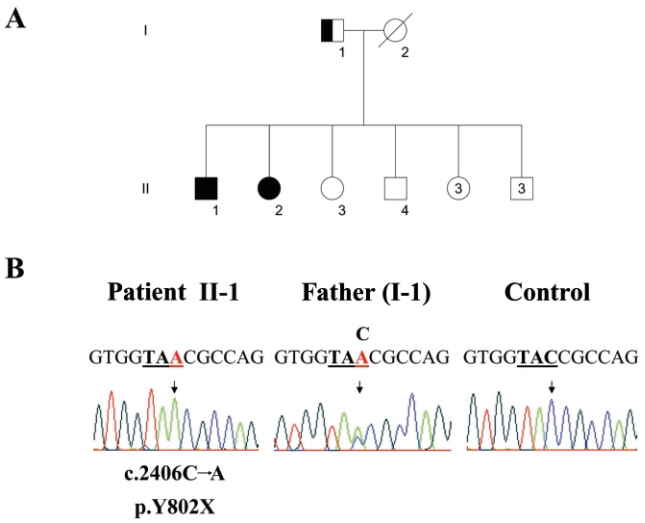


**Figure 1.** Mutation analysis of *CACNA2D4* of index patient II-1. *A,* Pedigree of the family of patient II-1. Blackened symbols indicate affected status, the half-blackened symbol indicates mutation carrier, unblackened symbols indicate unaffected status, squares represent males, and circles represent females. Sequence analysis revealed a homozygous c.2406C→A mutation in the index patient and in his affected sister (II-2) as well as the carrier status of the father (I-1). Mutation analyses of the two unaffected siblings (II-3 and II-4) displayed homozygosity for the wild-type allele. *B,* Electropherograms show the respective DNA sequences of exon 25 of *CACNA2D4*. The homozygous c.2406C→A mutation in patient II-1 (*left*), the heterozygous mutation in the unaffected father I-1 (*middle*), and the wild-type allele of a control DNA sample (*right*) are indicated. The position of the mutated nucleotide is indicated by an arrow.

in visual acuity (VA), noted only in the 3rd decade of life (table 1). They did not complain of night blindness or other visual problems, except for moderate photophobia since early childhood.

Index patient II-1 was aged 33 years at the time of examinations. In both eyes, it was determined that his VA was 0.7. Noteworthy, a decrease in VA was first noticed at age 23 years. In the Goldman kinetic perimetry, the visual field appeared almost normal, except for very mild concentric constriction in the midperiphery (isopter I/2e). The final threshold of the 30-min–dark adaptation was slightly elevated, by 0.3–0.5 log units. Color-vision discrimination, as tested by desaturated panel D15, showed four errors for each eye, (confusion index of 1.78 [right eye] and 1.81 [left eye]). The confusion index of ~2.0 provides 99% of probability of a defective color vision, in contrast to ~1.1 of unaffected color vision.[13] Fundus examination exhibited physiological reflexes of the macula and slight pigment mottling in the foveal area; otherwise, findings were normal (fig. 3). Full-field ERG, performed in accordance with the International Society for Clinical Electrophysiology of Vision (ISCEV), revealed well-preserved rod-photoreceptor responses. The rod response was just below the lower normal (5th) percentile (ISCEV) of unaffected controls (fig. 4*A*). The total amplitude of the maximal combined rod/cone responses was reduced to ~50% of the median and was dominated by an almost normal a-wave but indicated a remarkably attenuated b-wave (fig. 4*B*). The corresponding b/a-wave ratio accounted for 1.1 in both eyes and reflected a decline of the scotopic b-wave when compared with b/a values of >1.4 for unaffected eyes. Implicit times (IT) of all scotopic recordings were normal by ISCEV standards, except for a slight prolongation of oscillatory potentials of ~1 ms above the limit of the norm (fig. 4*C*). In photopic ERG, the amplitude of the single-flash cone response was not recordable (fig. 4*D*). Flicker amplitudes of 30-Hz, under light-adapted conditions, were below the lower (5th) percentile of unaffected individuals, with a phase shift of ~8 ms (fig. 4*E*). In addition, a frequency doubling of the 30-Hz flicker was evident, a feature observed elsewhere in patients with congenital stationary night blindness.[15] Vitamin A level in the serum was determined to be 0.8 mg/liter (normal range 0.2–1.2 mg/liter[16]). Retinol-binding protein contents were estimated to be 4.7 mg/dl (normal range 3.0–6.0 mg/dl[17]).

The sister (II-2) of patient II-1 presented similar ocular symptoms (table 1), except for the ERG b/a ratio of 1.2 of the maximal combined rod/cone response (fig. 4*B*). Her vitamin A and retinol-binding protein levels were 0.6 mg/liter and 3.4 mg/dl, respectively.

To summarize our clinical findings, the two patients are not experiencing night blindness; rather, they have a

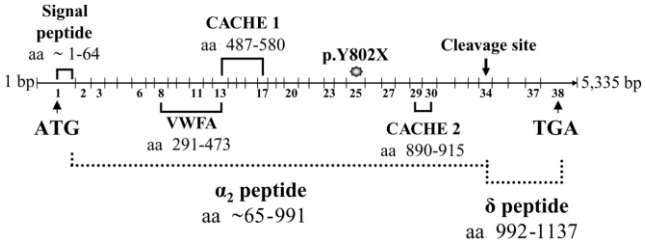


**Figure 2.** Schematic representation of *CACNA2D4* transcript. The schematic drawing shows the longest transcript variant of human *CACNA2D4* (GenBank accession number NM_172364.3) (fig. not to scale). Its ORF codes for a protein of 1,137 aa residues, posttranslationally cleaved into the $\alpha_2$ (aa 65–991; 232–3,204 bp) and the $\delta$ (aa 992–1137; 3,205–3,645 bp) peptides. The cleavage site between the $\alpha_2$ and $\delta$ peptides, encoded by an alanine residue at position 992 (exon 34), is indicated by an arrow. The locations of the conserved von Willebrand factor A (*VWFA*) domain and the calcium-channel and chemotaxis receptor (CACHE) domains are illustrated in the transcript scheme. The CACHE domains probably constitute binding sites for small ligands, whereas the *VWFA* domain is responsible for protein-protein interactions of the $\alpha_2\delta$ protein with $\alpha_1$ subunits. At the N-terminus, the CACNA2D4 protein also contains a putative signal peptide of 64 aa residues.[10,11]

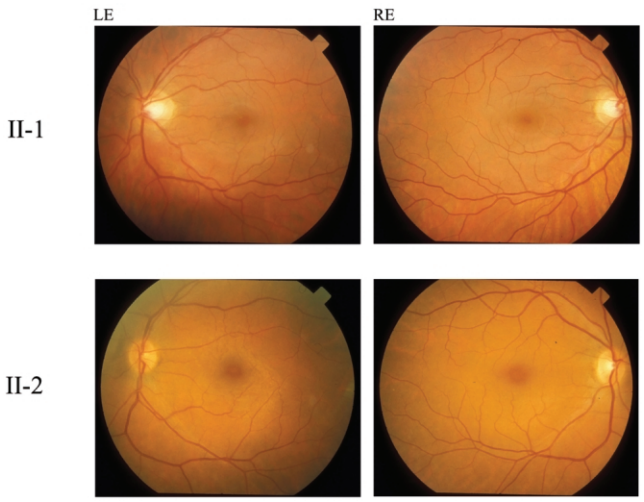


**Figure 3.** Fundus photographs of patients II-1 and II-2. Fundus of patient II-1 (*upper panels*) and patient II-2 (*lower panels*) of the left eye (LE) and right eye (RE), respectively, show nearly normal appearance.

mild form of cone dystrophy. Although the rod and cone ERG results are suggestive of incomplete stationary night blindness, the disease in our patients is progressive, not stationary.

Most likely, the nonsense mutation leads to CACNA2D4 deficiency. This involves the lack of auxiliary stimulation and a reduction of properly operating calcium channels in the retinal synaptic terminals of our two patients. In addition, the deficiency could result in an attenuation of calcium-channel densities at synaptic membranes. Translation of aberrant CACNA2D4 proteins might also contribute to the patients' retinal disease. If not efficiently degraded, anomalous CACNA2D4 proteins interact with the respective $\alpha_1$ subunits in cytosolic compartments and reduce their transport to the cell surfaces, which leads to decreased channel densities.[18] The reduction of functional CACNA2D4 protein in synaptic terminals may lead to inefficient photoreceptor-signal transmission and may account for the electronegative ERG. The synaptic dysfunction over years probably contributes to the slow progression of the disease. From the clinical point of view, the described CACNA2D4 protein mutation causes a functional alteration in the retina that is not noticed by the patient in early life but progresses, with symptoms of increasing photophobia, mildly decreasing VA, and moderate attenuated rod and markedly diminished cone ERG responses. In this sense, it is not a stationary but a progressive disease, with symptoms of such mildness that it may go unnoticed over the first 3 decades of life. The patients clearly display a mild cone dystrophy that is typically marked by progressive decline of the VA, increased photophobia, cone ERG loss, and mild morphological signs in the pigment epithelium. However, the possibility cannot be excluded that the mild rod affectation may be stationary and overlaid by a progressive cone dysfunction. Regarding the progression in patients with mutated *CACNA2D4,* recent studies also show that retinal channelopathies due to *CACNA1F* (MIM 300110) and *CABP4* (MIM 608965) mutations may underlie stationary as well as progressive disorders.[19–23] These results may have implications for diagnostic testing and genetics counseling of patients with mutations in these genes and their families.

**Table 1. Clinical Characteristics of Patients with the p.Y802X Mutation**

| | Patient | |
|---|---|---|
| Characteristics | II-1 | II-2 |
| Disease course | Slight progressive reduction of VA from 20/20 at age 18 years; first subjective changes at age 23 years | Slow progressive reduction of VA; first subjective changes at age 30 years |
| Age (in years) at examination | 33 | 46 |
| VA (RE; LE) | 20/32; 20/32 | 20/32; 20/32 |
| Refractive error[a] (sph/cyl) (RE; LE) | −.75/−.25; −.25/−.50 | −.50/−.50; −.25/−.50 |
| Color vision (confusion index) (RE; LE) | 1.78; 1.81 | 2.17; 1.95 |
| Dark adaptation | Slightly elevated (.5 log unit) final threshold | Normal final threshold |
| ERG: | | |
| Scotopic[b] | ISCEV rod response just below the lower (5th) percentile range of unaffected, mixed rod/cone response (SF), slightly reduced; IT normal, b/a-wave ratio markedly reduced ("negative" ERG) | ISCEV rod response just below the lower (5th) percentile range of unaffected, mixed rod/cone response (SF) slightly reduced, IT normal, b/a ratio markedly reduced ("negative" ERG) |
| Photopic | Markedly reduced, prolonged IT | Markedly reduced, prolonged IT |
| Anterior segment | Normal | Posterior synechiae after iridocyclitis (at age 33 years) |
| Fundus | Slight mottling of the pigment epithelium in the foveal region, otherwise inconspicuous | LE, slight mottling of the pigment epithelium in the foveal region, epiretinal gliosis LE, otherwise inconspicuous |

NOTE.—For both patients, the first symptom was glare sensitivity during early childhood, and isopter results were I/2e, with mild or very mild concentric constriction. LE = left eye; RE = right eye.

[a] sph = Spherical; cyl = cylindrical.

[b] SF = standard flash.

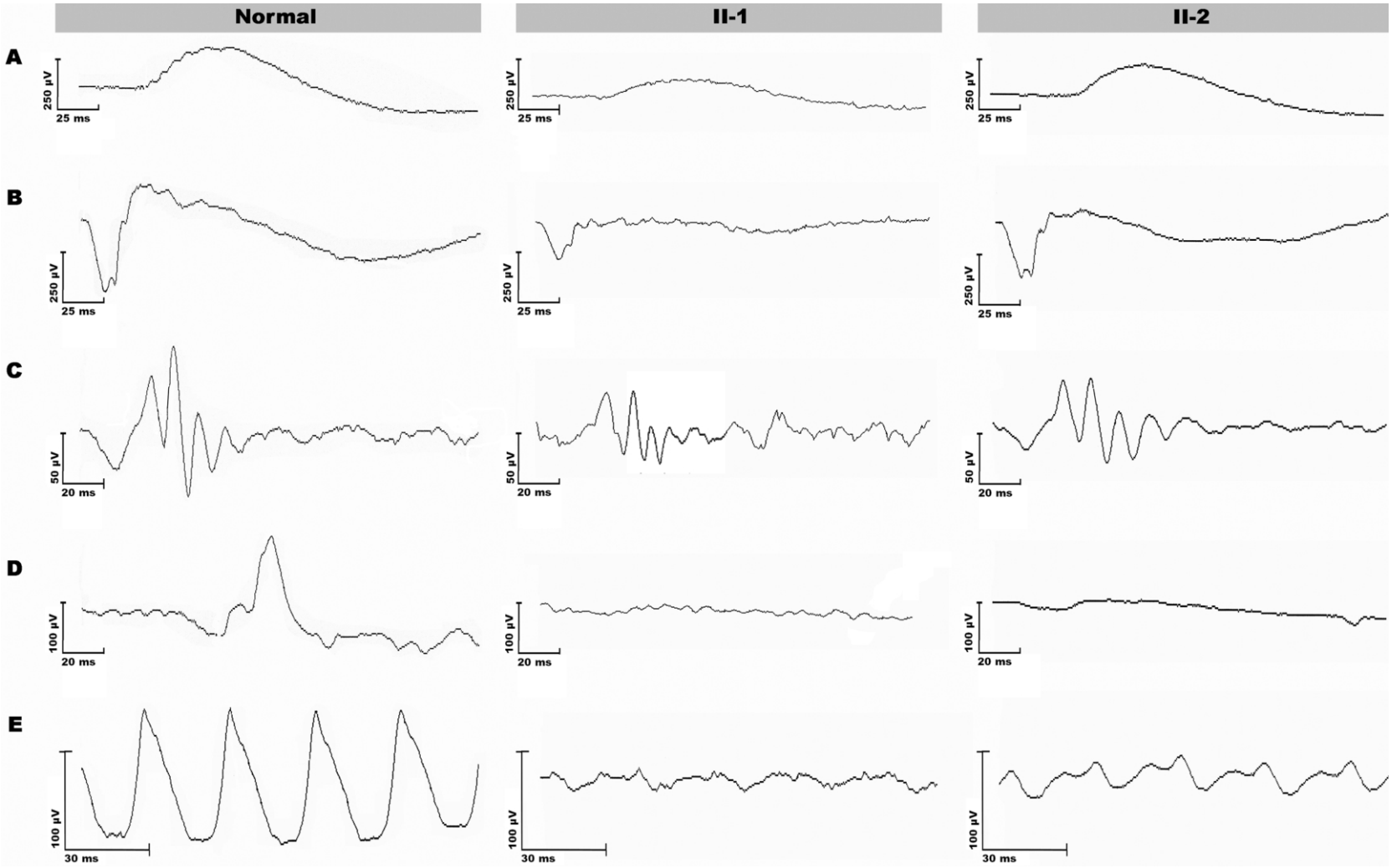


**Figure 4.** ERG of patients carrying the p.Y802X mutation and ERG recordings in the right eye of patients II-1 and II-2. The left panel shows representative recordings of an unaffected subject, the middle panel shows the recordings of patient II-1, and the right panel shows recordings of patient II-2. Rod responses (*A*), maximal combined responses (*B*), oscillatory potentials (*C*), single-flash cone responses (*D*), and 30-Hz flicker responses (*E*), each according to ISCEV standard,[14] are shown. The negative-oriented a-wave of an ERG response reflects the hyperpolarization of photoreceptors due to a flash of light. The positive-oriented b-wave represents a summation of responses evoked by activities of secondary neurons during photoreceptor synaptic signal transmission. Both patients share a frequency doubling of the 30-Hz flicker ERG.

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## Web Resources

The accession number and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for *CACNA2D4* [accession number NM_172364.3])
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *CACNA2D4, CACNA1F,* and *CABP4*)

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