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A novel GCAP1(N104K) mutation in EF-hand 3 (EF3) linked to autosomal dominant cone dystrophy

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ABSTRACT

The *GUCA1A* gene encodes a guanylate cyclase activating protein (GCAP1) that is involved in regulation of phototransduction in the vertebrate retina. We discovered a novel C312A transversion in exon 2 of the human *GUCA1A* gene, replacing Asn-104 (N104) in GCAP1 with Lys (K), in two affected members of a family with dominant cone dystrophy. The mutation N104K is located in the third EF-hand motif (EF3) shown previously to be instrumental in converting Ca^{2+}-free GCAP1 to a GC inhibitor in the Ca^{2+}-bound form. In one patient, rod ERGs were fairly stable over a 12-year-period whereas 30 Hz flicker ERG and single-flash cone ERGs declined. In both patients, double-flash ERGs showed that rod recovery from an intense test flash was significantly delayed. The EC_{50} for GC stimulation shifted from ~250 nM in wild-type GCAP1 to ~800 nM in the GCAP1(N104K) mutant suggesting inability of the mutant to assume an inactive form under physiological conditions. The replacement of N104 by K in GCAP1 is the first naturally occurring mutation identified in the EF3 loop. The rod recovery delays observed in double-flash ERG of affected patients suggest a novel dominant-negative effect that slows GC stimulation.

1. Introduction

Photoreceptors, using a process termed phototransduction, receive light and generate an electrical impulse that is sent to the visual cortex (Burns & Arshavsky, 2005; Ridge, Abdulaev, Sousa, & Palczewski, 2003; Stephen, Filipek, Palczewski, & Sousa, 2008). Key events in phototransduction are the hydrolysis of cGMP, the secondary messenger of phototransduction, and closure of cGMP-gated cation channels (Polans, Baehr, & Palczewski, 1996). Channel closure triggers a change in free [Ca^{2+}] (Nakatani, Chen, Yau, & Koutalos, 2002; Pugh, Nikonov, & Lamb, 1999) and re-synthesis of cGMP by membrane-associated guanylate cyclases (GCs) present in rod and cone outer segments (Baehr et al., 2007). Changes in cytoplasmic [Ca^{2+}] are monitored by GC-activating proteins (GCAPs) which are Ca^{2+}-binding proteins of the calmodulin superfamily (Palczewski, Sokal, & Baehr, 2004). Conformations of GCAPs change in response to Ca^{2+} binding. In dark-adapted outer segments, free Ca^{2+} is controlled by a light-insensitive NCKX and the cGMP-gated channel, and adjusts to about 250–600 nM (Woodruff et al., 2007). Under these conditions, high affinity Ca^{2+}-binding sites (EF-hands) on GCAPs are saturated, GCAPs are inactive and the GCs display a low basal activity (Gorczyca, Gray-Keller, Detwiler, & Palczewski, 1994a). In response to light, free [Ca^{2+}] drops to less than 50 nM, Ca^{2+} dissociates from Ca^{2+}-binding sites, and GCAPs convert into activators accelerating cGMP synthesis roughly 8- and 10-fold.

GCAPs are *N*-myristoylated neuronal Ca^{2+} sensors in which the acyl side chain is buried in the Ca^{2+}-bound as well as the Ca^{2+}-free state (Stephen, Bereta, Golczak, Palczewski, & Sousa, 2007). A hallmark of the GCAP structure are high affinity Ca^{2+}-binding sites termed EF-hands (Falke, Drake, Hazard, & Peerson, 1994; Gifford, Walsh, & Vogel, 2007; Persechini, Moncrief, & Kretsinger, 1989) consisting of a helix-loop-helix secondary structure that is able to chelate Ca^{2+} ions. EF-hands also have affinity for Mg^{2+} ions but the interaction is several orders of magnitude weaker (Gifford et al., 2007). In the canonical EF-hand, the loop consists of 12 amino acids rich in acidic residues providing oxygen ligands for Ca^{2+} coordination. The loop is flanked by hydrophobic residues (I, L, Y, W). GCAPs have two pairs of EF-hands, one each in the N-terminal and C-terminal half of the molecule. The first EF-hand in the N-terminal region is

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nonfunctional, as Ca^{2+} coordination is prevented by lack of acidic side chains providing oxygen for Ca^{2+} coordination (Palczewski et al., 2004). The N-terminal region including EF1 has a key role in interaction with the target protein GC (Ermilov, Olshevskaya, & Dizhoor, 2001; Krylov, Niemi, Dizhoor, & Hurley, 1999; Li, Sokal, Bronson, Palczewski, & Baehr, 2001). EF-hands 2–4 are fully functional, canonical EF-hand Ca^{2+}-binding sites. Their individual roles have been explored mostly by site-directed mutagenesis, and recording of conformational changes in the absence and presence of Ca^{2+} and/or Mg^{2+} (Otto-Bruc et al., 1997; Peshenko & Dizhoor, 2007; Rudnicka-Nawrot et al., 1998; Sokal et al., 1999).

Pathogenic mutations of residues flanking EF3 and EF4, as well as several residues of the EF4 loop of GCAP1, are associated with autosomal dominant cone dystrophy (adCD) or autosomal dominant cone-rod dystrophy (adCRD) (for review: (Baehr & Palczewski, 2007)). Four mutations are known to affect Ca^{2+}-binding of EF-hands (Y99C, I143NT, L151F, E155G) (Dizhoor, Boikov, & Olshevskaya, 1998; Jiang et al., 2005; Nishiguchi et al., 2004; Sokal et al., 1998, 2005; Wilkie et al., 2001). The Y99C mutation is located adjacent to EF3 (Payne et al., 1998) and I143NT adjacent to the EF4-hand motif (Nishiguchi et al., 2004). Two mutations (E155G, L151F) are located in EF4. These mutations alter the dissociation constant and coordination of Ca^{2+} to the mutant loop, and change the Ca^{2+} sensitivity of GCAP1. As a result, mutant GCAPs are not fully inactivated at dark Ca^{2+} levels, leading to the persistent stimulation of GC1 in the dark, elevated cGMP and Ca^{2+} levels, and cell death. To date, no naturally occurring mutations affecting one of the 12 amino acids of the Ca^{2+}-binding loop of EF3 had been identified. Here, we investigated a single family with dominant cone dystrophy and identified a novel mutation in the *GUCA1A* gene in which Asn-104 (N104), located in EF3, is replaced by lysine. It is predicted that the N104K substitution will dramatically affect the ability of the mutant GCAP1 to inhibit GC1 in dark-adapted cone photoreceptors.

2. Methods

2.1. Patients and mutation identification

This study was approved by each Institutional Review Board of the University of Utah Hospitals and Clinics, and the University of Texas Southwestern Medical Center-Dallas. All subjects provided informed consent prior to participation. Some subjects underwent complete ophthalmologic examination including visual acuity measurements and fundus examinations. Blood samples from two family members (#8279 and #4940, Fig. 1) were collected under consignment and genomic DNA was extracted using the Puregene DNA isolation kit. Each of the four exons of GCAP1 was amplified by PCR using flanking intron-specific primers (GCAP1_exon1F: 5′-GGCCTGTCCATCTCAGACGT; GCAP1_ exon1R: 5′-CCCCAGCTGGTC AGGCTTCCAG; GCAP1_ exon2F: 5′-GCCTGAGGCTGGAGTGAGCG; GCAP1_ exon2R: 5′-CTAACCCTGGGCTCTCAGTTCC; GCAP1_exon3F: 5′-CCTGAGATAGGATAAGGATGG; GCAP1_exon3R: 5′-ACCCCACATC CATGGTGACC; GCAP1_exon4F: 5′-CTGGACTGCAGAAATGAACAC CCTC; GCAP1_exon4R: 5′-GGCGAGCTAAGCCTCTGAGTTC) and screened for mutations by denaturing high performance liquid chromatography (DHPLC; WAVE® System, Transgenomic, Omaha, NE). Sequence alterations were identified by direct sequencing with a CEQ Dye Terminator Cycle Sequencing Kit on Beckman-Coulter CEQ 8000 Genetic Analysis System, according to the manufacturer's instructions and using established methods (Yang et al., 2005, 2006).

2.2. Electroretinography

Full-field ERGs were obtained following ISCEV standards (Marmor, Holder, Seeliger, & Yamamoto, 2004). Following pupil dilation (1% cyclopentolate hydrochloride and 2.5% phenylephrine hydrochloride) and 45 min dark-adaptation, ERGs were recorded as detailed (Birch & Fish, 1987). PC-based custom software was used for stimulus control and timing, data acquisition, averaging, and analysis.

Inactivation kinetics were probed with the "paired-flash" paradigm (Birch, Hood, Nusinowitz, & Pepperberg, 1995), using a test flash of 2.4 log sc td-s. The paired-flash ERG method involves the presentation of a bright probe flash at a defined time after the test flash, and determination of the prevailing response to the *test* stimulus by analysis of the *probe* flash response. Underlying the method

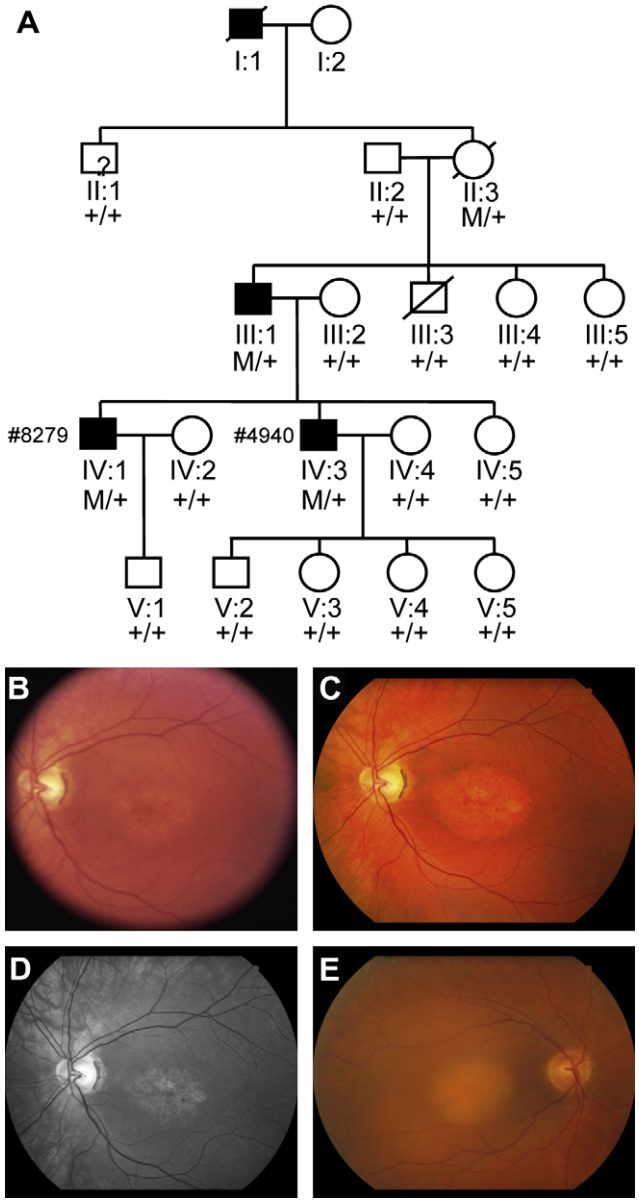

Fig. 1. (A) Pedigree of the study family with autosomal dominant cone dystrophy: Individuals are identified by pedigree number. Squares indicate males; circle, females; slashed symbols, deceased; solid symbols, affected; open symbols, unaffected; +/+, two copies of wild-type; M/+, one copy of wild-type, one copy of mutant. (B–D) Fundus photos of the posterior pole of the left eye of patient # 4940 at two separate visits, seven years apart. In June of 2000a Topcon 50 degree camera was used with Kodak Ektachrome 100 film and later digitized (B). In April of 2007, a Canon camera with EMI digital capturing system was used to take color posterior pole at 60° (C) and a "red-free" image at 40° for better contrast of the macular changes (D). (E) Fundus photo of the posterior pole in patient # 8279 taken with Canon camera. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)