

**Mutations in the gene *PDE6C* encoding the catalytic subunit of the cone photoreceptor phosphodiesterase in patients with achromatopsia**

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**Abstract**

Biallelic *PDE6C* mutations are a known cause for rod monochromacy, better known as autosomal recessive achromatopsia (ACHM), and early-onset cone photoreceptor dysfunction. *PDE6C* encodes the catalytic  $\alpha'$ -subunit of the cone photoreceptor phosphodiesterase, thereby constituting an essential part of the phototransduction cascade. Here, we present the results of a study comprising 176 genetically pre-selected patients who remained unsolved after Sanger sequencing of the most frequent genes accounting for ACHM, and were subsequently screened for exonic and splice site variants in *PDE6C* applying a targeted next generation sequencing approach. We were able to identify potentially pathogenic biallelic variants in 15 index cases. The mutation spectrum comprises 18 different alleles, 15 of which are novel. Our study significantly contributes to the mutation spectrum of *PDE6C* and allows for a realistic estimate of the prevalence of *PDE6C* mutations in ACHM since our entire ACHM cohort comprises 1074 independent families.

Keywords: Achromatopsia, *PDE6C*, cone phosphodiesterase, mutation spectrum and prevalence

Achromatopsia (ACHM; ACHM2 MIM# 216900, ACHM3 MIM# 262300, ACHM4 MIM# 613856, ACHM5 MIM# 613093, ACHM6 MIM# 610024, ACHM7 MIM# 616517) is a rare autosomal recessive cone disorder characterized by color vision defects, photophobia, nystagmus, and severely reduced visual acuity. To date, six genes have been linked to ACHM. In the Western population approximately 80% of the patients carry mutations in the genes *CNGA3* (MIM# 600053) (Kohl et al., 1998) and *CNGB3* (MIM# 605080) (Kohl et al., 2000; Sundin et al., 2000) encoding the two subunits of the cone photoreceptor cyclic nucleotide-gated channel. Much less frequently, causative mutations have been found in genes encoding other crucial components of the cone phototransduction cascade, namely *GNAT2* (MIM# 139340) (Aligianis et al., 2002; Kohl et al., 2002), *PDE6C* (MIM# 600827) (Chang et al., 2009; Thiadens et al., 2009), and *PDE6H* (MIM# 601190) (Kohl et al., 2012), or in *ATF6* (MIM# 605537) (Kohl et al., 2015), which is not involved in the phototransduction cascade, but in the unfolded protein response pathway. Larger case series or genetic screens of ACHM-associated genes are sparse (Kohl et al., 2005; Nishiguchi, Sandberg, Gorji, Berson, & Dryja, 2005; Wissinger et al., 2001; Zelinger et al., 2015; Mayer et al. 2017), therefore we lack a comprehensive view of the prevalence especially of the minor disease genes in ACHM.

In the present study, 176 patients diagnosed with ACHM who remained unsolved after Sanger sequencing of the most frequent genes accounting for ACHM, namely *CNGB3*, *CNGA3*, and *GNAT2*, were screened for exonic and splice site variants in the *PDE6C* gene. Samples from all patients and family members were recruited in accordance with the principles of the Declaration of Helsinki and were obtained with written informed consent accompanying the patients' samples. The study was approved by the institutional review board of the Ethics Committee of the University Hospital of Tuebingen.

While two patients were screened using a custom capture panel targeting 105 retinal disease genes including *PDE6C* (Glöckle et al. 2014), the others were analyzed by means of an amplicon-based next generation sequencing approach. Briefly, target enrichment of coding sequences including exon-intron boundaries of *PDE6C* (Supp. Table S1) was performed with Fluidigm 48.48 Access Arrays. Library capture was completed using the Nextera XT DNA Library Prep Kit and sequencing was performed on a MiSeq instrument at a core facility (c.ATG, Tübingen). Sequence data were aligned using the Burrows-Wheeler Aligner (Li & Durbin, 2009), and variants were called using an in-house bioinformatic pipeline. Only non-synonymous single nucleotide variants, nonsense variants, splice site ( $\pm 10$  bps) variants, insertions, duplications and deletions represented by more than 20 sequence reads were considered for further analysis. In addition, variants with a minor allele frequency (MAF)  $>1\%$  in the Genome Aggregation Database (gnomAD) Version r2.0.2 were excluded from further investigation. The potential pathogenicity of missense changes was assessed using five online prediction software tools, namely SIFT (<https://sift.jcvi.org/>) (Kumar, Henikoff, & Ng, 2009), PolyPhen-2 (<https://genetics.bwh.harvard.edu/pph2/>) (Adzhubei et al., 2010), Mutation Taster (<https://www.mutationtaster.org/>) (Schwarz, Cooper, Schuelke, & Seelow, 2014), Mutation Assessor (<http://mutationassessor.org/r3/>) (Reva, Antipin, & Sander, 2011) and Provean (<http://provean.jcvi.org>) (Choi & Chan, 2015). Prediction outcomes are given in Suppl. Table S2. The variant designation is based on the NCBI reference sequence for *PDE6C* (NC\_000010.11, NM\_006204.3; GRCh38) comprising 22 coding exons. We were able to identify potentially pathogenic biallelic variants in 15 index cases, thereby achieving a detection rate of 8.5%. All putative disease-associated variants in the *PDE6C* gene were validated and tested for segregation with the phenotype in available family members by conventional Sanger sequencing. The variants were seen in true

homozygous state in two patients and in apparent homozygous state in seven patients, respectively (Supp. Table S3). Compound heterozygosity was observed for two patients based on the analysis of paternal alleles. In one patient, *trans* configuration of variants was established by allelic cloning. Compound heterozygosity could not be demonstrated for three patients because DNA of family members was not available and the respective variants were located too far apart for allelic cloning. The mutation spectrum comprises 18 different alleles, 15 of which are novel (Table 1). All novel variants were deposited to the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) (Landrum et al., 2014) with accession codes provided in Table 1. The location of the variants identified in this study with respect to the PDE6C protein is depicted in Figure 1A.

All index patients harboured unique *PDE6C* genotypes with the exception of the missense variant p.I714N which was found in homozygous state in three independent patients. In gnomAD this variant is present in heterozygous state in only one single subject (1/245,508 alleles). The fact that we saw it in three independent patients was therefore indicative of a founder effect. Genotyping of microsatellite and SNP markers indeed revealed a common haplotype for the three patients (see Supp- Figure S1).

Potential pathogenicity of variants was determined on the basis of: (1) representing ultrarare alleles observed only in single cases or being absent in 277,264 general population alleles in gnomAD; (2) in the case of missense variants being predicted to be damaging by at least four out of five effect prediction programs listed above; (3) representing likely null alleles (nonsense, canonical splice site and frameshift variants); (4) having already been described to be pathogenic; and (5) analysed functionally (p.R29W; Grau et al. 2011). All variants were classified according to their pathogenicity based on the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al. 2015) (see Table 1). Since

nonsense, canonical splice site and frame-shifting variants have a strong weight in the ACMG scoring system, this class of variants are consequently classified either as likely pathogenic or pathogenic. An exception is the known non-canonical splice site variant c.939+5G>A (Abouelhoda et al., 2016), which is classified as a variant of unknown significance (VUS) since the +5 position is not invariable.

All missense variants we identified have an extremely low MAF or are even absent in gnomAD. In addition, their evolutionary conservation and localization as well as the type of the respective amino acid substitution are strong indicators of pathogenicity. However, following the ACMG guidelines, seven of the eight missense variants we identified are classified as VUS. This classification would only change to pathogenic if functional data (e.g. of an enzymatic activity assay) were supportive of a damaging effect as is the case for the p.R29W variant (Grau et al., 2011).

A summary of clinical findings is shown in Supp. Table S3 including all index patients and two affected siblings (patients 1-1 and 1-2). All patients were diagnosed in early childhood (ranging from birth to 5 years) and displayed characteristics of ACHM like photophobia, nystagmus and impaired color vision. Electroretinography (ERG) results were not available from every patient but generally revealed normal rod responses and either extinguished or severely reduced cone responses with the exception of patient 6 in whom ERG recordings also showed reduced b-waves in the scotopic ERG and an electronegative standard flash. We therefore re-classified his diagnosis from ACHM to cone-rod dystrophy. Optical coherence tomography (OCT) images were only available from six patients and revealed the typical disappearance of P2 (photoreceptor reflectivity) in patients with ACHM (Barthelmes, Sutter, Kurz-Levin, Bosch, Helbig, Niemeyer, & Fleischhauer, 2006).

The Human Gene Mutation Database currently lists 38 variants in *PDE6C* that explain the disease phenotype in the respective patients. Our study significantly contributes to the mutation spectrum of *PDE6C* and allows for a realistic estimate of the prevalence of *PDE6C* mutations in ACHM in the European population since our entire cohort comprises 1074 independent families mainly originating from Europe or being of European descent (USA, Canada, Australia, New Zealand). Considering an estimated prevalence of 1:30.000 to 1:50.000 for ACHM in Europe, this number is certainly high enough to give a comprehensive view on the spectrum and prevalence not only of the more common *CNGB3* and *CNGA3* mutations, but also on the four minor, non-CNG channel encoding ACHM genes. Taking together the results of the present study and a previous screening approach (Grau et al. 2011), we calculate a prevalence of 2.4% for *PDE6C* mutations in our cohort which is most probably representative for ACHM in the Western population (Figure 1B). As ACHM is in the focus of retinal gene therapy with four clinical trials ongoing, our study provides a valuable resource for putative gene therapy trials targeting *PDE6C*.

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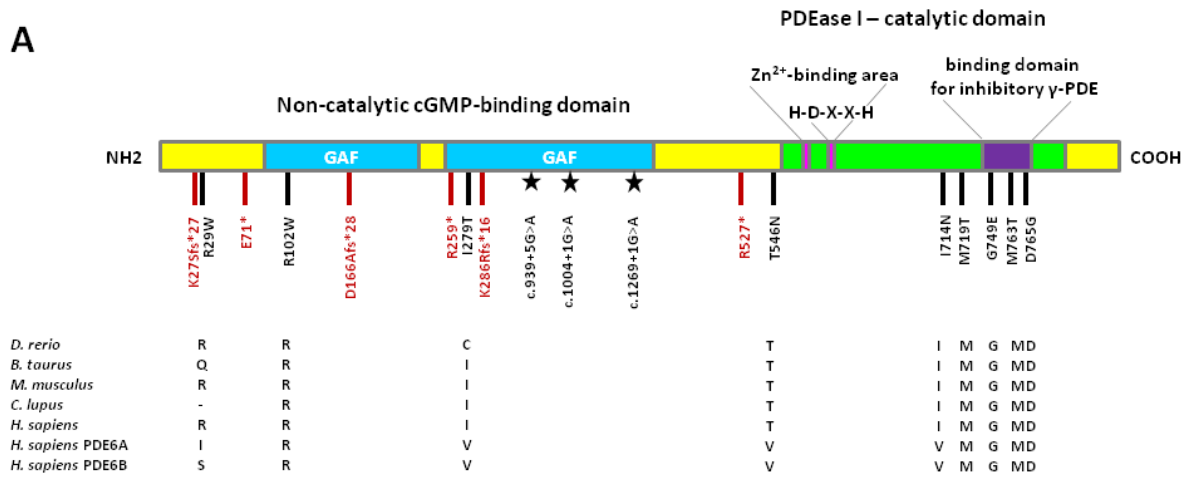
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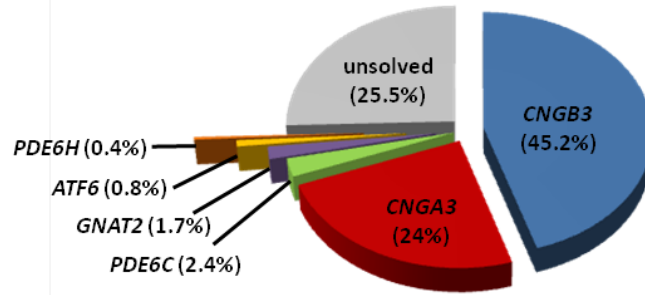
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**FIGURE 1** Localization and prevalence of *PDE6C* variants. **A:** Localization of protein truncating (nonsense, frame-shifting) (in red/gray) and missense variants (in black) identified in this study. The relative position of the three splice site variants is indicated with black stars. A comparative sequence analysis is given below the missense variants, showing conservation of the respective cone photoreceptor specific *PDE6C* amino acid residue according to HomoloGene (<https://www.ncbi.nlm.nih.gov/homologene/>). In addition, the respective amino acid residues in human rod photoreceptor *PDE6A* and *PDE6B* are shown. NCBI reference sequences are as follows: *D. rerio* (NP\_957165.1), *B. taurus* (NP\_776844.1), *M. musculus* (NP\_291092.1), *C. lupus* (XP\_543934.3), *H. sapiens* (NP\_006195.3), *H. sapiens* *PDE6A* (NP\_000431.2), *H. sapiens* *PDE6B* (NP\_000274.2) **B:** Prevalence of mutations in genes causing ACHM relating to our cohort of 1074 independent families. Mutations in approximately 25% of ACHM cases remain to be identified, or clinical diagnosis recapitulated.

**A**



**B**



Author M

**Table 1:** Sequence variants in PDE6C

Nucleotide (NM_006204.3)	PDE6C protein (NP_006195.3)	Reference(s)	ClinVar accession no.	Protein domain	Consensus prediction for missense variants <sup>a</sup>	ACMG <sup>b</sup>	ACMG prediction <sup>c</sup>	gnomAD MAF
c.939+5G>A	n.a. <sup>d</sup> (p.F290_E314del)	Abouelhoda et al., 2016	RCV000171185.1	n.a.		PM2;PM4;PP3	VUS	None
c.1004+1G>A	n.a. <sup>d</sup> (p.E314Dfs*11)	Huang et al., 2013	n.a.	n.a.		PM2;PVS1;PP3	Pathogenic (Ic)	0.00003228
c.1269+1G>A	n.a. <sup>d</sup> (p.K374_E423del)	This study	SCV000678420	n.a.		PM2;PVS1;PM4	Pathogenic (Ib)	None
c.211G>T	p.E71*	This study	SCV000678421	n.a.		PM2;PVS1	Likely pathogenic (I)	0.00000813
c.775C>T	p.R259*	This study	SCV000678422	n.a.		PM2;PVS1	Likely pathogenic (I)	0.00000813
c.1579C>T	p.R527*	This study	SCV000678423	n.a.		PM2;PVS1	Likely pathogenic (I)	0.0000447
c.78del	p.K27Sfs*27	This study	SCV000678424	n.a.		PM2;PVS1	Likely pathogenic (I)	None
c.497del	p.D166Afs*28	This study	SCV000678425	n.a.		PM2;PVS1	Likely pathogenic (I)	None
c.857del	p.K286Rfs*16	This study	SCV000678426	n.a.		PM2;PVS1	Likely pathogenic (I)	None
c.85C>T	p.R29W	Thiadens et al., 2009; Grau et al., 2011	SCV000086939.1	N terminal domain	Damaging	PM2;PM1;PP3;PS3	Pathogenic (II)	0.00002164
c.304C>T	p.R102W	This study	SCV000678427	GAF domain	Damaging	PM2;PM1;PP3	VUS	None
c.836T>C	p.I279T	This study	SCV000678428	GAF domain	Damaging	PM2;PM1;PP3;PM3	Likely pathogenic (IV)	0.00001804
c.1637C>A	p.T546N	This study	SCV000678429	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	None
c.2141T>A	p.I714N	This study	SCV000678430	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	0.00000407
c.2156T>C	p.M719T	This study	SCV000678431	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	0.00006457
c.2246G>A	p.G749E	This study	SCV000678432	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	None
c.2288T>C	p.M763T	This study	SCV000678433	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	None
c.2294A>G	p.D765G	This study	SCV000678434	Catalytic domain	Damaging	PM2;PM1;PP3	VUS	None

<sup>a</sup>Consensus determined as prediction of a damaging effect by at least 4 of 5 free web-based applications (SIFT, PROVEAN, PolyPhen-2, Mutation Taster, Mutation Assessor); <sup>b</sup>categories published in the ACMG guidelines; <sup>c</sup>Combination of scores leading to final classification in one of five pathogenicity categories; <sup>d</sup>*in silico* assessment using five algorithms embedded in the Alamut software (<http://www.interactive-biosoftware.com>) predicted that skipping of the respective exon is likely. The consequence of the putative exon skipping on protein level is given in brackets. n.a., not applicable. VUS, variant of uncertain significance; MAF, minor allele frequency.