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Genetics and Disease Expression in the CNGA3 Form of Achromatopsia

Steps on the Path to Gene Therapy

Lina Zelinger, PhD,[1] Artur V. Cideciyan, PhD,[2] Susanne Kohl, PhD,[3] Sharon B. Schwartz, MS, CGC,[2] Ada Rosenmann, MD, PhD,[1] Dalia Eli, PhD,[1] Alexander Sumaroka, PhD,[2] Alejandro J. Roman, MS,[2] Xunda Luo, MD, PhD,[2] Cassondra Brown, BS,[2] Boris Rosin, MD, PhD,[1] Anat Blumenfeld, PhD,[1] Bernd Wissinger, PhD,[3] Samuel G. Jacobson, MD, PhD,[2] Eyal Banin, MD, PhD,[1,] Dror Sharon, PhD[1,*]*

Purpose: Achromatopsia (ACHM) is a congenital, autosomal recessive retinal disease that manifests cone dysfunction, reduced visual acuity and color vision, nystagmus, and photoaversion. Five genes are known causes of ACHM. The present study took steps toward performing a trial of gene therapy in ACHM by characterizing the genetics of ACHM in Israel and the Palestinian Territories and analyzing retinal function and structure in *CNGA3* ACHM patients from the Israeli–Palestinian population and US patients with other origins.

Design: Case series study.

Participants: Patients with clinically suspected ACHM, cone dysfunction phenotypes, and unaffected family members were included. The protocol was approved by the local institutional review board and informed consent was obtained from all participants.

Methods: Genetic analyses included homozygosity mapping and exome sequencing. Phenotype was assessed with electroretinography (ERG), optical coherence tomography, psychophysics, and photoaversion testing.

Main Outcome Measures: Single nucleotide polymorphism microarray, exome analysis, DNA sequence analysis, visual function testing including ERG, and photoaversion.

Results: We identified 148 ACHM patients from 57 Israeli and Palestinian families; there were 16 *CNGA3* mutations (5 novel) in 41 families and 5 *CNGB3* mutations (1 novel) in 8 families. Two *CNGA3* founder mutations underlie >50% of cases. These mutations lead to a high ACHM prevalence of ~1:5000 among Arab-Muslims residing in Jerusalem. Rod ERG abnormalities (in addition to cone dysfunction) were detected in 59% of patients. Retinal structure in *CNGA3* ACHM patients revealed persistent but abnormal foveal cones. Under dark- and light-adapted conditions, patients use rod-mediated pathways. Photoaversion was readily demonstrated with transition from the dark to a dim light background.

Conclusions: Among Israeli and Palestinian patients, *CNGA3* mutations are the leading cause of ACHM. Retinal structural results support the candidacy of *CNGA3* ACHM for clinical trials for therapy of cone photoreceptors. Efficacy outcome measures would include chromatic light-adapted psychophysics, with attention to the photoreceptor basis of the response, and quantitation of photoaversion. *Ophthalmology 2015;■:1–11 © 2015 by the American Academy of Ophthalmology.*

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Inherited retinal degenerations can be classified by different criteria, such as the pattern of disease progression and type of retinal cells affected.[1,2] Diseases affecting predominantly the cone photoreceptors are visually disabling owing to abnormal visual acuity, impaired color vision, visual field disturbances, and sensitivity to ambient light levels. Cone disorders can be progressive, such as various molecular forms of cone–rod degeneration (CRD) and cone dystrophy (CD), or symptoms are mostly stationary, showing no major progression, such as blue cone monochromatism and achromatopsia (ACHM).

Achromatopsia is a heterogeneous group of inherited congenital, autosomal recessive (AR) retinal diseases characterized by severely reduced visual acuity, severely impaired or complete color blindness, nystagmus, photoaversion, the absence of cone function as measured by full-field (FF) electroretinography (ERG), and minimal findings on clinical ophthalmoscopic examination. Five genes encoding proteins that are involved in cone phototransduction are currently known to cause the disease. These genes encode the 2 subunits of the cone cyclic guanosine monophosphate–regulated cation channel (*CNGA3*[3] and

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CNGB3[4,5]), the alpha subunit of the cone-specific G-protein transducin (*GNAT2*[6]), and the active and inhibitory subunits of the cone-specific phosphodiesterase (*PDE6C*[7] and *PDE6H*,[8] respectively). Mutation analysis of these genes in European subjects revealed that *CNGB3* is the major cause of ACHM, mainly owing to a single frameshift mutation (c.1148delC), which is found in approximately 40% of ACHM cases.[9]

Although ACHM is considered a relatively nonprogressive disease affecting only cone function, 2 recent studies showed that retinal structure and rod function may be affected to some extent in some ACHM patients.[10,11] Although no cure for ACHM is currently available, gene therapy performed on animal models for 3 ACHM genes (*CNGA3*, *CNGB3*, and *GNAT2*) show promising results.[12–14] An accurate genetic and clinical diagnosis of patients with ACHM might therefore enable therapeutic intervention in the near future.[15]

We reported previously genetic findings in a number of ACHM patients from the United States[16,17] and identified a founder mutation in the *CNGA3* gene causing ACHM in Muslim and Jewish populations representing 10 families living in Israel.[18] That observation prompted this study, which shows that ACHM is a relatively common retinal phenotype in the Israeli and Palestinian populations. A comprehensive genetic and clinical analysis revealed the identification of 21 disease-causing mutations in the *CNGA3* and *CNGB3* genes in 49 families (including 130 patients). Mutations in *CNGA3* are the most common cause of ACHM in these populations, mainly owing to 2 founder mutations that can be identified on >50% of disease-causing alleles.

The components of a clinical trial are thus coming together for the *CNGA3* form of ACHM: identification of a large population of these patients in the current study, clarification that there is abnormal but detectable cone structure in human cross-sectional imaging across a wide age range, devising and testing of feasible outcome measures in the present work, and identifying and characterizing small and large animal models of *CNGA3* disease,[12,19–23] and proof of concept of gene augmentation therapy efficacy in a murine model with a *CNGA3* mutation.[13,24]

Materials and Methods

Subjects

Patients with clinically suspected ACHM as well as patients with cone dysfunction as determined by FF ERG testing were included. The tenets of the Declaration of Helsinki were followed, and informed consent and assent were obtained from all patients who participated in this study before donation of a blood sample for DNA extraction and before phenotype assessment. The research was approved by the institutional review board at the Hadassah Medical Center and the University of Pennsylvania. Samples were obtained from the index patient and other affected and unaffected family members upon availability. Genomic DNA was extracted from peripheral blood using the FlexiGene DNA kit (QIAGEN).

Mutation Analysis

Primers flanking all coding *CNGA3* exons and *CNGB3* exon 10 were either used as previously described[3,5] or designed using Primer3 (http://primer3.ut.ee). The primer sequences are available by request. Polymerase chain reaction was performed in 30 μl reaction with 35 cycles. Mutation analysis was performed by direct sequencing of polymerase chain reaction products. The possible pathogenicity of missense changes was evaluated using PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) and MutationTaster (http://www.mutationtaster.org/).

Microarray Analysis

Whole-genome single nucleotide polymorphism (SNP) analysis was performed by either the 10K or 250K Affymetrix system. A region of homozygosity was determined if the hybridization quality had a P value >0.05 and if 500 consecutive homozygote markers (Affymetrix 250K platform) or ≥5 cM of homozygous markers (Affymetrix 10K platform) were identified.

Exome Sequencing

Whole-exome sequencing was performed on the index case of family MOL0332 using the Roche NimbleGen V2 preparation kit and the HiSeq2000 sequencer (Illumina). The average exome coverage was 49. The DNAnexus software package was used for aligning reads to the human reference sequence (UCSC Genome Browser hg19) and variant calling. We used the wANNOVAR web server for sequence variant annotations. We identified 178 420 sequence variants; 23 746 of them were within the exons or splice sites. We found 233 variants in retinal genes. After filtering for variant frequency, effect on protein, and coverage, 11 variants remained. Of the 11 variants, 2 (c.1640T>G [p.F547C] and c.904A>G [p.R302G]) were located in the *CNGA3* gene.

Phenotype Assessment

All patients underwent a full ophthalmologic examination. Kinetic perimetry, color vision testing using the Ishihara 38 plate and Farnsworth D-15 tests, and FF ERG were also performed. We performed FF ERG according to the International Society for Clinical Electrophysiology of Vision standard.[25] A subset of patients from the Israeli–Palestinian population and the US population were further evaluated with specialized methods. Retinal structure was assessed with spectral domain optical coherence tomography and analyzed according to published methods.[26] In brief, retinal layers corresponding to the outer nuclear layer (ONL), inner segment ellipsoid (ISe), cone outer segment (COS) tips, and rod outer segment (ROS) tips were segmented manually using backscatter intensity and slope information. Tests were also devised to quantify the aversion to light in these patients. White background light at different intensities was presented to both eyes using a Ganzfeld stimulator (Colordome, Diagnosys LLC, Lowell, MA) and custom programs. The subject's reaction to the light was recorded using 10-s-long videos of both eyes from the internal camera of the instrument. Two photoaversion tests were performed. In the first test, subjects were presented with a dim background of 0.6 $cd \cdot m^{-2}$ immediately after a dark adaptation period of 10 minutes. Videos were obtained in the dark as well as in the dim background. In the second test, 7 higher background intensities in the range of 1.6 to 400 $cd \cdot m^{-2}$ were presented in increasing steps of 0.4 log units. The transitions between intensities were gradual (2 s) and each intensity level lasted 5 s. For both tests, the average distance between the eyelids (palpebral fissure height) was measured on a vertical line