The Spectrum of Retinal Diseases Caused by NR2E3 Mutations in Israeli and Palestinian Patients

Dikla Bandah, MSc; Saul Merin, MD; Munther Ashhab, MD; Eyal Banin, MD, PhD; Dror Sharon, PhD

Objectives: To evaluate the involvement of NR2E3 in inherited retinal degenerative diseases in the Israeli and Palestinian populations and to study phenotypic variability in patients who are homozygous for the same mutation.

Methods: Patients from 35 families underwent clinical evaluation, including a full ophthalmologic examination and electroretinography. Genetic analyses included direct sequencing of polymerase chain reaction products and haplotype reconstruction.

Results: We recruited 6 consanguineous Muslim families and 2 Jewish families with enhanced S-cone syndrome. Patients from 4 of the Muslim families were homozygous for the same NR2E3 mutation, c.194-2A>G, but showed considerable variability in fundus appearance and retinal function, even among patients of comparable ages. Both Jewish patients were compound heterozygotes for the c.932G>A mutation in combination with c.194-202del9bp or a novel splice-site mutation, c.747+1G>C. Homozygosity analysis in 27 consanguineous families with retinitis pigmentosa revealed a homozygous mutation, c.932G>A, in 2 families. The electroretinographic responses in these patients were compatible with retinitis pigmentosa and did not show the characteristic enhanced S-cone syndrome pattern.

Conclusion: Our results demonstrate the involvement of NR2E3 in enhanced S-cone syndrome and retinitis pigmentosa phenotypes in our populations.

Clinical Relevance: Patients with NR2E3 mutations may manifest variable phenotypes. Moreover, patients who are homozygous for the same NR2E3 mutation have variable expression of retinal disease, suggesting the involvement of modifier genes.


Photoreceptor differentiation is a precisely controlled developmental process regulated by the function of transcription factors, some of which (eg, CRX, NRL, NOTCH1, and NR2E3) have been extensively studied during the last decade.1-5 Mutations in 3 of these genes (NR2E3, NRL, and THRB) can cause a unique retinal phenotype, enhanced S-cone syndrome (ESCS), in which the retina is enriched with blue (short wavelength) cone photoreceptors while rod photoreceptors are depleted. Enhanced S-cone syndrome is an autosomal recessive retinopathy in which patients have increased sensitivity to blue light, reduced visual acuity, night blindness that begins early in life, varying degrees of red (long wavelength) and green (middle wavelength) cone-mediated vision, and retinal degeneration (RD). Enhanced S-cone syndrome has been described in patients with NR2E3 mutations,6,8 in the rd7 mouse due to a homozygous NR2E3 mutation,10 in a family with NRL mutations,11 in a knockout mouse model for Nrl,1 and in a knockout mouse model for Thrb.12

The NR2E3 gene, also known as PNR (photoreceptor-specific nuclear receptor), encodes a retinal nuclear receptor that is a ligand-dependent transcription factor. The NR2E3 protein is part of a large family of nuclear receptor transcription factors involved in signaling pathways.13 Mutations in NR2E3 were initially described in patients with ESCS6 but later were associated with other retinal diagnoses.7,14,15 Mutations in NR2E3 were associated with Goldmann-Favre syndrome (GFS),6,7,14 a vitreoretinopathy characterized by liquefied vitreous body with preretal band-shaped structures (veils), macular changes in the form of retinoschisis or cystoid edema, and pigmentary degeneration of the retina with nystagm and extinguished electretinograms (ERGs). In addition, about 50% of cases with clumped pigmentary retinal degeneration (CPRD) are due to NR2E3 mutations.7 Recently, specific NR2E3 mutations were shown to cause autosomal dominant retinitis pigmentosa in European families.15

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There is no clear genotype-phenotype correlation among the different autosomal recessive retinal dystrophies caused by NR2E3 mutations. The same mutations can cause either phenotype, suggesting that ESCS, GFS, and CPRD might represent different stages or expressions of NR2E3 disease, as suggested previously by psychophysical, electrophysiological, and genetic studies.6–10

The purposes of this study were to evaluate the role of NR2E3 mutations in different forms of retinal disease among Israeli and Palestinian patients and to study phenotypic variability in patients who are homozygous for the same mutation.

PATIENTS AND MOLECULAR ANALYSES

The tenets of the Declaration of Helsinki were followed and informed consent was obtained from all patients who participated in this study before donation of a blood sample. We included 35 families with different clinical phenotypes of hereditary retinal disease. Genomic DNA was extracted using a commercially available kit (FlexiGene DNA kit; Qiagen, Hilden, Germany). Genotyping of single-nucleotide polymorphism (SNP) markers within NR2E3 (rs8178 and rs12898728) was performed by means of restriction analysis using the enzymes BclI and MnlI, respectively. Genotyping of the microsatellite marker D15S131 was performed on a DNA sequencer (ABI 3700; Applied Biosystems, Foster City, California). Primers flanking NR2E3 exons were designed using Primer3 (available at http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3 www.cgi). Polymerase chain reaction (PCR) was performed in a 20-µL reaction with 35 cycles. Mutation analysis of NR2E3 was performed first for the 2 common mutations (c.119-2A>C and c.932G>A) by restriction enzymes (Ddel and HpaII, respectively), then by direct sequencing of PCR products of all the exons. Gene accession numbers of the studied genes (all at NCBI Entrez Gene) are as follows: for NR2E3, NM_006177.3; and for THRB1, NM_000461.4.

CLINICAL EVALUATION

A full ophthalmologic examination, including visual acuity, ocular motility, and pupillary reaction assessments plus biomicroscopic slitlamp and dilated fundus examinations, was performed in all patients. Optical coherence tomography (OCT-3; Zeiss Humphrey Systems, Jena, Germany) was performed when macular edema or thickening was suspected. Subsequently, perimetry, color vision testing, full-field ERG, and electro-oculography were performed according to patient ability and level of cooperation. Full-field ERGs were recorded using corneal electrodes and a computerized system (UTAS 3000; LKC Technologies Inc, Gaithersburg, Maryland) as previously described.11 Briefly, in the dark-adapted state, a rod response to a dim blue flash and a mixed cone-rod response to a white flash were acquired. Cone responses to 30-Hz flashes of white light were acquired under a background light of 21 candelas/m². All ERG responses were filtered at 0.3 to 500 Hz, and signal averaging was used.

RESULTS

MUTATION ANALYSIS OF NR2E3

We recruited 6 consanguineous Muslim families and 2 nonconsanguineous Jewish families with a clinical diagnosis of ESCS or GFS. Disease-causing mutations in NR2E3 were identified in 6 of the families (Figure 1). The only mutation we identified among Muslim patients was a previously described splicing mutation, c.119-2A>C (IVS1-2A>C), found homozygously in 4 of the 6 Muslim families we studied (Table). In the remaining 2 Muslim families (MOL0521 and MOL0599), no disease-causing mutations were identified in the NR2E3 gene. A screen for the c.119-2A>C mutation in 57 healthy control subjects matched for ethnicity (114 chromosomes) did not reveal any mutant chromosomes. Patients from the 2 Jewish families were compound heterozygotes for NR2E3 mutations (Table and Figure 1). Patient MOL0528 II.1, from an Ashkenazi Jewish family, was heterozygous for the previously described mutation c.932G>A (Arg311Gln) and for a novel splicing mutation c.747+1G>C (IVS5+1g>c) (Figure 2). This novel mutation is likely to cause a frameshift due to exon 5 skipping (176 nucleotides). Patient MOL0581 II.1 was a compound heterozygote for the c.932G>A mutation and a 9–base pair deletion within exon 2 (c.194-
In this study, we examined the possible involvement of the NR2E3 gene in ARRP by screening for mutations in 27 consanguineous families with ARRP. We identified mutations in the NR2E3 gene in 2 patients from 2 families, and we also studied 111 healthy controls.

**Table. Clinical Data of Patients With NR2E3 Mutations**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Initial Diagnosis</th>
<th>Patient's Origin</th>
<th>Consanguinity</th>
<th>Mutations 1 and 2</th>
<th>Refraction</th>
<th>Visual Acuity</th>
<th>FFERG Rod a/b Waves, μV</th>
<th>FFERG Mixed Cone-Rod a/b Waves, μV</th>
<th>FFERG 30-Hz Cone Response, μV/ms</th>
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</thead>
<tbody>
<tr>
<td>MOL0002</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>+3.50/−1.00</td>
<td>20/20</td>
<td>16</td>
<td>98,203</td>
<td>±193/25 ±286/51</td>
</tr>
<tr>
<td>II:1/M:16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/20</td>
<td></td>
<td>27</td>
<td>238,309</td>
<td>±134/25 ±192/51</td>
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<tr>
<td>MOL0046</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>+6.00/−1.50</td>
<td>20/20</td>
<td>23</td>
<td>28,48</td>
<td>Severely reduced (BE)</td>
</tr>
<tr>
<td>II:7/F:22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/20</td>
<td></td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
</tr>
<tr>
<td>MOL0560</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>−0.75 sph</td>
<td>20/20</td>
<td>20</td>
<td>298,369</td>
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</tr>
<tr>
<td>II:1/F:19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/20</td>
<td></td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
</tr>
<tr>
<td>MOL0461</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>+0.50/−1.50</td>
<td>20/20</td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
</tr>
<tr>
<td>II:1/F:12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20/20</td>
<td></td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
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<tr>
<td>MOL0461</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>+0.75/−1.50</td>
<td>20/20</td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
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<td></td>
<td></td>
<td></td>
<td>20/20</td>
<td></td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
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<tr>
<td>MOL0528</td>
<td></td>
<td></td>
<td></td>
<td>c.119-2A&gt;C, c.932G&gt;A</td>
<td>+1.00/−1.50</td>
<td>20/20</td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
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<tr>
<td>II:1/M:32</td>
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<td></td>
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<td>±138/13 ±241/42</td>
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<tr>
<td>MOL0581</td>
<td></td>
<td></td>
<td></td>
<td>c.932G&gt;A</td>
<td>−0.50/−0.50</td>
<td>20/20</td>
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<td>298,369</td>
<td>±138/13 ±241/42</td>
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<td>II:1/M:7</td>
<td></td>
<td></td>
<td></td>
<td>c.932G&gt;A</td>
<td>−0.50/−0.50</td>
<td>20/20</td>
<td>20</td>
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<td>±138/13 ±241/42</td>
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<td></td>
<td></td>
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<td>c.932G&gt;A</td>
<td>−0.50/−0.50</td>
<td>20/20</td>
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<td>298,369</td>
<td>±138/13 ±241/42</td>
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<td>II:1/F:31</td>
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<td></td>
<td>20/20</td>
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<td>20</td>
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<td>MOL0368</td>
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<td></td>
<td></td>
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<td>c.932G&gt;A</td>
<td>−0.50/−0.50</td>
<td>20/20</td>
<td>20</td>
<td>298,369</td>
<td>±138/13 ±241/42</td>
</tr>
</tbody>
</table>

Abbreviations: BE, both eyes; ESCS, enhanced S-cone syndrome; Ext, extinguished; FC, finger counting; FFERG, full-field electroretinogram; GFS, Goldmann-Favre syndrome; NA, not available; NP, not performed; RP, retinitis pigmentosa; sph, sphere.

Indicates mixed cone-rod responses under photopic and scotopic conditions.

Indicates 30-Hz cone flicker response (reference amplitude value, >60 μV; reference implicit time, ≤33 ms).

Indicates severe impairment of the rod response with atypical supernormal cone responses under photopic and scotopic conditions (Table). However, among Muslim patients homozygous for the same c.119-2A>C mutation, clinical findings varied. Patient MOL0560 II:1, at a young age, had relatively mild funduscopic changes with elongated deep flecks and preserved foveal structure (Figure 3 A, D, E, and H). Patient MOL0045 (a microsatellite marker, D15S131, located 0.9 megabase toward the 3' end of the NR2E3 gene) was negative. The mutation phase is unknown. The c.747C>A mutation, located in the NR2E3 gene, was not detected.

**Clinical Characteristics of Patients With NR2E3 Mutations**

Most of the patients with NR2E3 mutations had clinical findings within the spectrum previously described in ESCS and GFS. They manifested decreased visual acuity early in life, hypermetropia, clumped pigment in their peripheral retina, and characteristic ERG findings. These include severe impairment of the rod response with atypical supernormal cone responses under photopic and scotopic conditions (Table). However, among Muslim patients homozygous for the same c.119-2A>C mutation, clinical findings varied. Patient MOL0560 II:1, at a young age, had relatively mild funduscopic changes with elongated deep flecks and preserved foveal structure (Figure 3 A, D, E, and H). Patient MOL0045 (a microsatellite marker, D15S131, located 0.9 megabase toward the 3' end of the NR2E3 gene) was negative. The mutation phase is unknown. The c.747C>A mutation, located in the NR2E3 gene, was not detected.
II:2, at 20 years of age, manifested perimacular atrophy, classic pigment clumps beyond the arcades, and severe cystoid macular edema (Figure 3C, G, and I). Two siblings, also homozygous for the c.119-2A>H11022C mutation (MOL0461 II:1 and II:2), were clinically diagnosed as having GFS in view of significant vitreous involvement. The full-field ERG findings in these 2 patients differed markedly from those of the other patients with the same mutation and from the classic ESCS findings, with very markedly reduced responses under all types of stimulus conditions (Table). These results suggest widespread and severe RD involving all photoreceptor types, including blue (short wavelength) cones.

With the aim of studying the possibility that other genes that play roles in determination of photoreceptor fate might modulate retinal disease expression caused by NR2E3 mutations, we sequenced the open reading frame of NRL and THRB1 in the 4 index patients who were homozygous for c.119-2A>H11022C. The analysis did not reveal any sequence changes.

Two additional patients who were homozygous for the c.932G>H11022A mutation had similar severely reduced full-field ERG responses that differ from those usually seen in ESCS and were initially clinically diagnosed as having retinitis pigmentosa (patients MOL0099 II:1 and MOL0363 IV:1) (Table).

**COMMENT**

We report herein the first NR2E3 analysis, to our knowledge, in patients with RD from Israel and the Palestinian territories. Our analysis revealed previously described mutations occurring in multiple families of the same origin and a novel splice-site mutation. In addition, we include data showing that NR2E3 mutations are associated with variable retinal phenotypes, even among patients who are homozygous for the same NR2E3 mutation.

Among Israeli and Palestinian Muslim patients with ESCS, c.119-2A>C was the most common NR2E3 mutation identified. This mutation has been reported thus far as 1 of the 2 most common NR2E3 mutations.6-9 We predict that the carrier frequency of this mutation is not high because none of our 124 control chromosomes was mutant. Only 2 Jewish families (both of Ashkenazi origin) with ESCS were recruited for this study, and both had compound heterozygosity for NR2E3 mutations, sharing the c.932G>H11022A mutation. The c.932G>H11022A mutation has been previously reported to be the cause of retinal disease in multiple European Jewish families: patients from a large Crypto-Jewish family with retinitis pigmentosa and CPRD were reported to be homozygous for this mutation.18 In a later report,8 the c.932G>H11022A mutation was found in 7 of 9 Ashkenazi Jewish patients with ESCS (5 homozygotes and 2 compound heterozygotes). These results indicate that c.932G>H11022A is the most common NR2E3 mutation in the Ashkenazi Jewish population. Interestingly, a retinitis pigmentosa–like rather than an ESCS phenotype was present in 1 Muslim and 1 Jewish patient in our cohort who were homozygous for the c.932G>H11022A mutation.

Patients from 4 Muslim families were homozygous for a single NR2E3 mutation, c.119-2A>C. This allowed us a rare opportunity to compare the retinal phenotype of similarly aged patients with an identical NR2E3 geno-
type. Recently, a comprehensive clinical analysis of pa-
tients with ESCS by Audo et al9 revealed variability in the
fundus appearance (from normal to pigment clumping
and foveal or peripheral schisis) and the severity of ERG
abnormalities in patients with a variety of
NR2E3

mutations. Our analysis extends the observed phenotypic vari-
ability and shows that patients who are homozygous for the same mutation (c.119-2A

/H11022

C) and share the same eth-
nic origin can manifest variable funduscopic and ERG
phenotypes. Why such relatively wide variation in reti-
nal phenotype exists among a relatively genetically ho-
mozygous group of patients is intriguing. A search for
additional mutations in possible modulator genes (NRL
and
THRB1

) that would perhaps explain this variability
was negative. An alternative explanation could be vari-
able expression of the protein product among patients,
despite their harboring of the same homozygous muta-
tion. Indeed, the effect of the c.119-2A>C mutation on
the transcribed NR2E3 messenger RNA was studied pre-
viously using transient transfection assay of COS7 cells.19
The mutation was shown to produce the normal tran-
script and a mutant transcript in which exon 2 is skipped,
resulting in the generation of a premature stop codon.19
The observation that a mutation in the second intronic
base of the donor splice site does not result in a total splic-
ing defect is rare and might cause a variable amount of
normal protein among a homozygous set of patients, and
hence a variable phenotype. In addition, interaction with
other genetic and/or environmental factors among dif-
ferent individuals may also underlie the observed phe-
notypic variability.

To date, only a few studies have reported on the degree
of variability of retinal phenotype among patients with au-
tosomal recessive RD who are homozygous for the same

Figure 3. Variability of retinal funduscopic appearance (A-G) and degree of foveal involvement as seen on optical coherence tomography (H and I) in patients who are homozygous for the c.119-2A>C NR2E3 mutation. Findings ranged from relatively mild retinal pigmentary epithelial changes with elongated deep flecks in patient MOL00560 II:1 at 19 years of age (A, D, and E) through discrete areas of chorioretinal atrophy in patient MOL0002 II:1 at 16 years of age (B and F) to the more classic appearance of clumped pigment and atrophy in patient MOL0045 II:2 at 22 years of age (C and G). Foveal involvement also markedly differed, with preserved structure in patient MOL00560 II:1 (H) as opposed to severe cystoid edema and schisis in patient MOL0045 II:2 (I).
disease-causing mutation. Patients of Ashkenazi Jewish origin who were homozygous for the N48K mutation in the USH3A gene were reported to have a variable expression of hearing loss and vision impairment.\(^{20}\) We have previously reported homozygosity for splice-site mutations in the CERKL and the ABCA4 genes to cause unique but much less variable RD phenotypes in Yemenite Jewish patients\(^{21}\) and Muslim patients.\(^{17}\)

In summary, we report herein, to our knowledge, the first mutation analysis of the NR2E3 gene among Israeli and Palestinian Muslim patients. The most common NR2E3 mutation identified in this population was c.119-2A>C, which was associated with variable clinical manifestations. Our results add to the disease spectrum associated with NR2E3 and exemplify phenotypic variability among patients who are homozygous for the same autosomal recessive mutation.

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REFERENCES


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