Phenotypic Features of Patients With NR2E3 Mutations

Sophia I. Pachydaki, MD; Carolyn C. Klaver, MD; Irene A. Barbazetto, MD; Monique S. Roy, MD; Peter Gouras, MD; Rando Allikmets, PhD; Lawrence A. Yannuzzi, MD

Objective: To describe the phenotypes of 5 patients with NR2E3 mutations.

Methods: Two patients with familial and 3 with sporadic early-onset nyctalopia and retinal pigment abnormalities were screened for mutations in the NR2E3 gene (OMIM 604485). The clinical course, fundus features, visual field test results, and fluorescein angiographic and electrophysiologic findings were compared.

Results: Three different mutations in NR2E3 were identified: R311Q and 2 novel mutations—missense change Q350R and an in-frame deletion of phenylalanine at position 71 (delF71) in exon 2. Three patients who were homozygous for R311Q had posterior subcapsular cataracts and a concentric ring of round pigment clumps. Electroretinograms were extinguished. A fourth patient, a 24-year-old man who was heterozygotic for R311Q and Q350R, had Goldmann-Favre syndrome. A fifth patient, a 10-year-old boy with heterozygotic mutations R311Q and delF71, had diminished foveal reflexes and subtle pigmentary changes, perhaps a forme fruste of Goldmann-Favre syndrome. Both of these patients had an identical spectral electroretinographic pattern characteristic of enhanced S-cone syndrome.

Conclusions: Molecular genetic testing is essential for establishing the correct diagnosis in patients with NR2E3 mutations because of the variable phenotype associated with these degenerations. Two novel NR2E3 mutations are described that are associated with Goldmann-Favre syndrome and enhanced S-cone syndrome.

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The NR2E3 gene codes for a nuclear receptor that is specific to photoreceptors.\(^1\)\(^2\) It is thought that one of its principle functions is to repress the expression of cone-specific genes in rods.\(^4\) Mutations in this gene have been associated with a variety of autosomal recessive retinal degenerations, including enhanced S-cone syndrome (ESCS), Goldmann-Favre syndrome, and clumped pigmented retinal degeneration.\(^3\)\(^8\) A common feature of these syndromes is a uniquely abnormal electroretinographic pattern in which there is maximal response to short wavelengths and virtually no change in response waveform to light and dark adaptation; this is invariably associated with nyctalopia and some long- and middle-wavelength-sensitive cone dysfunction. The unique ERG syndrome was first thought to be due to abnormal rods responding at photopic levels.\(^3\)\(^4\)\(^11\) Subsequently, high subjective S-cone spectral sensitivity was demonstrated, and the term ESCS became associated with this particular ERG syndrome, although some patients maintain rod function at threshold.\(^12\)\(^13\) In addition to a variety of mutations in the NR2E3 gene that have been linked to autosomal recessive retinal abnormalities, a recently described dominant mutation produces a severe form of retinitis pigmentosa.\(^16\)\(^17\) This vast diversity of retinal abnormalities related to the NR2E3 gene warrants further probing of the relationship of phenotype to genotype associated with this gene.

Author Affiliations:
Department of Ophthalmology, Columbia University, New York, New York (Drs Pachydaki, Klaver, Barbazetto, Gouras, and Allikmets); Institute of Ophthalmology and Visual Science, University of Medicine and Dentistry, New Jersey Medical School, Newark (Dr Roy); Vitreous-Retina-Macula Consultants of New York and the LuEsther T. Mertz Retinal Research Center, Manhattan Eye, Ear, and Throat Hospital, New York (Dr Yannuzzi).

METHODS

CLINICAL EVALUATION

Four patients were recruited from a tertiary ophthalmologic referral center (Vitreous-Retina-Macula Consultants of New York) with a suspected diagnosis of Goldmann-Favre syndrome and 1 patient (patient 5) was recruited from the ophthalmology department of Columbia University. Two patients were blood relatives (patients 1 and 2 are cousins). These 2 patients had the same 4 grandparents. In addition, we studied 3 individuals not related by blood (patients 3, 4, and 5). The clinical pre-
presentation, fundus features, visual field perimetry results, and findings from fluorescein angiography, ocular coherence tomography, and ERG were compared. Spectral ERGs were obtained in patients 4 and 5 using a technique that has been shown to reveal the typical ESCS pattern. Informed consent was obtained from participants after explanation of the procedures. All the studies conformed to institutional guidelines and the Declaration of Helsinki.

**MUTATION SCREENING**

The entire coding region of the NR2E3 gene was screened by means of direct sequencing. The DNA of all patients was extracted from peripheral leukocytes and was amplified by means of polymerase chain reaction with oligonucleotide pairs designed in exon-flanking regions of NR2E3. The polymerase chain reaction products were directly sequenced from both strands.

**RESULTS**

**PATIENT 1**

The patient was a 60-year-old man in good general health with a history of nystagmus since childhood. His vision had remained relatively stable during his disease. His best-corrected visual acuity was 20/40 OD and 20/60 OS with moderate myopia OU. There was no afferent pupillary defect. He had right esotropia with right preferential fixation. On slitlamp examination, the corneas were clear and the anterior chamber was normal bilaterally. There was +1 and +2 nuclear sclerosis in the right and left eyes, respectively, with significant posterior subcapsular lens opacities bilaterally. Intraocular pressure was normal in both eyes. Fundus examination showed a bilaterally symmetrical appearance of the retina (Figure 1). His optic discs had mild pallor. The retinal vessels were mildly attenuated. There was a ring of markedly dark, round, clumped pigment in the midperiphery and mild retinal pigment epithelium depigmentation in the macula in both eyes. No vitreous abnormalities were noted. Ocular coherence tomography revealed no cystoid macular edema or macular schisis. Fluorescein angiography showed slight hyperfluorescence in the macula secondary to window defects. Electroretinography performed using a white light stimulus revealed undetectable scotopic and photopic responses (performed at New York University Medical Center, New York, New York).

The patient’s best-corrected visual acuity at age 39 years was recorded as 20/40 OU. Fundus examination at that time showed incipient midperipheral pigmentation. Cystoid edema or retinoschisis was not observed. The ERG was undetectable (performed at New York University Medical Center). Mutation analysis of the NR2E3 gene showed a homozygous G>A change at position 932 in exon 6 that resulted in the amino acid change R311Q.

**PATIENT 2**

This 60-year-old male patient was a cousin of the proband (patient 1). He was a 60-year-old man with a history of nystagmus since childhood. Visual acuity was 20/60 OU. Slitlamp examination showed posterior subcapsular cataracts in both eyes. Fundus examination revealed a prominent ring of dark, round, clumped pigment in the midperiphery bilaterally. Ocular coherence tomography and fluorescein angiography showed an intact macula without cystic changes or schisis. Visual field testing showed constriction within 15° of fixation bilaterally. The patient did not consent to ERG. Sequencing of the NR2E3 gene revealed the same mutation as in the proband: a homozygous R311Q change.

**PATIENT 3**

A 63-year-old man experienced a gradual decline in vision of a few months’ duration. His ocular history was notable for poor night vision since birth. He was diagnosed as having atypical retinitis pigmentosa at age 17 years. His medical history was significant for hypertension and hypothyroidism. His family history was unremarkable for ocular diseases. Best-corrected visual acuity was 20/80 OD and 20/200 OS, with moderate myopia in both eyes. Motility examination findings were unremarkable. There was no afferent pupillary defect. On slitlamp examination, the corneas were clear and the ante-
rior chamber was normal bilaterally. There were bilateral nucleosclerotic and posterior subcapsular changes. Intraocular pressure was normal in both eyes. On fundus examination, no vitreous abnormalities were noted. The fundus appearance was bilaterally symmetrical; a well-defined ring of heavy clumped pigmentation was noted in the midperiphery in both eyes around an intact macular area (Figure 2). Mild sheathing of the retinal vessels was noted in the periphery. Fluorescein angiography showed mild cystoid macular changes. Visual field testing showed symmetrical severe constriction bilaterally of less than 20° of fixation. The ERG with a white light stimulus revealed undetectable scotopic and photopic responses (performed at New York University Medical Center). Mutation analysis of the NR2E3 gene showed the homozygous amino acid change R311Q.

PATIENT 4

A 24-year-old man had a diagnosis of juvenile retinoschisis. He had decreased vision at age 11 years. Bilateral cryopexy was performed at age 16, and a vitrectomy was performed in the right eye to flatten macular schisis at age 17. Before surgery, his visual acuity was 20/200 OD and 20/300 OS. His current visual acuity is 20/400 OD and 20/200 OS, both improving to 20/100 with a pinhole. Slitlamp examination revealed posterior subcapsular lens opacities bilaterally (Figure 3). Intraocular pressures were normal. Fundoscopic examination of the right eye revealed that the macula was anatomically flat, with atrophic and pigmentary disturbances. In the left eye, he had a large confluent schisis cavity in the posterior pole (Figure 4). Peripheral retinoschisis was noted inferiorly in both eyes. The vitreous was optically empty in both eyes; a prominent vitreous band was present inferiorly. Visual fields were constricted to within 20° of fixation symmetrically in both eyes. Ocular coherence tomography revealed a flat macula in the right eye and extensive cystic changes in the macula in the left eye (Figure 5). His ERG showed an ESCS pattern that changed nonsignificantly across 4 years (Figure 6). In Figure 6, the ERGs of the right eye are shown in the light-adapted and dark-adapted states; those of the left eye are identical. The different spectral stimuli were obtained from calibrated Wratten filters (Eastman Kodak Company, Rochester, New York). In the light-adapted state, the arrows in Figure 6 point out that yellow light elicited a corneal positive β-wave and that blue light elicited mainly a negative α-wave, typical of ESCS. In the dark-adapted state, the response to blue was larger than to any other spectral stimulus, although the green stimulus is strongest for rods. Screening of the NR2E3 gene revealed compound heterozygous mutations—R311Q and a novel heterozygous 1049A>G variant in exon 7, resulting in the amino acid change Q350R.

PATIENT 5

A 10-year-old boy had a 2-year history of poor vision and nyctalopia. His family history was noncontributory. His visual acuity was 20/25 OD and 20/20 OS. Anterior segment examination findings were unremarkable. Fundus examination revealed a similar picture in both eyes. The foveal reflex was absent in the right eye but not in the left eye. There was macular pigment heterogeneity in both eyes. In the midperiphery, there was retinal pigment epithelium hypopigmentation and some retinal pigment epithelium mottling. No pigmentary deposits were observed. His optic discs and vessels were normal. His ERG responses were identical to those of patient 4 (Figure 6). Sequencing of the NR2E3 gene revealed compound heterozygous mutations—R311Q and a novel in-
frame deletion of 3 nucleotides, 211-213delTTC in exon 2, resulting in the absence of the amino acid phenylalanine at position 71 (delF71).

These 5 patients illustrate a variety of phenotypes associated with autosomal recessive NR2E3 mutations, including Goldmann-Favre syndrome, ESCS, and clumped pigment retinopathy. Patients 1, 2, and 3 had global retinopathy with nyctalopia, constricted visual fields, profoundly reduced ERGs, and clumped pigment. The retinitis-pigmentosa–like picture was caused by autosomal recessive NR2E3 mutations. All 3 of these patients had R311Q, the most common mutation associated with NR2E3, in homozygous form. The only clinical clue that their retinal degeneration was due to an NR2E3 mutation was the presence of clumped pigment. The key evidence for the diagnosis, however, came from genotyping.

Patients 4 and 5 are different from the previous 3 patients in having compound heterozygous mutations. The clinical pictures of these 2 patients were similar to each other but very different from those of the other 3 patients. They have nyctalopia but do not have undetectable ERGs. One patient had Goldmann-Favre syndrome with retinoschisis, and the other may have a forme fruste of this syndrome. Both patients are identical in having the typical ESCS pattern in their ERGs. Both patients also have the common R311Q mutation on one allele but different and novel mutations on the other allele. These 2 patients could be suspected of having NR2E3 mutations because of their unique ERGs, and this suspicion was confirmed by means of genotyping. This compound heterozygosity of their mutations has produced a change in the phenotype compared with the other 3 patients with homozygous R311Q mutations.

The fact that there can be complex interactions between different heterozygous mutations in the NR2E3 gene has recently been highlighted by the discovery of mutation R56G in the NR2E3 gene, which exhibits dominant inheritance. The phenotype of the dominant mutation produces a typical retinitis pigmentosa–like retinopathy with nyctalopia, restricted visual fields, attenuated retinal vessels, and a profoundly reduced ERG signal.
The variability of clinical features and the severity of retinal degeneration produced by NR2E3 mutations may often complicate the diagnosis. Few patients with NR2E3 mutations show schisis characteristic of Goldmann-Favre syndrome or have the ERG signature of ESCS or clumped pigment. Most mutations lead to autosomal recessive disease, but one causes a dominant disease. Therefore, molecular studies of this gene are required to make the diagnosis that is most relevant for understanding the pathogenesis of this spectrum of condition and potentially provide some form of therapy for affected patients.

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Correspondence: Lawrence A. Yanuzzi, MD, Vitreous-Retina-Macula Consultants of New York, 460 Park Ave, 5th Floor, New York, NY 10022.

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REFERENCES

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According to Greeff interstitial keratitis attacks persons below the age of forty, females more frequently than males. It usually depends upon hereditary syphilis and occasionally upon acquired. Next to syphilis the most frequent cause is tuberculosis, and rheumatic influences also may bring it about. There is doubtles some connection between interstitial keratitis and malaria, diabetes, influenza, and affections of female genit organs.