Retina

Double Concentric Autofluorescence Ring in NR2E3p.G56R-Linked Autosomal Dominant Retinitis Pigmentosa

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PURPOSE. We reported an unusual appearance of fundus autofluorescence (FAF) associated with *NR2E3*-p.G56R-linked autosomal dominant retinitis pigmentosa (ADRP).

METHODS. Patients were enrolled among three generations in a Swiss family. Molecular diagnosis identified a c.166G > A (p.G56R) mutation. Ophthalmic examination included fundus photography, FAF near-infrared autofluorescence (NIA), optical coherence tomography (OCT), and visual fields (VF).

RESULTS. Fundus examination revealed a wide range of features from unremarkable to attenuated arterial caliber, clumped and spicular pigment deposits in the mid-periphery and optic nerve pallor. FAF showed a double concentric hyperautofluorescent ring: an inner perimacular ring that tended to be smaller in older patients, and an outer ring located along the vascular arcades, which appeared to extend over time toward the periphery and eventually became hypoautofluorescent. The inner and outer hyperautofluorescent rings were seen both on NIA and FAF at a similar localization. There was also a spatial correspondence between the loss of photoreceptor inner segment and outer segment junction on OCT and the area delimited by both double FAF and NIA rings. VF showed either mid-peripheral annular scotoma or constricted visual field loss in advanced cases, correlating with dystrophic nonfunctional retinal regions demarcated by the hyperautofluorescent annuli. A double ring of hyperautofluorescence was observed in all but one patient of two additional families, but not in patients harboring mutations in other ADRP genes, including PRPF3, RHO, RP1, PRPH2, PROM1, and CTRP5.

CONCLUSIONS. The presence of a double concentric hyperautofluorescent ring of FAF may represent a highly penetrant early phenotypic marker of *NR2E3*-p.G56R-linked ADRP. (*Invest Ophthalmol Vis Sci.* 2012;53:4754-4764) DOI: 10.1167/iovs.11-8693

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Corresponding author: Francis L. Munier, Hôpital Ophtalmique Jules Gonin, Avenue de France 15, CH-1007 Lausanne, Switzerland; francis.munier@fa2.ch. **N**R2E3 [MIM# 604,485], also called photoreceptor-specific nuclear receptor (PNR), is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors, uniquely restricted to photoreceptor cells.¹ The physiological functions of NR2E3 identified so far are: repression of cone generation program in retinal progenitor cells²; repression of cone-specific transcription in adult rod photoreceptor cells³⁻⁵; and activation of rhodopsin expression in adult rod photoreceptor cells.^{3,5} This dual function of NR2E3 is developmentally regulated by SUMOylation (small ubiquitin-related modifier-posttranslational modification), which converts NR2E3 into a repressor.⁶ In the absence of NR2E3, short wavelength (S-) cones continue to proliferate, disrupting the retinal architecture by formation of rosettes and whorls.⁷ Rods become nonfunctional hybrid photoreceptors ("cods"), expressing both rod- and cone-specific genes.⁸

The 33 recessive and one dominant mutation identified so far in the NR2E3 gene have been listed in a public database at www.lovd.nl/eye.9 Recessive mutations in NR2E3 caused Goldmann-Favre syndrome (GFS), also called enhanced short wavelength (S-) cone sensitivity syndrome (ESCS: enhanced Scone syndrome; MIM# 268100).10,11 Initial diagnosis of a substantial number of patients was clumped pigmentary retinal degeneration (CPRD) or autosomal recessive RP (ARRP).^{12,13} A high phenotypic variability has been observed in patients affected with recessive NR2E3-linked retinal degenerations, but because of the presence of nonfunctional rod photoreceptors, all patients suffered from night blindness early in life. On fundus photography, a mid-peripheral RPE atrophy with nummular pigment deposits along the vascular arcades was a characteristic clinical sign.^{9,14,15} The proliferation of S-cones was detected by spectral ERG as a pathognomonic hyperfunction in response to blue light.¹⁶

Intriguingly, a unique dominantly inherited c.166G > A (p.G56R) mutation located in the DNA-binding domain (DBD) of NR2E3 caused ADRP (MIM# 611131]).¹⁷ So far, a total of 11 families have been reported, and this mutation may account for approximately 1.5%-2% of ADRP cases.¹⁷⁻²¹ In contrast to all recessive *NR2E3* mutations located in the DBD, the NR2E3-p.G56R mutant protein remained bound to a master regulator of photoreceptor development, the cone-rod homeobox (CRX) transcription factor.²² By acting as a trans-repressor of CRX, the NR2E3-p.G56R mutant protein might recapitulate some aspects of the severe cone-rod dystrophy and Leber's congenital amaurosis observed in the presence of dominant mutations located in the *CRX* gene.^{23,24}

Among the reported families affected by *NR3E3*-p.G56R-linked ADRP, only two have been clinically described in detail so far.¹⁷ This study reports in detail the clinical characteristics of a new Swiss kindred, with emphasis on the identification of distinctive clinical features by lipofuscin-related fundus auto-fluorescence (FAF)²⁵ and melanin-related near-infrared auto-fluorescence (NIA).²⁶

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TABLE 1. Primers Used for Molecular Diagnosis of NR2E3

NR2E3	Forward Primer (5'-3')	Reverse Primer (5'-3')	Length (bp)
Exon 1	TTGGTAATGCTGCAGGTGG	ATTCTGGGTTTACCCACAGG	554
Exons 2-3	TTCGTTCAAATGCGGGTGAGC	GTGTTGGACTCCATGCTGTC	662
Exon 4	GCTGAAGAAGTGCCTGCAGG	GTTGTGATCTTAGCGCCTGC	489
Exon 5	GGGGCTCCAAGTACTCCCTG	TCACCATCCCTGAGATGCAC	428
Exon 6	TCTGAGCCTCTGGCTGATGTCA	AGAAGGGAGTCCAGCCTCAC	545
Exon 7	CACTCCTGGTTGACTGTGAG	CTGGGACACCATAGATGTTG	468
Exon 8	TCCTCGAAATTCCTCCTGAC	TACCACAACTTGTTAATTCA	485
Exon 8	CATAATAGCCCCCAAACTGTA ACTCAAAGACCTTGCGCTCA		479

The reference genomic sequence used for molecular diagnosis of *NR2E3* is NW_001838218.2, because the NCBI reference human genomic sequence NT_010194.17 of GRCh37.p2 contains an additional C at location c.960.

PATIENTS AND METHODS

Patients and Clinical Assessment

This study followed the tenets of the Declaration of Helsinki (1983 Revision) and was approved by the Ethics Committee of the University of Lausanne. Informed consent was obtained from all subjects.

Complete clinical examination included refraction, best corrected Snellen visual acuity (BCVA), slit lamp assessment, and dilated funduscopic examination. Visual fields (VF) were determined by Goldmann perimetry on an all-in-one perimeter system (Octopus 900; Haag-Streit AG, Bern, Switzerland). Color vision testing was performed with Ishihara plates. Ganzfeld ERG was performed with an electrophysiological diagnostic system (RETIport 32; Roland Consult, Wiesbaden, Germany) and incorporated recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV).²⁷ FAF and NIA were performed on a confocal scanning laser ophthalmoscope (cSLO) (Heidelberg Retina Angiograph 2; Heidelberg Engineering, Heildelberg, Germany). For FAF, lipofuscin autofluorescence was excited with an Argon laser light at 488 nm. A band-pass filter with a cut-off at 500 nm included in the system was inserted in front of the detector. For NIA, a diode laser light at 787 nm was used to excite melanin autofluorescence and a band-pass filter at 800 nm was inserted. SD-OCT images were obtained using a spectral domain technology instrument (Cirrus HD-OCT; Carl Zeiss Meditec AG, Jena, Germany).

Molecular Genetic Analysis

This study was approved by the Swiss Federal Department of Health (Authorization # 035.0003-48) and follows the principles of the Declaration of Helsinki. Blood samples were collected after informed consent. Genomic DNA was extracted from peripheral blood using a genomic DNA extraction kit (Nucleon BACC3; GE Healthcare, Glattbrugg, Switzerland). Mutation screening of the entire coding sequence and intron-exon junctions of NR2E3 was undertaken. Primers covered the eight exons of transcript variant 2 (NM_014249.2) encoding the full-length human NR2E3 protein (Table 1). These primers replaced previously used ones covering a misspliced transcript variant 1 that retains part of the intron 7 but does not contain exon 8 (NM_016346.2). The polymerase chain reaction (PCR) was performed in a total volume of 20 µL, containing 100 ng genomic DNA, 1 µM of each primer (Eurogentec, Liège, Belgium), and 10 µL of a premixed solution (peqGold PCR Master Mix Y; Peqlab, Erlangen, Germany). Amplification was performed in a thermal cycler (GeneAmp 9700; Applied Biosystems, Carlsbad, CA) as follows: 1 minute at 95°C, 35 cycles of 1 minute at 94°C, 1 minute at 60°C, 1 minute at 72°C, and a final elongation step at 72°C for 10 minutes. PCR-amplified products were purified with a PCR purification kit (Invitek MSB Spin PCRapace kit; STRATEC Molecular GmbH, Berlin, Germany). Bidirectional Sanger sequencing was done in a final reaction volume of 10 µL, using a cycle sequencing kit (BigDye Terminator v3.1; Applied Biosystems). Fragments were separated on a genetic analyzer (ABI PRISM 3100; Applied



FIGURE 1. Swiss kindred affected by *NR2E3*-p.G56R-linked ADRP. (A) Pedigree over six generations of the affected family. No ophthalmic history had been reported for individuals I.1 and I.2, but inheritance was clearly dominant. The proband V.2 is indicated by an *arrow*. (B) Electropherogram of the heterozygous substitution c.166G > A (p.G56R) in patient IV.2. Exon 2 was sequenced with the reverse primer, resulting in a C > T substitution for this figure. The electropherogram of unaffected family member VI.3 showed the wild-type G residue in a homozygous state.

TABLE 2.	Sun	mmary	7 of Clinical Findings								
Patient	Sex	Age (y)	Symptoms	BCVA OD-OS	OCT (µM) OD-OS	Fundus	FAF	NIA	ERG	Visual Fields OD-OS	Color Vision Ishihara
IV.2	М	65	Night blindness since age 40 y, no photophobia, sensitive to intense light	6/10-6/7.5	214-210	Rare peripheral, clumped pigments	Double ring	Central 5°, double ring	No rod function, low a/b ratio on maximal scotopic ERG, moderate cone dysfunction	Partial isopters V4e-I4e dissociation, 40° annular scotoma	12/13
IV.4	ц	63	Night blindness since childhood, photophobia, cataract OU (60 y)	6/15-6/10	240-249	Optic nerve pallor, attenuated arterial caliber, scattered, clumped and bone-spicule- shaped pigments along vascular arcades and periphery	Peri-foveal ring	Central 5°	Not recordable	Restricted to the central 5°	0/13
V.2	μ.	37	Night blindness since childhood, cataract OS (36 y)	6/7.5-6/7.5	199-204	Optic nerve pallor, attenuated arterial caliber, perimacular and mid-peripheral clumped and bone spicule-shaped pigments	Peri-foveal ring	Central 5°	Not recordable	Isopters V4e-14e dissociation, bi-nasal scotoma, with 40° annular scotoma	12/13
9'A	Ц	41	Night blindness since age 20 y, no photophobia, sensitive to intense light	6/7.5-6/7.5	227-222	Physiologic	Double ring	Central 5°, double ring	No rod function, low a/b ratio on maximal scotopic ERG, moderate cone dysfunction	Partial isopters V4e-I4e dissociation, 40° annular scotoma	12/13
VI.4	Μ	16	None	9/9-9/9	207-212	Physiologic	Double ring	Central 10°, double ring	Rod dysfunction, low a/b ratio on maximal scotopic ERG	Incomplete inferior 40° annular scotoma	13/13
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OCT was used to determine macular thickness. OD, right eye; OS, left eye; OU, both eyes; y, years. Unaffected family members IV.3, V.5, V.7, and VI.3 were also clinically examined and presented with normal status.



FIGURE 2. Composite fundus photographs of patients of the affected Swiss family (OD: *left panel*; OS: *right panel*). (**A**) In the 65-year-old patient IV.2, a few peripheral clumped pigments were detected in the periphery, but fundus was otherwise unremarkable. (**B**) At age 63 years, patient IV.4 presented a late-stage ADRP clinical phenotype in both eyes with optic nerve pallor, attenuated blood vessels, clumped and bone spicule-like pigment deposits in the mid-periphery along the vascular arcades, but very few clumped pigments in the periphery. (**C**) Fundus examination of the proband V.2 revealed optic nerve pallor, attenuated blood vessels, perimacular and mid-peripheral clumped and bone spicule-like pigments, and rare peripheral clumped pigments. (**D**) Patient V.6 at age 41 years. (**E**) Patient VI.4 at age 16 years. Neither patient showed detectable signs of retinal degeneration on fundus examination.

Biosystems). Sequences were analyzed using a chromatogram sequencing software (Chromas 2.23; Technelysium, Tewantin, QLD, Australia) and aligned with the reference genomic *NR2E3* sequence NW_001838218.2. Information for molecular diagnosis for mutations located in precursor mRNA-processing factor 3 (*PRPF3*), rhodopsin (*RHO*), retinitis pigmentosa 1 (*RP1*), peripherin 2 (*PRPH2/RDS*), C1qand tumor necrosis factor-related protein 5 (*CTRP5/C1QTNF5*) and prominin-1 (*PROM1*) genes are available on request.

RESULTS

Molecular Genetic Analysis

ADRP patients were enrolled among three generations in a previously non-described kindred originating from the Swiss Jura (Fig. 1A), unrelated to a previously reported Swiss family.¹⁹ Among 10 examined family members, five were affected. The youngest and oldest affected patients were 16 and 65 years old, respectively. Direct sequencing of exon 2 of the *NR2E3* gene revealed a G to A transition at position 166 (c.166G > A) of the coding sequence (NM_014249.2), resulting in the previously reported ADRP-linked NR2E3-p.G56R mutant protein¹⁷ (Fig. 1B). This mutation segregated heterozygously in all patients affected with ADRP and was absent in the unaffected family members. Because variable expression in clinical phenotypes had been linked to the presence of both the dominant and a

recessive mutation,¹⁹ study authors tested for additional *NR2E3* mutations in all eight exons, but none were found. The proband's two young children VI.1 and VI.2 were not available for molecular diagnosis.

Clinical Features in a Swiss Family over Three Generations

Patient Information. This Swiss family has a reported ophthalmic history over six generations. Individual II.2 was reportedly blind at the end of his life, as well as his two sisters, II.4 and II.6. His son, patient III.2, suffered from the characteristic tunnel vision of advanced RP at the time of his death at age 63. The clinical findings of the examined living family members are summarized in Table 2. Clinical examination of the unaffected individuals V.7 and VI.3 was unremarkable.

Patient IV.2. Disease progression was milder compared with the younger sister (IV.4) and daughter (V.2). The patient started to notice night blindness in his fifth decade. At age 65 years, he was still driving and working as a technician. Fundus examination showed rare peripheral pigment deposits (Fig. 2D). FAF examination showed a double ring of hyperauto-fluorescence, an inner perimacular ring, and an outer ring located within the vascular arcades. The area of demarcation between the two was diffusely hyperautofluorescent (Figs. 4B,

patient	rod specific	scotopic 5 dB	30 Hz Flicker	transient photopic
IV.4	300 200 100 -100 0 50 100	200 -200 -400 0 50 100 150	100- 0	100 - 0 - -100 - 0 -50 100 150
V.6	300 200 100 -100 0 50 100	200 -200 -400 0 50 100 150	100- 0- -100- <u>50 100 150</u>	100- 0- -100- 0 50 100 150
VI.4	300 200 100 0 -100 0 50 100	200 -200 -400 0 -400 0 50 100 150	100- 0- -100- 50 100 150	100 - 0 - -100 - 0 -50 100 150
normal control	300 200 100 0 -100 0 50 100	200 -200 -400 0 -400 0 50 100 150	100- 0- -100- 50 100 150	100 0 -100 0 50 100 150

FIGURE 3. Characteristic full-field ERG findings of the Swiss kindred, illustrated over three generations. For the most severely affected patient, IV.4, neither rod nor cone responses could be recorded. No rod-specific nor severely diminished scotopic maximal responses with a low a/b ratio were recorded for her daughter, patient V.6. The photopic 30-Hz flicker was delayed and of decreased amplitude, the transient photopic response diminished. For the grandson, patient VI.4, rod-specific responses were absent and the scotopic maximal response also showed an electronegative ERG. Photopic responses were close to normal. For comparison, normal control traces are indicated in the *bottom panel*. For the *x*-axis, time units are msec; and for the *y*-axis, amplitudes are indicated in μV .

4C). NIA also showed a double hyperautofluorescent ring colocalizing with both the exterior rim of the outer ring and the perimacular ring detected by FAF, while the area demarcated by both rings was hypoautofluorescent (Figs. 4A, 4D). OCT scans were compared with FAF images over corresponding retinal locations (Figs. 5A-5D). Macular structure and thickness was preserved; however, there was a loss of the inner segment/outer segment (IS/OS) band within the surface delimited by the two FAF rings. VFs were within normal limits for isopter I4e and V4e (Fig. 6A). However, in the left eye, there was an absolute scotoma in the mid-periphery extending to a relative annular scotoma. This corresponded to areas delimited by FAF and NIA rings (Figs. 4A-4D) and the region of photoreceptor loss identified by OCT (Figs. 5A-5D). ERG showed severe rod dysfunction with a low a/b ratio on maximal scotopic ERG with unremarkable photopic examination (Table 2).

Patient IV.4. This patient had experienced night blindness since childhood. She had been noticing a visual field constriction for the past 20 years. At age 52 years, she was no longer able to fulfill her professional duties. At age 60 years, cataract surgery was performed on both eyes. At age 63 years, fundus examination showed optic nerve pallor, normal macula, attenuated retinal vasculature and numerous pigment deposits along the vascular arcades, mainly temporal superior and inferior (Fig. 2A). Central visual acuity was severely affected, color vision was undetectable (Table 2), and ERG was not recordable (Fig. 3). FAF examination revealed an innerperifoveal hyperautofluorescent ring and hyperautofluorescence in the far periphery (Figs. 4F, 4G). Hypoautofluorescent signals of clumped, nummular, and bone spicule-like shapes were extensively detected not only in the mid-periphery, but also in the far periphery. NIA examination showed a hyperautofluorescent area restricted to the central fovea (Figs. 4E, 4H). OCT revealed a normal macula (Figs. 5E-5H). There was a

loss of the IS/OS lamina, with multiple small hyperreflective debris at the level of the inner retina, outside the perimacular FAF ring. VFs were constricted to the central 10 degrees when tested with isopter V4e, with inferior perimacular sparing in the left eye (Fig. 6B). ERG revealed severe rod dysfunction with a low a/b ratio on maximal scotopic ERG, associated with severe cone dysfunction (Fig. 3).

Patient V.2. Ophthalmic examination of the proband was first performed at the age of 5 years, when the first signs of impaired dark adaptation were noticed. At age 20 years, VFs started to constrict, especially in the left eye. Fundus examination showed normal macula and retinal vasculature. along with numerous diffuse grevish pigment alterations, predominantly in the mid-periphery. Anterior segment examination detected a cortical cataract in the left eye. Scotopic ERG was not recordable and the photopic ERG was severely reduced. At age 37 years, fundus examination showed optic nerve pallor, normal macula, attenuated retinal vasculature, and numerous perimacular and mid-peripheral pigment deposits (Fig. 2E). ERG was not recordable. On FAF examination, hyperautofluorescence was detected as an inner perifoveal ring in both eves (Figs. 4J, 4K). Hypoautofluorescent signals of clumped, nummular, and specular pigment deposits were detected in the mid- and far-periphery. NIA examination showed a normal signal restricted to the central fovea surrounded by a hyperautofluorescent ring (Figs. 4I, 4L). OCT showed a preserved neuroretina only in the area demarcated by the perifoveal ring (Figs. 5I-5L). VF correlated with the FAF ring diameters and was constricted to 10 degrees when tested with isopter I4e (Fig. 6C). There was an annular scotoma with sparing in the mid-periphery of the nasal part.

Patient V.6. The 41-year-old first cousin of the proband had been noticing night blindness for the past 20 years. Fundus examination revealed no abnormalities, except slight optic



FIGURE 4. FAF and NIA examinations of the NR2E3-p.G56R-linked ADRP patients. FAF of the youngest and least affected patient VI.4 showed two hyperautofluorescent rings, an inner perimacular ring and an outer ring, located within the vascular arcades and demarcating a diffuse hyperautofluorescent annular surface area (**R**, **S**). A comparable FAF was observed in the less affected patient IV.2 (**B**, **C**). In the more affected patient V.6, the inner ring of AF was perifoveal, whereas the outer ring was located along the vascular arcades (**N**, **O**). In the advanced disease patients, IV.4 and V.2, only an inner perifoveal hyperautofluorescent ring was observed (**F**, **G**, **J**, **K**). Some diffuse hyperautofluorescence was detected in the far periphery of patient IV.4 (**F**, **G**), but was restricted to a small temporal region of the left eye in the proband V.2 (**K**). In these two

patients, hypoautofluorescent signals of clumped, nummular, and bone spicule-like shapes were extensively detected, not only in the midperiphery, but also in the far periphery (**F**, **G**, **J**, **K**). NIA examinations showed macular hyperautofluorescence in the less affected patients IV.2 (**A**, **D**) and VI.4 (**Q**, **T**). This hyperautofluorescent signal became progressively restricted to the central fovea in the more severely affected patients IV.4 (**E**, **H**), V.2 (**I**, **L**) and V.6 (**M**, **P**). The outer hyperautofluorescent ring observed by NIA colocalized with the exterior rim of the outer hyperautofluorescent ring observed by FAF in patients IV.2 (**A**-**D**), V.6 (**M**-**P**), and VI.4 (**Q**-**T**). The annular area delimited by the two NIA rings was hypoautofluorescent.



FIGURE 5. Optical coherence tomography (OCT) of the *NR2E3*-p.G56R-linked ADRP patients. For each patient, the scanning sections were indicated by a *green line* on FAF images of the right (**B**, **F**, **J**, **N**, **R**) and left eye (**C**, **G**, **K**, **O**, **S**). In all patients, the structure and thickness of the fovea was conserved. Photoreceptor inner segment/outer segment band was undetectable within the area delimited by the two AF rings (demarcated by *yellow bars*). Multiple small hyperreflective foci were present in the inner retina.



FIGURE 6. Visual fields of the *NR2E3*-p.G56R-linked ADRP patients. In both eyes of patient IV.4 (**B**), the VF was restricted to the most central retina with some inferior paramacular sparing in the left eye. In patient V.2 (**C**), constricted visual field loss was also present with some VF left in the temporal far periphery, due to an extensive annular scotoma of the mid-periphery. A mid-peripheral annular scotoma was present in patient V.6 (**D**) and, to a lesser extent, in the youngest patient VI.4 (**E**). V4e and I4e isopters dissociation was noted in all but one patient, VI.4. Isopter V4e is in *blue*, III4e in *red*, and I4e in *green*.

nerve pallor (Fig. 2B). Visual acuity was preserved and VF showed annular scotoma in the mid-periphery, with a partial dissociation of isopters V4e and I4e. Rod-specific ERG was at the limit of detection. The maximal responses of the scotopic ERG showed a low a/b ratio (Fig. 3). Amplitudes of both photopic 30 Hz Flicker and transient photopic ERG responses were reduced. FAF examination revealed two hyperautofluorescent rings, an inner perifoveal ring, and a broader outer ring located beyond the vascular arcades (Figs. 4N, 4O). Macular hyperautofluorescence observed by NIA was restricted to the central fovea, and an outer hyperautofluorescent ring colocalized with the exterior rim of the outer hyperautofluorescent ring observed by FAF (Figs. 4M, 4P). OCT showed preserved IS/ OS lamina within the perifoveal ring and immediately external to the outer ring (Figs. 5M-5P). The area between the two FAF rings showed loss of IS/OS band and correlated to the annular scotoma observed on VF (Fig. 6D).

Patient Vi.4. The 16-year-old son of patient V.6 did not complain of visual loss, but VFs revealed the beginning of an annular scotoma in both eyes (Fig. 6D). Fundus examination was unremarkable (Fig. 2C). Scotopic ERG responses were reduced, but photopic ERG was still within normal limits (Fig. 3). Similar to patients IV.2 and V.6, FAF examination revealed a double hyperautofluorescent ring (Figs. 4R, 4S), the outer one colocalizing with the hyperautofluorescent ring detected by NIA (Figs. 4Q-4T). Again the IS/OS band was undetectable in the diffusely hyperautofluorescent zone demarcated by the two FAF rings, leaving a preserved retina beyond these (Figs. 5Q-5T).

FAF Findings in Additional NR2E3-p.G56R-Linked ADRP Families

Two hyperautofluorescent rings were also observed in the four FAF-examined patients of the originally described *NR2E3*p.G56R-linked ADRP Swiss family.^{19,28} An outer ring initially located along the vascular arcades and an inner one that became progressively constricted to a perifoveal location (Figs. 7A, 7B). In addition, an unrelated 21-years-old Swiss index patient was recently examined at the Jules-Gonin Eye Hospital and found to display a double ring of hyperautofluorescence (Fig. 7C). Subsequent molecular diagnosis identified the *NR2E3*-p.G56R mutation in the affected family members. However, no double ring of hyperautofluorescence was observed in the proband's 18-year-old sister (Fig. 7D).

FAF Findings in Patients Affected with Dominant Retinal Dystrophies

To test whether a double concentric hyperautofluorescent ring was specific to *NR2E3*-p.G56R-linked ADRP, study authors reexamined all available FAF data of patients affected by dominant retinal dystrophies with known underlying genotype. A single ring of hyperautofluorescence was observed in families affected with ADRP caused by *PRPF3*-p.T494M (Fig. 7E),²⁹ *RHO*-p.C110W (Fig. 7F), *RHO*-p.C110Y, and *RP1*-p.L762Yfs17 mutations (data not shown). No hyperautofluorescent rings were observed in ADRP families harboring *PRPH2*-p.R46X and *PRPH2*-p.N54fsX63 mutations, nor in families affected with dominantly inherited *CTRP5*-p.S163R-linked lateonset retinal degeneration (LORD) and *PROM1*-p.R373C-linked bull's eye maculopathy (BEM).³⁰

DISCUSSION

The detailed cross-sectional phenotypic description of a Swiss kindred affected with *NR2E3*-p.G56R-linked ADRP revealed in



FIGURE 7. FAF examinations of additional ADRP patients. The originally described family with NR2E3-p.G56R-linked ADRP²⁸ was illustrated with a 50-year-old female patient (**A**) and a 64-year-old severely affected male patient, with a constricted perifoveal inner hyperautofluorescent ring and numerous hypoautofluorescent pigment deposits in the periphery (**B**). In the newly identified Swiss family, the outer hyperautofluorescent ring was located outside the vascular arcades in the 21-year-old female proband, thus allowing observation with a 30° lens instead of the 55° lens (**C**). In her 18-year-old sister, a single perimacular hyperautofluorescent ring was present (**D**). The hyperautofluorescent ring was perifoveal in a previously described family affected with *PRPF3*-p.T494M-linked ADRP, as illustrated by a 45-year-old male patient (**E**).²⁹ In other patients affected with dominant retinal dystrophies, the hyperautofluorescent ring was perimacular, as exemplified by a 24-year-old patient affected by *RHO*-p.C110W-linked ADRP (**F**).

the mid-peripheral zone a double concentric hyperautofluorescent ring on FAF at an early stage of the disease. These FAF findings were also present in the two additional Swiss kindred and in two other FAF-investigated kindred of Belgian and French origin with the same mutation.¹⁷ In patients with advanced disease, study authors observed a trend toward constriction of the inner perimacular ring to a perifoveal location. The outer ring initially located within the vascular arcades extended toward the periphery and eventually became hypoautofluorescent. NIA hyperautofluorescence colocalized with the outer and inner rims of the area demarcated by the double concentric hyperautofluorescent ring, consistent with the common initial stage of photoreceptor degeneration (i.e., increased accumulation of the main antioxidant melanin in RPE cells due to increased RPE phagocytotic activity).^{26,31} In contrast, FAF hyperautofluorescence due to lipofuscin accumulation within RPE cells had been associated with a more advanced degenerative disease process with further increased RPE phagocytosis.³¹ Absence of NIA in the area demarcated by

the double hyperautofluorescent concentric ring suggested RPE cell death by loss of melanin, but presence of residual FAF indicated some remaining RPE phagocytosis.^{26,31,32} This is consistent with centripetal and centrifugal photoreceptor loss in the progressively enlarged area demarcated by both hyperautofluorescent concentric rings, correlating with the loss of IS/OS band on OCT and VF restriction.^{33–38}

In the three-generation branch of the kindred, an agedependent disease progression was observed for patients IV.4, V.6, and VI.4. However, in the other branch of the family, visual function was relatively preserved in the oldest patient IV.2, but severely reduced in his daughter V.2. This intrafamilial phenotypic variability suggested variable expressivity, with additional genetic and environmental factors involved.

Incomplete penetrance of the double concentric hyperautofluorescent ring provided further circumstantial evidence of intrafamilial variability. However, because longitudinal FAF data was missing for this youngest patient of the newly described Swiss family, one cannot rule out whether the double ring ever existed or had already started its centrifugal/ centripetal migration.

With respect to dominant retinal dystrophies, the presence of a double hyperautofluorescent ring would appear so far to be restricted to *NR2E3*-p.G56R-linked ADRP, whereas a perifoveal or perimacular ring of hyperautofluorescence is a common FAF finding in RP patients.^{33–35,37,39,40} Based on available data, a double hyperautofluorescent ring has not been reported in recessive RP patients so far.

Interestingly, FAF in *NR2E3*-p.G56R-linked ADRP patients is strikingly different from those affected by recessive *NR2E3*-linked retinal degenerations. Indeed, in ESCS (GFS) patients, FAF examination showed hyperautofluorescent spots in both the macular area and mid-peripheral retina,^{41,42} and OCT analysis correlated these hyperautofluorescent spots with the rosette-like lesions characteristic of GFS.⁴²

In conclusion, study findings suggest that this double concentric hyperautofluorescent ring, where the inner ring appears smaller and the outer ring larger in patients with more advanced disease, to be a highly penetrant FAF feature of *NR2E3*-p.G56R-linked ADRP. Its absence, however, does not exclude definitively the presence of the *NR2E3*-p.G56R mutation.

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