Quantitative Autofluorescence as a Clinical Tool for Expedited Differential Diagnosis of Retinal Degeneration

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Homozygous and compound heterozygous mutations in *ABCA4* are associated with multiple phenotypes, including bull's-eye maculopathy (BEM).[1] The BEM fundus patterning can also be conferred by other genes such as the retinitis pigmentosa guanosine triphosphatase regulator gene (*RPGR*), a gene that encodes a protein involved in trafficking to the outer segment.[2] The carrier frequency of disease-causing mutations in *ABCA4* is 1 in 20. Even after complete sequencing of the *ABCA4* exons and adjacent intronic sequences in patients with a clinical diagnosis of Stargardt disease 1 (STGD1), 15% to 20% of cases still have

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only 1 identified disease-causing mutation and no mutations are found in 10% to 15% of individuals.[3] Thus, the phenotypic and allelic variability of *ABCA4*-related disease remains a challenge.

Due to reduced protein function, *ABCA4* mutations trigger elevated accumulation of bisretinoid lipofuscin in retinal pigment epithelial cells. This increase is revealed in most but not all cases as augmented fundus autofluorescence (AF), the inherent AF of the retina that is emitted with 488-nm excitation.[4] Using a standardized method for quantification of fluorescence intensities in fundus AF images (quantitative fundus AF[qAF]), increased qAF levels have been observed in patients with STGD1[4] even at young ages and in fundus areas that qualitatively appear to be unaffected. Herein, we show that qAF is a clinical tool that may help in the management of inherited retinal diseases.

Report of a Case

A man in hismid-40s had blurred central vision, photophobia, and nyctalopia since childhood. Examination showed a BEM that was evident in fundus AF images, but there were no flecks, intraretinal pigment migration, retinal vessel attenuation, or optic nerve pallor (Figure 1).He had previously been clinically diagnosed as having STGD1 and genetic screening with an *ABCA4* array had revealed a single copy of an *ABCA4* gene variant (rs1800548, p.E471K). Sanger sequencing confirmed this change. Retinal disease was reported in his brother and maternal grandfather. As previously described,[2,5] the proband's mother had reported being asymptomatic but was found to exhibit decreased visual acuity, BEM in the right eye, and tapetal reflex in both eyes. Whole-exome sequencing confirmed the *ABCA4* variant and detected a novel mutation in the open reading frame 15 domain of *RPGR* (c.3070G>T, pGlu1024×, OMIM #312610). The mean qAF values of the patient were found to be within the range observed in healthy eyes. The patient's mean values were also lower than in many patients of the same age with STGD1[4] (Figure 1 and Figure 2).

Discussion

X-linked retinitis pigmentosa accounts for up to 20% of families with retinitis pigmentosa, and *RPGR* is the most frequently mutated gene in X-linked retinitis pigmentosa.[6] In our proband, the inheritance pattern was typical of X-linked disease and the report of a tapetal reflex in the mother was consistent with X-linked retinitis pigmentosa. It was ultimately apparent that the BEM phenotype had also been conferred by the mutation in *RPGR*. Nevertheless, given that disease caused by mutations in *ABCA4* can manifest as BEM, that disease-causing variants in*ABCA4* are copious (approximately 800 sequence variants), and that a second mutation is frequently not detected in the presence of clinical disease, it was initially reasonable to consider *ABCA4* pathogenicity on the basis of a single allele and the clinical presentation. Here was a case in which an additional clinical tool, qAF, could guide genetic screening so as to potentially avoid the pitfalls of phenotypic and genetic heterogeneity. Subsequent to the identification of the *ABCA4* variant p.E471K by array screening, data available from the Exome Variant Server[7] indicated that this allele is unlikely to be disease causing.

Acknowledgments

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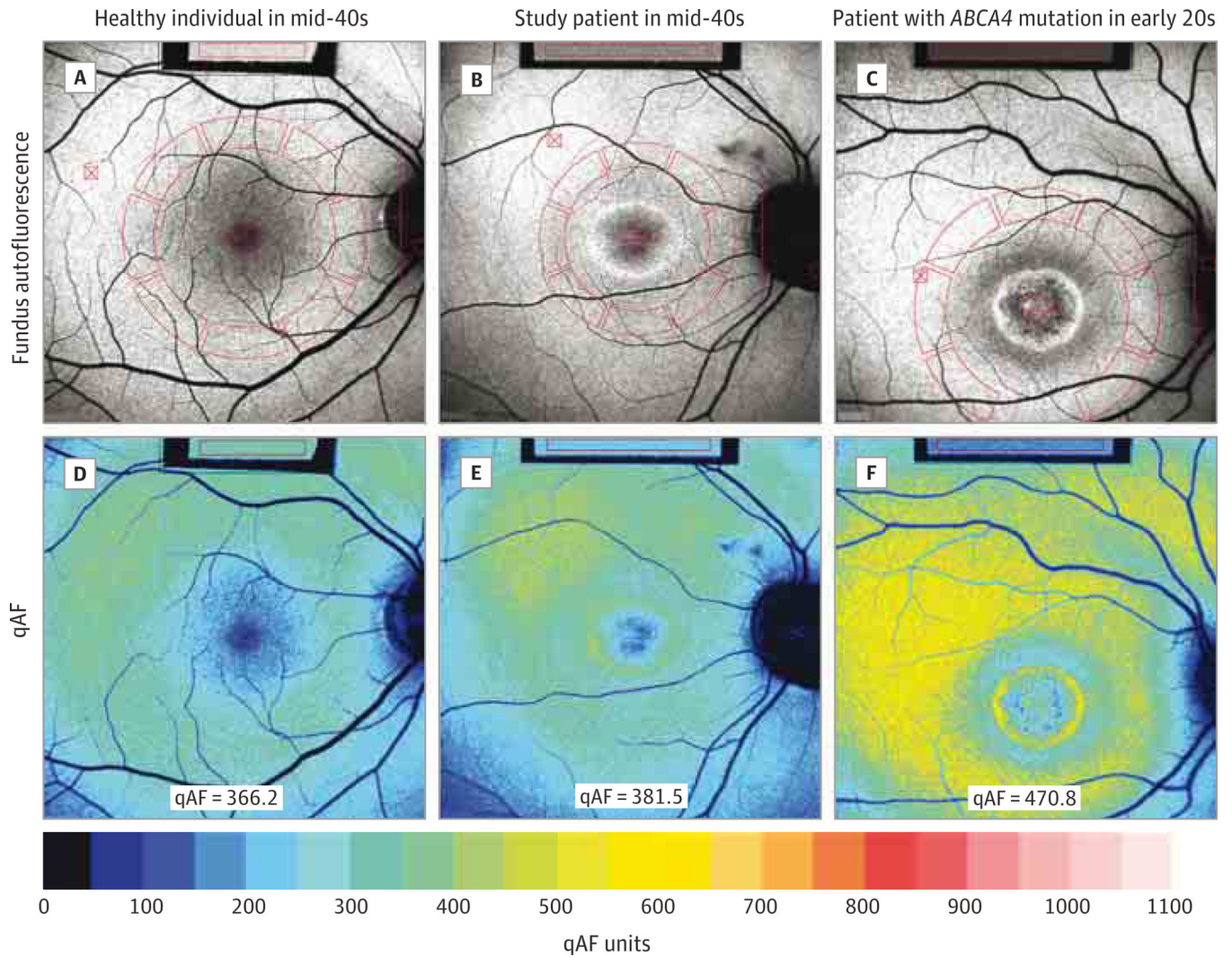7. National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project. Exome Variant Server. http://evs.gs.washington.edu/EVS/.

Figure 1. Fundus Autofluorescence and Color-Coded Quantitative Fundus Autofluorescence (qAF)

Mean grayscale levels in the circularly arranged segments (red) normalized to the reference (red rectangle) (A–C) provide the qAF values (arbitrary grayscale units),[4] which are color coded (D–F).
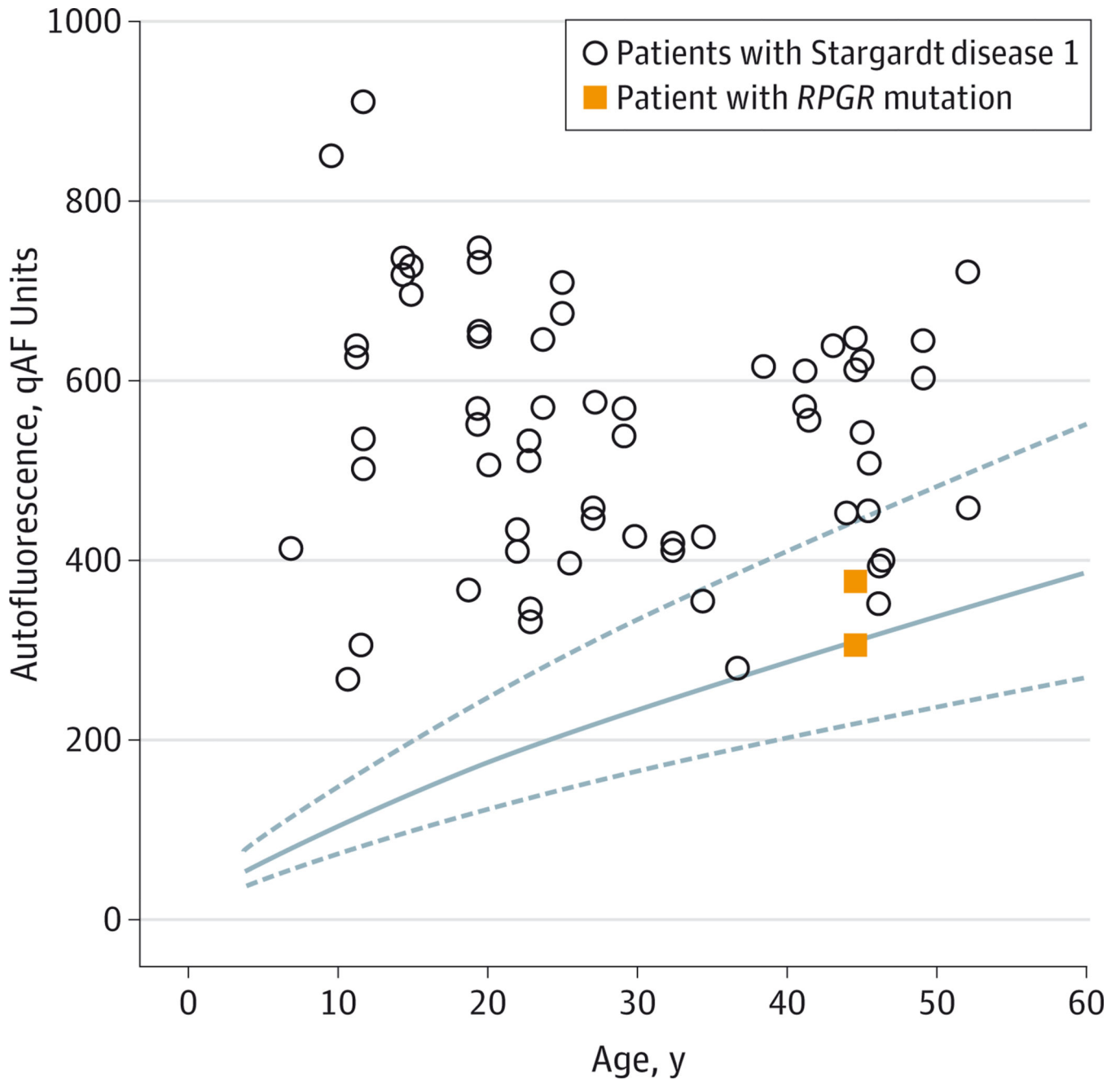
Figure 2. Quantitative Fundus Autofluorescence (qAF) Values by Age for Study Patient With *RPGR* Mutation vs Patients With Stargardt Disease 1 and Healthy Individuals

The qAF values (arbitrary grayscale units) acquired from each eye of our patient with an *RPGR* mutation and qAF values from patients with Stargardt disease 1 are plotted by age.[4] Solid line indicates mean qAF values from 277 healthy individuals; dashed lines, 95% confidence interval.[4]