Relationship Among CFH and ARMS2 Genotypes, Macular Pigment Optical Density, and Neuroretinal Function in Persons Without Age-Related Macular Degeneration

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Objectives: To determine whether there is a difference in neuroretinal function and in macular pigment optical density between persons with high- and low-risk gene variants for age-related macular degeneration (AMD) and no ophthalmoscopic signs of AMD, and to compare the results on neuroretinal function to patients with manifest early AMD.

Methods: Neuroretinal function was assessed with the multifocal electroretinogram for 32 participants (22 healthy persons with no AMD and 10 patients with early AMD). The 22 healthy participants with no AMD had either high- or low-risk genotypes for CFH (rs380390) and/or ARMS2 (rs10490924). Trough-to-peak response densities and peak-implicit times were analyzed in 5 concentric rings. Macular pigment optical density was assessed by use of customized heterochromatic flicker photometry.

Results: Trough-to-peak response densities for concentric rings 1 to 3 were, on average, significantly greater in participants with high-risk genotypes than in participants with low-risk genotypes and in persons with early AMD after correction for age and smoking (P < .05). The group peak-implicit times for ring 1 were, on average, delayed in the patients with early AMD compared with the participants with high- or low-risk genotypes, although these differences were not significant. There was no significant correlation between genotypes and macular pigment optical density.

Conclusions: Increased neuroretinal activity in persons who carry high-risk AMD genotypes may be due to genetically determined subclinical inflammatory and/or histological changes in the retina. Neuroretinal function in healthy persons genetically susceptible to AMD may be a useful additional early biomarker (in combination with genetics) of AMD before there is a clinical manifestation.

cone function when rod activity was silenced but that the combined rod and cone function was reduced in high-risk genotypes. In the present study, we investigate a subset of this group to determine the effect of genotypes on neuroretinal function. We compared neuroretinal function in healthy persons with high- and low-risk genotypes but no clinical signs of AMD with neuroretinal function in patients with early AMD, with the aim to provide a genotype-phenotype neuroretinal function signature that may reflect the electrophysiological dynamics in subclinical and early AMD. Because a lower macular pigment optical density (MPOD) has been associated with AMD risk genotypes in a small group (n = 4) of patients homozygous for the \textit{CFH} and \textit{ARMS2} genes, we were also interested in the relationship between MPOD and genotypes in our cohort of healthy participants.

**METHODS**

We investigated the right eyes of 22 healthy persons (mean [SD] age, 56.1 [4.8] years) who have been previously genotyped for the common AMD \textit{CFH} (rs380390) and \textit{ARMS2} (rs10490924) risk gene variants using optimized gene-expression assays (TaqMan Gene Expression Assay; Applied Biosystems). Of these 22 participants, 14 were either homozygous or heterozygous for one or both of the high-risk genotypes, and 8 did not carry any of the determined high-risk genes (Table 1). The odds ratios, and thus risks, were assigned to each genotype according to published data. For \textit{CFH} (rs380390), the genotype CC had an odds ratio of 7.4, and the genotypes GG and CG had an odds ratio of 1; for \textit{ARMS2} (rs10490924), the genotype TT had an odds ratio of 6.09, the genotype GT had an odds ratio of 1.35, and genotype GG had an odds ratio of 1.

The investigator was masked to the genotyping results. All healthy participants had a visual acuity better than 6/6 and no refractive errors more than ±3 diopters. The participants had no ophthalmoscopic signs of AMD, and all had a normal central retinal thickness measured with optical coherence tomography (OCT) (Stratus III; Carl Zeiss) as determined by an ophthalmologist (B.F.). For comparison with the healthy participants without clinical signs of AMD, we included 10 participants (mean [SD] age, 73.1 [2.7] years) with early AMD (graded according to the Age-Related Eye Disease Study) who had been previously tested in our laboratory and who had not been genotyped (Table 2). Fundus grading was based on color fundus photographs (Zeiss).
Jena Mydriatic Fundus Camera) of the central 30°, and retinal changes were graded independently by 2 experienced observers who were masked to the results. All patients with early AMD had a visual acuity of 6/15 or better, a drusen size of greater than 63 µm, and/or retinal pigment abnormalities but no late geographic or neovascular AMD. Written informed consent was obtained from all participants, and our study was approved by the Human Research Ethics Committee of the Queensland University of Technology and followed the tenets of the Declaration of Helsinki.

MULTIFOCAL ELECTRORETINOGRAM

We performed neuroretinal function testing with the mfERG (VERIS; Electro-Diagnostic Imaging). The stimulus array of the mfERG was presented on a calibrated 22.9-cm (9-in) cathode-ray tube monitor (screen refresh rate, 13.3 milliseconds; frame rate, 75 Hz); the display consisted of 103 hexagons (ranging in size from about 3.2° to 5° in horizontal extent and subtended approximately 25°) (Figure 1A). The binary m-sequence was $2^{13}-1$ frames long. A slow-flash mfERG paradigm was used for better detection of subclinical changes in adaptation compared with the conventional fast-flicker mfERG. However, the positive (trough-to-peak [N1-P1]) waveform component of both paradigms, the fast-flicker mfERG and the slow-flash mfERG, contains mainly contributions from on and off bipolar cells.

The stimulus sequence was slowed down by inserting 3 blank frames after the initial m-sequence frame in which the hexagons had a 50% probability of being white (200 candela [cd]/m²) or black (3 cd/m²), and the next 3 frames remained black (3 cd/m²), resulting in a time-average luminance of 26 cd/m². The surround luminance was always 52 cd/m². The recordings followed the International Society for Clinical Electrophysiology of Vision standard and were made monocularly and in ambient room lighting conditions. The recordings were divided into 16 segments, resulting in a total recording time of approximately 8 minutes. We used DTL electrodes as active electrodes. The participant’s right pupil was dilated with tropicamide, 0.5%; a pupil diameter greater than 8 mm was achieved in all participants. Refractive errors were corrected using the VERIS eye refractor/camera unit so that optimal acuity could be achieved. During the recording, the VERIS system displayed a video image of the eye and the ERG signal. The segments that were contaminated owing to fixation loss or repeated blinks were discarded and rerecorded. Retinal signals were band-pass–filtered between 10 and 300 Hz, amplified 100 000 times, and sampled every 0.83 milliseconds. A single spatial averaging was performed as per the International Society for Clinical Electrophysiology of Vision/VERIS recommendations. We determined the N1-P1 response density and P1 implicit time (time from onset of stimulus to first peak) after averaging the 103 hexagons into 5 concentric rings (ring 1, 0°-5°; ring 2, 5°-9°; ring 3, 9°-13.5°; ring 4, 13.5°-19°; and ring 5, 19°-25°) (Figure 1A). The concentric ring averaging was based on the predilection of the first histological and functional changes in AMD being central and paracentral.

MACULAR PIGMENT OPTICAL DENSITY

The MOPD was estimated with the Macular Pigment Densitometer (Macular Metrics II, LLC) in 20 of 22 healthy participants (2 participants were unavailable to return for MPOD measurements). In brief, the system is based on heterochromatic flicker photometry. A 0.5° spot is presented in the fovea, and a 2° spot is presented 7° paracentrally. The stimulus is a flickering light that alternates between 460 nm (which is absorbed...
The slow-flash mfERG results of 4 participants were excluded from analysis owing to poor fixation.\textsuperscript{66} The mfERG results of the remaining 18 participants (11 with high-risk genotypes and 7 with low-risk genotypes) for the N1-P1 response density and the P1-implicit time are shown in Table 1. The slow-flash mfERG results for the 10 patients with early AMD are outlined in Table 2. Figure 1B-D shows 3 representative mfERG trace arrays: a healthy, funduscopically normal participant with a high-risk genotype (Figure 1B); a healthy, funduscopically normal person with a low-risk genotype (Figure 1C); and a patient with early AMD (Figure 1D). The person who is genetically at high risk (Figure 1B) demonstrates lower responses and has delayed central responses compared with the persons with either a high-risk (Figure 1B) or a low-risk genotype (Figure 1C). The patient with early AMD (Figure 1D) shows lower responses and has delayed central responses compared with the persons with either a high-risk (Figure 1B) or a low-risk genotype (Figure 1C). The mean mfERG data (ie, the N1-P1 response densities and P1-implicit times) of the 3 groups are shown in Figure 2, they show the same response patterns as the individual observers. The MPOD results for each of the 19 healthy participants are shown in Table 2 (the MPOD was not obtained for 2 participants, and so they were excluded from the 21 enrolled healthy persons), and these were within the normal range reported in the literature.\textsuperscript{69} Statistical analysis was performed, and a significant difference was detected among the 3 groups for the N1-P1 response density (\(F_{2,15}=5.1, P=.01\)) demonstrating that healthy persons with high-risk genotypes had, on aver-

### Table 3. Macular Pigment Optical Densities and Genotypes for the 20 Healthy Participants

<table>
<thead>
<tr>
<th>Age, y</th>
<th>MPOD</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>51</td>
<td>0.36</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>48</td>
<td>0.31</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>55</td>
<td>0.43</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>56</td>
<td>0.48</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>57</td>
<td>0.53</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>58</td>
<td>0.58</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>59</td>
<td>0.63</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>60</td>
<td>0.68</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>61</td>
<td>0.73</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>62</td>
<td>0.78</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>63</td>
<td>0.83</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>64</td>
<td>0.88</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>65</td>
<td>0.93</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>66</td>
<td>0.98</td>
<td>CC/GG (low risk)</td>
</tr>
<tr>
<td>67</td>
<td>1.03</td>
<td>CC/GG (low risk)</td>
</tr>
</tbody>
</table>

Abbreviation: MPOD, macular pigment optical density.

between the MPOD and haplotype combinations. \(P \leq .05\) was considered statistically significant.
Correlation between the genotype and the MPOD (P groups for the P1-implicit time for each ring (P values for the comparison between the high-risk genotypes and patients with early AMD were significant) was found. The AMD group demonstrated, on average, decreased values, which is reflected in the statistical model, there was a significant difference among the 3 groups. The model suggests that, in stage 1 (subclinical), persons with high-risk genotypes show supernormal responses in rings 1 to 3. As the disease becomes manifest (stage 2), responses decrease to "normal (low-risk)" values, which are more pronounced in the parafoveal and perifoveal areas (rings 2 and 3) where there are the greatest changes in retinal topography (photoreceptor/bipolar cell loss) in age-related macular degeneration (AMD). In stage 3 of early AMD, neuroretinal responses have decreased further and below normal values, which is reflected in progressive fundus changes (drusen size >125 µm).

Figure 3. Multifocal electroretinogram N1-P1 response densities shown for an individual person of each of the 3 groups. The model suggests that, in stage 1 (subclinical), persons with high-risk genotypes show supernormal responses in rings 1 to 3. As the disease becomes manifest (stage 2), responses decrease to "normal (low-risk)" values, which are more pronounced in the parafoveal and perifoveal areas (rings 2 and 3) where there are the greatest changes in retinal topography (photoreceptor/bipolar cell loss) in age-related macular degeneration (AMD). In stage 3 of early AMD, neuroretinal responses have decreased further and below normal values, which is reflected in progressive fundus changes (drusen size >125 µm).

Our study identified significantly greater central neuroretinal function in healthy participants with high-risk genotypes compared with those with low-risk genotypes with no clinical signs of AMD (as determined ophthalmoscopically according to the Age-Related Eye Disease Study classification and with OCT). These findings and our previous research have important implications for studies on "normal ageing" because they provide a way to differentiate neuroretinal and psychophysical function among healthy older persons. Based on our findings, we infer that there are clinically "normal" subgroups whose neuroretinal function may be differentiated on the basis of their genetics. In healthy participants, however, high-risk genotypes were not significantly associated with lower MPOD levels, which suggests that further investigations into a larger sample of homozygous AMD risk genotypes are required. Supernormal responses in electrophysiology are rare, but they are not novel observations. They are thought to be of genetic, ischemic, inflammatory, or toxic origin and can be found in patients with cone dystrophy, retinal vein occlusion, uveitis, and sferosis bulbii and can affect the cone-mediated pathways and rod-mediated pathways. In particular, supernormal responses have been described as the earliest detectable signs in inflammatory disease before disease is ophthalmoscopically manifest. Once inflammatory disease is manifest, the neuroretinal responses decrease to normal and subnormal values. One of the main risk factors of AMD is age, and ageing itself has been linked with chronic inflammation. Moreover, the pathomechanisms in AMD involve a dysregulated inflammatory/immune response due to gene variants in the complement factor H (CFH) region. It is thought that an uncontrolled inflammation occurs because of this gene variant. Drusen, the hallmark and first clinical signs of AMD, are considered as by-products of this local, chronic inflammation. Our findings of supernormal neuroretinal responses in people with high-risk genotypes related to the gene variants in the CFH region may therefore reflect a genetically determined subclinical and low-grade inflammation before drusen are ophthalmoscopically evident. However, drusen may be apparent on high-resolution OCT scans but not on ophthalmoscopic/fundus photography or conventional time-domain OCT scans. Therefore, beginning pathology cannot be completely excluded in our healthy cohort of participants. It would be interesting to determine whether healthy participants with high-risk genotypes and supernormal ERGs show corresponding changes with the high-resolution OCT.

We link our results with previous findings in our laboratory where we demonstrated reduced mesopic vision in persons genetically at risk for AMD. Under mesopic conditions, both cone-mediated and rod-mediated pathways are active. Although the stimulus mean luminance conditions with the slow-flash mfERG were low photopic, rod-driven ERG responses can be obtained at photopic illuminances and with stimuli containing low temporal frequencies, as with the mfERG recording used in our study. Normal rod function is crucial for cone survival, and the role that rods play with regard to cones has been suggested to be protective. Cone function may be altered when operating in the presence of defective rods, which results in supernormal photopic mfERG responses. Our working hypothesis is that increased neuroretinal function in persons genetically at risk for AMD would occur because their retina is genetically determined to age faster and because they are susceptible to chronic inflammation. An early histological correlate of this process (before there are clinically detectable drusen) may be the expansion of cone photoreceptor inner segments to fill the spaces where there is rod loss, as is found in early AMD and in normal ageing. The larger photoreceptor inner segments contain a higher number of mitochondria, which may give them greater capacity for enhanced output.

We propose a hypothetical model that links neuroretinal function with subclinical and clinical changes that occur in AMD. Figure 3 illustrates the individual N1-P1 re-
response densities for the 5 rings of 1 representative of the healthy high-risk genotype group, 1 representative of the low-risk genotype group, and 2 representatives of the early AMD group (1 patient with less progressed early AMD and a drusen size of <125 µm and 1 patient with early AMD with more progressed funduscopic changes and with a drusen size of >125 µm). The data from the group of individuals in this example are not significantly different from the mean group data (Figure 2). In stage 1 (subclinical stage, with normal ophthalmoscopy and OCT finding but histological signs of cell loss), healthy persons genetically at risk for AMD have supernormal neuroretinal responses in the central, paracentral, and pericentral retinal areas (rings 1-3).

In stage 2 (clinical stage, with early AMD drusen size of <125 µm), in which the first funduscopic signs become apparent, a pronounced decrease of neuroretinal function (in particular, in paracentral and pericentral areas [rings 2 and 3]) is evident where changes in retinal topography occur first in AMD. In that sense, neuroretinal responses may decrease once the first clinical signs of inflammation occur (eg, small drusen). In stage 3 (AMD drusen size ≥125 µm), a further decrease in the central, paracentral, and pericentral regions (rings 1-3) below normal values occurs as the disease progresses to more advanced forms of AMD. This model is in accord with electrophysiological and histopathological findings in experimental inflammatory disease in which a decrease in neuroretinal responses coincides with histological changes. The subnormality of the ERG correlates with the photoreceptor and bipolar loss in ageing and with drusen in early AMD.

A potential confounding factor of this model is that the genotypes of the early AMD group were not known, and there is an approximately 40% risk of having AMD without these high-risk gene variants. Some of the patients with early AMD may have had other genes and/or environmental risk factors involved, and different electrophysiological features may apply. The conflicting findings from mERG studies that report either normal or decreased responses in early AMD could therefore be explained by patients having different genotypes in addition to technical and/or analysis differences. Nevertheless, patients with manifest disease can be considered to undergo the pathological processes that are encapsulated by the final stage of this model, irrespective of their individual gene-environment exposure.

In summary, we have identified supernormal neuroretinal responses in healthy persons with high-risk AMD genotypes without clinical signs of the disease. Given that our findings may appear counterintuitive to results that might be expected if high-risk genotypes were hypothesized to decrease neuroretinal activity, we consider our results as interesting preliminary data that should be further investigated in larger longitudinal studies. In support of our findings is a recent animal study suggesting that retinal function is genetically determined but that there are numerous phenotypic variances in retinal function, even in the healthy retina. The electrophysiological findings of supernormality may be linked with genetically determined inflammatory and subclinical ageing processes at the histological level that are established factors in the pathomechanisms of AMD. The multifocal electroretinogram is now being established as a robust technology with low variability in healthy participants. In disease, neuroretinal deficits can be detected in retinal locations before there is clinical evidence of disease, as has been shown in diabetic retinopathy, and owing to discrete changes in oxygenation that may occur via chorioidal blood flow disturbances in AMD. It is further recognized as a valuable tool for documenting treatment effects in AMD. Our findings suggest that neuroretinal function may have a value as a biomarker, in addition to genetic prediction models for the detection of people at risk of AMD before there is funduscopic evidence of AMD.

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Ophthalmic Images

Chlorpromazine-Induced Corneal Toxicity
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Wei-Boon Khor, MRCS(Ed)
Li Lim, FRCS(Ed)

A 62-year-old schizophrenic patient on long-term chlorpromazine treatment
presented with crystalline deposition on bilateral cornea in the posterior
stroma and endothelium (Figure, A; arrow). Sclerotic scatter illumination
demonstrates that the stromal deposits are more dense centrally and
involving the visual axis (Figure, B).