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The relationship among *CFH* and *ARMS2* genotypes, macular pigment optical density and neuroretinal function in persons without age-related macular degeneration

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Purpose: 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 and Participants: Neuroretinal function was assessed with the multifocal electroretinogram (mfERG) for 32 participants (22 healthy persons with no AMD and 10 early AMD patients). The 22 healthy participants with no AMD had high- or low-risk genotypes for either *CFH* (rs380390) and/or *ARMS2* (rs10490924). Trough-to-peak response densities and peak-implicit times were analyzed in 5 concentric rings. Macular pigment optical densitometry was assessed by 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 ($p<0.05$). 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.

Conclusion: 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) before there is clinical manifestation.

Psychophysical and electrophysiological measures of central retinal function have been repeatedly shown to be reduced in patients with manifest early age-related macular degeneration (AMD).[1-13] Psychophysical tests of dark adaptation,[14] glare recovery[2, 15] and flicker sensitivity[16, 17] were identified as markers of visual function deficits before loss of visual acuity. The introduction of multifocal electroretinography (mfERG) ,[18] enabled a detailed determination of local neuroretinal function in cone and rod-mediated pathways in AMD.[6, 7, 13, 17, 19-22] These findings have been replicated in recent studies.[23] Reasons for conflicting results regarding neuroretinal function deficits in early AMD have been discussed and these include different paradigms, analysis methods and phenotypes,[5, 23-25] however, the effect of genotype on neuroretinal function has not been studied.

Both the rod and cone-mediated visual pathways can be affected in early stages of AMD, with evidence of greater rod than cone vulnerability.[6, 26] The current understanding is that the pathomechanisms of AMD involve genetic and environmental risk factors.[24, 27] While the genetics cannot fully explain AMD,[28] gene variants account for more than 50% of the risk. This has led to the use of statistical models that have included high-risk genotypes and environmental risk factors with the aim to better predict AMD.[29-33] These models may be extended by including visual function tests as biomarkers for vision loss before there are clinical signs of AMD. [28, 34]

We have shown in our previous investigations that persons with no signs of AMD but who are genetically at high-risk, have on average, worse combined rod and cone function (mesopic vision) than those with low-risk gene variants.[34] Our results indicated that there was normal cone-function when rod activity was silenced but the combined rod and cone

function was reduced in high–risk genotypes. In this study we investigate a subset of this group to determine the effect of genotypes on neuroretinal function. We compared neuroretinal function between healthy persons with high and low-risk genotypes but no clinical signs of AMD and early AMD patients, with the aim to provide a genotype-phenotype neuroretinal function signature that may reflect the electrophysiological dynamics in subclinical and manifest early AMD. Given lower macular pigment optical density (MPOD) has been associated with AMD risk genotypes in a small group (n=4) of patients homozygous for the *CFH* and *ARMS2*,[35] we were also interested in the relationship between MPOD and genotypes in our cohort of healthy participants.

Participants and Methods

We investigated the right eye of 22 healthy persons (mean age 56.1 yrs ± 4.8 SD) who have been previously genotyped for the common AMD *CFH* (rs380390) and *ARMS2* (rs10490924) risk gene variants using optimized gene-expression assays (TaqMan Gene Expression Assay; Applied Biosystems, Inc., Foster City, CA).[34] Of these, 14 participants were either homozygous or heterozygous for one or both of the high-risk genotypes and 8 participants 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:[36, 37] *CFH* (rs380390), CC OR = 7.4, GG + CG OR = 1; *ARMS2* (rs10490924), TT OR = 6.09, GT OR = 1.35, GG OR = 1. The investigator was masked to the genotyping results. All healthy participants had visual acuity better than 6/6 and no refractive errors more than ± 3 diopters.[38] The participants had no ophthalmoscopic signs of AMD and all had normal central retinal thickness measured with optical coherence tomography (OCT) (Stratus III, Carl Zeiss Pty Ldt, Oberkochen, Germany) as determined by an ophthalmologist (BF).

For comparison with the healthy participants without clinical signs of AMD, we included ten participants (mean age 73.1 yrs ±2.7SD) with manifest early AMD (grading according to AREDS)[39] who had been previously tested in our laboratory[22] and who had not been genotyped (**Table 2)**. Fundus grading was based on colour fundus photographs (Zeiss Jena Mydriatic Fundus Camera) of the central 30º and retinal changes were graded independently by two experienced observers who were masked to the results. All early AMD patients had visual acuity of 6/15 or better, drusen > 63μm and/or retinal pigment abnormalities but no late geographic or neovascular AMD.

Written informed consent was obtained from all participants and the study was approved by the Human Research Ethics Committee of the Queensland University of Technology and which followed the tenets of the Declaration of Helsinki.

Multifocal electroretinogram

We performed neuroretinal function testing with the multifocal electroretinogram (mfERG) (VERIS, EDI, Redwood City, CA). The stimulus array of the mfERG was presented on a calibrated 9 inch CRT monitor (screen refresh rate 13.3 ms, frame rate 75 Hz); the display consisted of 103 hexagons (ranging in size from about 3.2 to 5 deg in horizontal extent) and subtended approximately 25 degrees (Figure 1A). The binary m-sequence was 2^{13}-1 frames long. A slow flash mfERG (sf-mfERG) paradigm was used for better detection of subclinical changes in adaptation compared to the conventional fast-flicker mfERG.[40, 41] The positive (N1P1) waveform component of both paradigms, the fast flicker (ff)mfERG[42] and sf-mfERG[43] however, contains mainly contributions from ON and OFF bipolar cells.

The stimulus sequence was slowed by inserting 3 blank frames after the initial m-sequence frame where the hexagons had a 50% probability of being white (200 cd/m^2) or black (3 cd/m^2), and the next 3 frames remained dark (3 cd/m^2) resulting in a time-average luminance of 26 cd/m^2. The surround luminance was always 52 cd/m^2. Recordings followed the ISCEV standard[44] and were made monocularly and in ambient room lighting conditions. Recordings were divided into sixteen segments resulting in a total recording time of ~8 minutes. We used DTL electrodes as active electrodes. The participant's right pupil was dilated with 0.5% tropicamide; 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 recording, the VERIS system displayed a video image of the eye and the ERG signal. Contaminated segments due to fixation loss or repeated blinks were discarded and re-recorded. Retinal signals were band-pass filtered between 10 and 300 Hz, amplified 100,000 x and sampled every 0.83 ms. A single spatial averaging was performed as per ISCEV/VERIS recommendations.[44] We determined the trough N1 to peak P1 response density (RD), N1P1-RD and peak P1 implicit time (time from onset of stimulus to first peak), P1-IT after averaging the 103 hexagons into 5 concentric rings (ring 1: 0º -5º, ring 2: 5º -9º, ring 3: 9º -13.5º, ring 4: 13.5º -19º, ring 5: 19º -25º) (Figure 1A).[22] The concentric ring averaging was based on the predilection of first histological and functional changes in AMD being centrally and paracentrally.[26, 45]

Macular pigment optical density

Macular pigment optical density (MOPD) was estimated with the Macular Pigment Densitometer (Macular Metrics II, LLC, Providence, USA) in 20 out of 22 healthy participants (two participants were unavailable to return for MPOD measurements). In brief, the system is based on Heterochromatic Flicker Photometry (HPF).[46] A 0.5º spot is

presented in the fovea and a 2º spot is presented 7º paracentrally. The stimulus is a flickering light that alternates between 460 nm which is absorbed by macular pigment and 520 nm which is not absorbed by macular pigment. The participant's task is to adjust the stimulus radiance to minimize or eliminate any flicker in the stimulus. The amount of 460 nm light required to achieve minimum flicker is used to calculate the MPOD. The Macular Pigment Densitometer allows individual adjustment of the flicker frequency: at the start of a practise run, the flicker frequency is determined where the participant finds a zone of no flicker by increasing or decreasing the flicker frequency. All participants performed four measurements at both central and peripheral locations. The MPOD was derived by subtracting the log foveal sensitivity from the log peripheral (7º) sensitivity using the manufacturer's software. MPOD was normal in all healthy participants (mean 0.52 ± 0.2SD).

Statistical analyses

We used PASW Statistics 18 (SPSS Inc Chicago, IL) for analysis. All data were normally distributed and therefore parametric analyses were performed. The mfERG N1P1-response density and P1-implicit time data were analysed using mixed design repeated measures analysis of variance to determine effects between the three groups (high-risk AMD genotype, low-risk AMD genotype and early AMD groups). *Post-hoc* analysis was performed when significant effects were found. Age and smoking status were included as confounding variables and entered as covariates in the statistical analysis. Pearson's correlation was used to determine a relationship between the MPOD and haplotype combinations. A p-value of 0.05 or less was considered statistically significant.

Results

The slow flash (sf)-mfERG results of four participants were excluded from analysis due to poor fixation.[44] The mfERG results of the remaining 18 participants (11 with high-risk and 7 with low-risk genotypes) for N1P1-RD and P1-IT are shown in Table 1. The sf-mfERG results for the ten early AMD patients are outlined in Table 2. Figure 1B-D shows three representative mfERG trace arrays: a healthy funduscopically normal participant with a high-risk genotype (B), a healthy funduscopically normal person with a low-risk genotype (C) and a patient with early AMD (D). The person who is genetically at high-risk (B) demonstrates larger (supernormal) N1P1 response amplitudes compared to the healthy person with the low-risk genotype (C). The early AMD patient (D) shows lower responses and has delayed central responses compared to the high-risk (B) and low-risk genotype (C). The mean mfERG data (N1P1-RD and P1-IT) of the three groups are demonstrated in Figure 2 (upper and lower panels) and show the same response patterns as the individual observers. The MPOD results for each of the 19 healthy participants are shown in **Table 3** (two participants could not perform the MPOD and were excluded from the 21 enrolled healthy persons) and these where within the normal range reported in the literature.[47]

Statistical analysis was performed and a significant difference was detected between the three groups for the N1P1-RD ($F_{2, 25}$ = 5.1, p=0.01) demonstrating that healthy persons with high-risk genotypes had on average significantly larger N1P1-RD for rings 1-3 compared to the participants with low-risk genotype (*post-hoc* p<0.01*)* and early AMD patients (*post-hoc* p<0.05*)*. After correction for age, the difference between the three groups was still significant ($F_{2, 25}$ = 3.6, p=0.04). In the cohort of (healthy and early AMD) participants there were no current smokers. However, after including past smokers as a covariate in the statistical model, there was a significant difference between the three groups ($F_{2,24}$ = 4.1, p=0.03).

There was no significant difference between any of the groups for the P1-IT for each ring ($F_{2, 25} = 0.6$, p=0.5) but the AMD group demonstrated on average delayed implicit times for ring 1 group compared to the other two groups (Figure 2, Table 1). There was no significant correlation between the genotype and macular pigment optical density ($r = 0.14$, $p = 0.5$).

Discussion

Our study identified significantly greater central neuroretinal function in healthy participants with high-risk genotypes compared to those with low-risk genotypes with no clinical signs of AMD (as determined ophthalmoscopically according to AREDS classification and with the Stratus OCT). These findings and our previous research[34] in healthy observers have important implications for studies on "normal ageing" as they provide a way to differentiate neuroretinal and psychophysical function between healthy older persons. Based on our findings we infer that there are clinically "normal" subgroups whose function may be differentiated on the basis of their genetics. Healthy high risk genotypes however, were not significantly associated with lower MPOD levels suggesting further investigations into a larger sample of homozygous AMD risk genotypes are required.[35]

Supernormal responses in electrophysiology are rare, but not novel observations. They are thought to be of genetic, ischemic, inflammatory or toxic origin and can be found in patients with cone dystrophy,[48] retinal vein occlusion,[49] uveitis [50-52] and in siderosis bulbi [53] and can affect the cone [49] and rod-mediated pathways.[54] In particular, supernormal responses have been described as the earliest detectable signs in inflammatory disease before disease is ophthalmoscopically manifest.[50-52] Once inflammatory disease is manifest, the neuroretinal responses decrease to normal and subnormal values.[50] One of the main risk factors of AMD is age,[55] and ageing itself has been linked with chronic inflammation.[56]

Moreover, the pathomechanisms in AMD involve a dysregulated inflammatory/immune response due to gene variants in the complement factor H *(CFH)* region.[57] It is thought that 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.[58] 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 definition (HD) OCT but not on ophthalmoscopy/fundus photography[59] and Stratus OCT, therefore beginning pathology cannot be completely excluded in our healthy cohort of participants. It would be interesting to determine if healthy participants with high risk genotypes and supernormal ERGs show corresponding changes with the HD-OCT.

We link our results with previous findings in our laboratory where we demonstrated reduced mesopic vision in persons genetically at-risk for AMD.[34] Under mesopic conditions both cone and rod pathways are active.[60] Although the stimulus mean luminance conditions with the slow-flash mfERG were low photopic,[61] rod-driven ERG responses can be obtained at photopic illuminances and with stimuli containing low temporal frequencies[62] as with the mfERG recording used in this study. Normal rod function is crucial for cone survival and a protective role of rods over cones has been suggested.[63] 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 (and 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 found in early AMD as well as in normal ageing.[64, 65] The larger photoreceptor inner segment contain a higher number of mitochondria[66] 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 demonstrates individual N1P1 response densities for the five rings of one representative for each of the healthy high-risk genotype group and the low-risk genotype group, and two representatives of the early AMD group (one with less progressed early AMD and drusen size less than 125 μm, and one early AMD patient with more progressed funduscopic changes and with drusen size greater than 125 μm). The individuals in this example are not significantly different from the mean group data (Figure 2). In stage 1 (subclinical stage, normal ophthalmoscopy and Stratus OCT but histological signs of cell loss) healthy persons genetically at-risk for AMD have supernormal neuroretinal responses in the central, para- and pericentral retinal areas (rings 1-3). In stage 2 (clinical, early AMD drusen <125 μm) where first funduscopic signs become apparent, a pronounced decrease of neuroretinal function in particular in paracentral and pericentral areas (rings 2-3) is evident where changes in retinal topography occur first in AMD.[67] In that sense, neuroretinal responses may decrease once the first clinical signs of inflammation[68, 69] occur (e.g small drusen). In stage 3 (AMD drusen ≥ 125 μm), a further decrease for central, para- and pericentral regions (rings 1-3) below normal values occurs as the disease progresses to more advanced forms of AMD. This model is in accordance with electrophysiological and histopathological findings in experimental inflammatory disease where a decrease in neuroretinal responses coincides with histological changes.[51] The subnormality of the ERG

correlates with the photoreceptor and bipolar loss in ageing[70, 71] and with drusen in early AMD.[72]

A potential confounding factor of this model is that the genotypes of the early AMD group were not known and there is a ~40% risk of having AMD without these high-risk gene variants.[73] Some of the early AMD patients may have had other genes or/and environmental risk factors involved and different electrophysiological features may apply. The conflicting findings from mfERG studies that report either normal or decreased responses in early AMD[5, 74] could therefore be explained by patients having different genotypes in addition to technical/analysis differences. Nevertheless, patients with manifest disease can be considered to undergo the pathological processes which 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 our findings may appear counter-intuitive 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.[75] 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.[76] In disease, neuroretinal deficits can be detected in retinal locations

before there is clinical evidence of disease as has been shown in diabetic retinopathy,[77] in early AMD[20] and due to discrete changes in oxygenation[78] that may occur via choroidal blood flow disturbances in AMD.[79] It is further recognized as a valuable tool for documenting treatment effects in AMD.[80, 81] 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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Legends:

Figure 1: Slow-flash multifocal electroretinogram (mfERG) arrays: hexagonal mfERG stimulus array of 5 concentric rings (**A**) and mfERG trace arrays showing the results of a representative sample of healthy participants with a high-risk genotype (**B**), or a low-risk genotype (**C**) and patients with early AMD (**D**) who demonstrated supernormal, normal and centrally delayed responses, respectively.

Figure 2: Mean response densities for rings 1-5 of the three groups of individuals. The densities demonstrate significantly larger N1P1-RD for the healthy participants with high-risk genotypes compared to the healthy participants with low-risk genotype and the patietns with early age-related macular degeneration (AMD) **(A)**. No significant difference was found in P1-implicit times for rings 1 to 5 among the three groups, but those were on average delayed for ring 1 in the early AMD group (lower panel) **(B)**. Error bars indicate SD,*P≤.05

Figure 3. Multifocal electroretinogram N1P1-RD are shown for an individual person of each of the three 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 para- and pericentral area (rings 2 and 3) where there are greatest changes in retinal topography (photoreceptor/bipolar cell loss) in 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 1

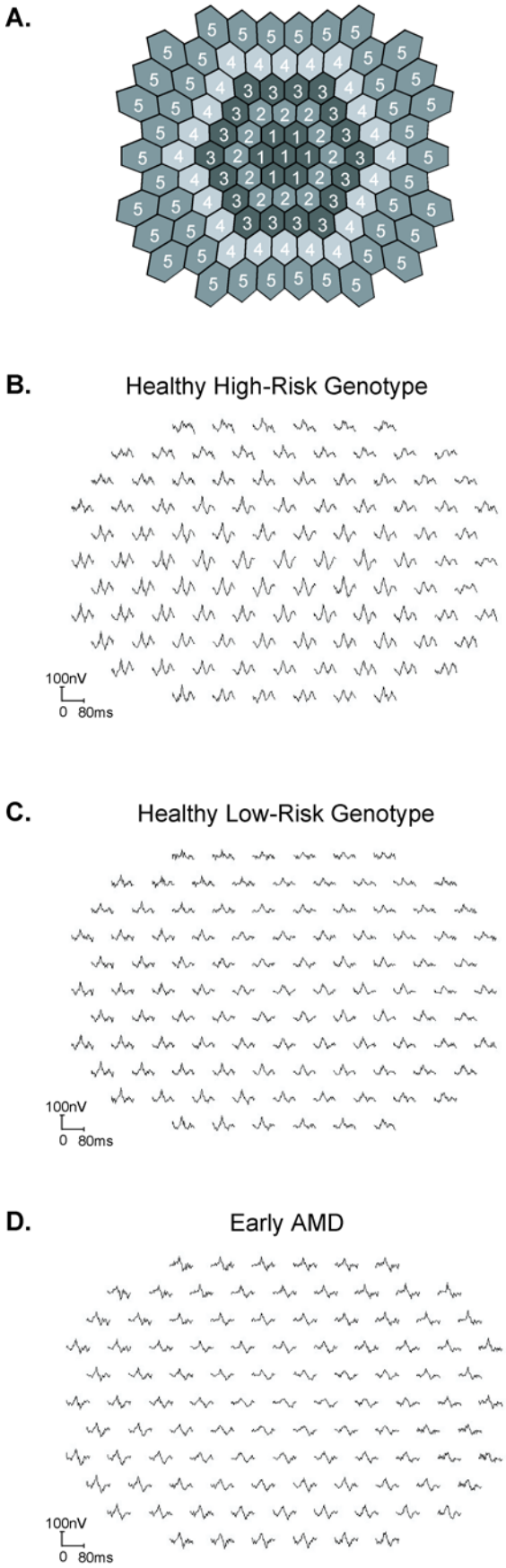
A.
B.
Healthy High-Risk Genotype
100nV
0 80ms
C.
Healthy Low-Risk Genotype
100nV
0 80ms
D.
Early AMD
100nV
0 80ms

Figure 2

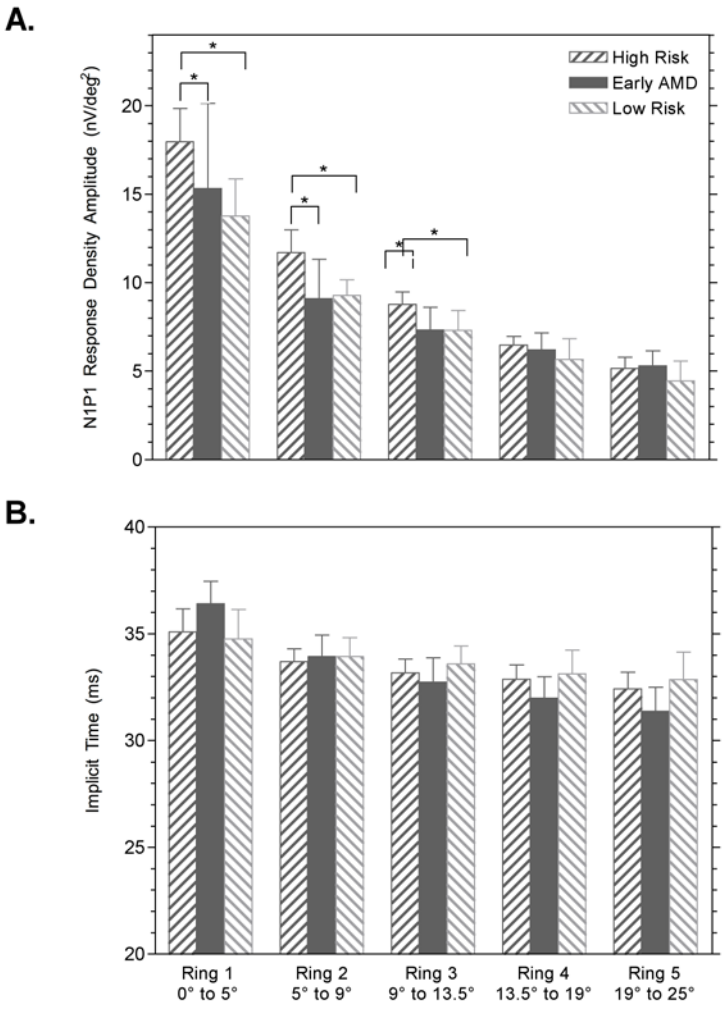
A.
N1P1 Response Density Amplitude (nV/deg²)
High Risk
Early AMD
Low Risk
0
5
10
15
20
B.
Implicit Time (ms)
20
25
30
35
40
Ring 1
0° to 5°
Ring 2
5° to 9°
Ring 3
9° to 13.5°
Ring 4
13.5° to 19°
Ring 5
19° to 25°

Figure 3

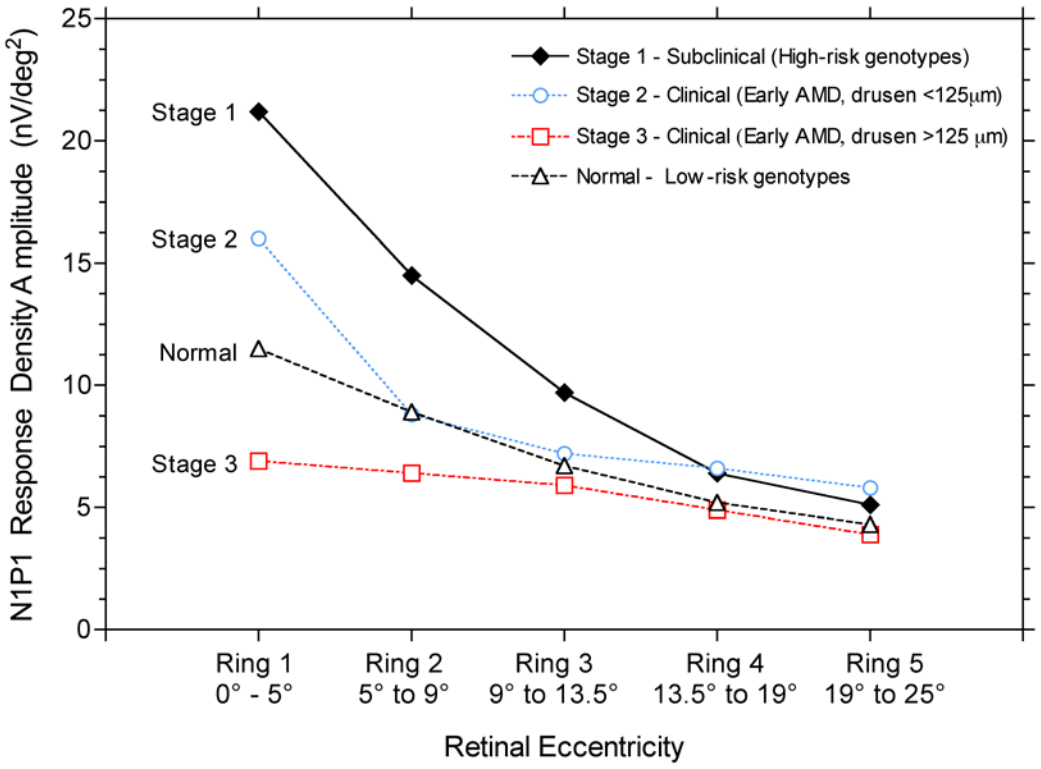
N1P1 Response Density Amplitude (nV/deg²)
25
20
15
10
5
0
Stage 1 - Subclinical (High-risk genotypes)
Stage 2 - Clinical (Early AMD, drusen <125μm)
Stage 3 - Clinical (Early AMD, drusen >125 μm)
Normal - Low-risk genotypes
Stage 1
Stage 2
Normal
Stage 3
Ring 1
0° - 5°
Ring 2
5° to 9°
Ring 3
9° to 13.5°
Ring 4
13.5° to 19°
Ring 5
19° to 25°
Retinal Eccentricity

Table 1. The demographic characteristics of healthy participants with high and low-risk genotypes and multifocal ERG response densities (in nV/deg^2) and implicit times (in ms)

age	gen	*CFH/ARMS2* genotype	Risk	RDR1	RDR2	RDR3	RDR4	RDR5	ITR1	ITR2	ITR3	ITR4	ITR5
57	f	CC/GT	High risk	21.2	14.5	9.7	6.4	5.1	33.3	33.3	32.5	32.5	32.4
48	m	CC/GT	High risk	20.1	13.6	9.5	6.9	5.1	35.8	34.2	34.2	34.2	32.5
62	f	CC/GT	High risk	15.4	11.3	8.8	6.2	4.7	34.2	34.2	32.5	32.5	31.7
57	m	CC/GG	High risk	18.6	11.5	8.3	5.8	4.6	33.3	33.3	33.3	33.3	32.5
51	m	CC/GG	High risk	15.8	10.2	8.4	6.2	4.8	35.8	34.2	34.2	33.3	33.3
53	f	CC/GG	High risk	18.4	11.2	9.2	6.7	4.9	35.0	33.3	32.5	32.5	31.7
53	m	CG/GT	High risk	16.9	11.2	9.8	7.5	6.3	34.2	33.3	32.5	32.5	32.5
52	f	CG/GT	High risk	16.6	10.6	8.7	6.4	4.8	35.0	33.3	33.3	33.3	33.3
52	f	CG/GT	High risk	18.7	11.4	8.4	5.9	4.6	35.0	33.3	32.5	31.7	30.8
57	m	CG/GT	High risk	19.5	12.3	8.2	6.9	6.3	36.7	35	33.3	33.3	33.3
55	f	CG/GT	High risk	16.5	10.9	7.6	6.3	5.5	36.7	33.3	33.3	32.5	32.5
56	m	CG/GG	Low risk	13.9	9	6	4.3	3.7	34.2	34.2	34.2	34.2	34.2
63	f	CG/GG	Low risk	12.7	9.1	7.5	5.6	4.4	36.7	34.2	34.2	34.2	34.2
61	f	CG/GG	Low risk	15.9	10.5	8.6	6.4	5	33.3	32.5	3.5	32.5	32.5
52	m	CG/GG	Low risk	11.5	8.9	6.7	5.2	4.3	33.3	33.3	33.3	32.5	32.5
60	m	CG/GG	Low risk	11.5	8.3	7	4.8	2.8	35.8	35	34.2	34.2	33.3
63	f	CG/GG	Low risk	16.2	10.4	8.9	7.6	6.1	34.2	33.3	32.5	31.7	30.8
53	m	CG/GG	Low risk	14.7	8.7	6.4	5.7	4.8	35.8	35	34.2	32.5	32.5

Abbreviations: gen: gender, m: male, f: female, RD: response density, IT: implicit time, R1-R5: ring 1-ring 5

Table 2. The characteristics of early AMD patients and multifocal ERG response density (in nV/deg^2) and implicit time (in ms) results

age	gender	RDR1	RDR2	RDR3	RDR4	RDR5	ITR1	ITR2	ITR3	ITR4	ITR5
73	m	22.8	13.7	10	7.5	5.7	36.6	34.2	32.5	31.7	30.8
74	f	18.1	10.1	6.5	5.1	4.6	35.8	35	34.2	1.7	31.7
75	m	15.2	9.2	7.5	6.3	6.2	36.6	33.3	32.5	31.7	31.7
73	m	13.9	8.5	8.1	7	6.6	37.5	32.5	32.5	31.7	31.7
70	f	16	8.8	7.2	6.6	5.8	35	35	33.3	32.5	32.5
69	m	13.7	7.9	7.1	6.5	5	36.6	32.5	31.7	31.7	30.8
75	f	9.5	5.8	5.3	4.7	4.3	36.6	33.3	31.7	30.8	30
75	f	6.9	6.4	5.9	4.9	3.9	34.2	34.2	34.2	34.2	33.3
77	f	15.9	9.9	7.9	6.7	5.5	37.5	35	34.2	33.3	32.5
69	f	21.7	10.6	7.7	6.8	5.4	36.6	33.3	30.8	30.8	30

Abbreviations: m: male, f: female, RD: response density, IT: implicit time, R1-R5: ring 1-ring 5

Table 3. The MPOD results and genotypes for the healthy participants

Age	MPOD	genotype
57	0.49	CC/GT (high risk)
48	0.71	CC/GT (high risk)
62	0.43	CC/GT (high risk)
57	0.36	CC/GG (high risk)
51	0.72	CC/GG (high risk)
53	0.97	CC/GG (high risk)
53	0.46	CG/GT (high risk)
52	0.55	CG/GT (high risk)
52	0.3	CG/GT (high risk)
53	0.58	CG/GT (high risk)
56	0.3	CG/GT (high risk)
57	0.03	CG/GT (high risk)
54	0.53	CG/GT (high risk)
56	0.39	CG/GG (low risk)
63	0.33	CG/GG (low risk)
61	0.48	CG/GG (low risk)
52	0.89	CG/GG (low risk)
67	0.78	CG/GG (low risk)
60	0.47	CG/GG (low risk)
53	0.57	CG/GG (low risk)