



Article

Exploratory Longitudinal Study of Ocular Structural and Visual Functional Changes in Subjects at High Genetic Risk of Developing Alzheimer’s Disease

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Abstract: This study aimed to analyze the evolution of visual changes in cognitively healthy individuals at risk for Alzheimer’s disease (AD). Participants with a first-degree family history of AD (FH+) and carrying the E4+ allele for the ApoE gene (ApoE ε4+) underwent retinal thickness analysis using optical coherence tomography (OCT) and visual function assessments, including visual acuity (VA), contrast sensitivity (CS), color perception, perception digital tests, and visual field analysis. Structural analysis divided participants into FH+ ApoE ε4+ and FH– ApoE ε4– groups, while functional analysis further categorized them by age (40–60 years and over 60 years). Over the 27-month follow-up, the FH+ ApoE ε4+ group exhibited thickness changes in all inner retinal layers. Comparing this group to the FH– ApoE ε4– group at 27 months revealed progressing changes in the inner nuclear layer. In the FH+ ApoE ε4+ 40–60 years group, no progression of visual function changes was observed, but an increase in VA and CS was maintained at 3 and 12 cycles per degree, respectively, compared to the group without AD risk at 27 months. In conclusion, cognitively healthy individuals at risk for AD demonstrated progressive retinal structural changes over the 27-month follow-up, while functional changes remained stable.

Keywords: Alzheimer’s disease; genetic risk; familiar history; ApoE ϵ 4; optical coherence tomography; visual acuity; contrast sensitivity

1. Introduction

By 2050, dementia is expected to affect nearly 152 million people worldwide [1], with Alzheimer’s disease (AD) being the most common cause, responsible for 50–70% of all cases [2]. AD pathophysiology is known to be initiated several years before the first clinical signs appear [3]. Traditionally, studies have focused on describing the clinical stages of the disease, from mild cognitive impairment (MCI) to established disease [4]. However, there are very few studies that have addressed the changes that may occur in subjects at significant genetic risk for developing AD [5–7]. These very early stages would be key to understanding the early onset of AD which would be the stage at which modulating intervention would be possible [8].

Among the genetic risk factors for the development of AD are [6]: (i) presence of the ApoE ϵ 4 allele [9], where the number of copies of ϵ 4 influences the level of risk and is related to the age of presentation of the clinical disease [10]; and (ii) a first-degree family history of AD, which increases the probability of developing this neurodegeneration in the future by 2.9 to 6.1 times compared to subjects with no family history [11]. In this respect, descendants of individuals with AD propensity display decreased brain metabolism of cerebral areas that are affected in neurodegenerative pathology such as the posterior cingulate, precuneus, medial temporal and parietal cortex [12,13].

In addition to brain changes, given the close relationship of this structure with the retina, retinal changes observed by optical coherence tomography (OCT) have opened up new research paths. Previously, in subjects with significant genetic risk factors for the development of AD (family history and carrier of the ApoE ϵ 4 allele), we demonstrated that thickness changes appear in different retinal layers [7], as well as in the choroidal [14] and retinal vasculature [15]. Furthermore, in these subjects, the sectors of the retina that have volume changes have been correlated with brain areas involved in AD such as the lingual gyrus, the entorhinal cortex, and the hippocampus [16].

Visual function has also been shown to be impaired in AD [17]. In fact, in cognitively healthy subjects with two genetic risk factors for the development AD (FH+ and ApoE ϵ 4+), it has been shown that there is an increase in VA and that this correlates with a lower M100 latency and a higher power time–frequency cluster measured by magnetoencephalography (MEG) [18].

Most of the studies that analyze the retina and visual function have been performed as a descriptive observational study design and in most studies when cognitive decline already exist or when there is an AD diagnosis [19–21]. Nevertheless, there is still a requirement for future longitudinal trials involving individuals who are at a higher risk of developing dementia caused by AD. This type of study is of special interest to know how subjects without memory complaints and at high risk of developing AD evolve over time. Therefore, the purpose of this study is to analyze how structural and functional alterations of the visual pathway progress at 27-month follow-up in a population of cognitively healthy subjects, without neurological or ophthalmological diseases, but with significant genetic risk of developing AD (family history positive and carriers of ApoE ϵ 4+).

2. Materials and Methods

2.1. Participants

Subjects were enrolled from the study “The cognitive and neurophysiological characteristics of participants at high genetic risk of developing dementia: a multidimensional approach” (COGDEM study) which was subsidized by the Spanish Ministry of Economy and Competitiveness with project code PSI2015-68793-C3-2-R. All participants had an age of 40–75 years; they did not have psychiatric or neurological disorders or, structural

alterations on MRI. Furthermore, these subjects were free of addictions or chronic use of neuroleptic, anxiolytic, anticonvulsant, narcotic, or sedative–hypnotic sedative medications and were cognitively healthy. All subjects had undergone a complete neuropsychological screening and did not present any abnormalities.

Two cohorts were subjected to analysis. On one hand, there was a group comprising individuals lacking a familial history of sporadic Alzheimer’s disease (AD) with senile onset. These participants demonstrated the following characteristics: (i) normal cognitive function; (ii) absence of a first-degree family history of AD (FH–); (iii) non-carriers of the ApoE ε4 allele; and (iv) absence of memory recall complaints. On the other hand, the second study group consisted of individuals at a significant genetic risk of developing AD, and they were matched with the control group in terms of age and socioeconomic status. This group consisted of participants with the following attributes: (i) normal cognitive function; (ii) a first-degree family history of senile AD (FH+) (either the father or mother having AD); (iii) carrying one or more ε4 alleles for the ApoE gene (ApoE ε4+); and (iv) no memory-related complaints and no presence of vascular or other types of dementia. All participants in both cohorts obtained scores exceeding 26 on the Mini-Mental State Examination (MMSE).

Informed written consent was obtained from the participants for their inclusion in the study, and the study protocol received approval from the Local Ethics Committee (Hospital Clínico San Carlos) under the internal code 18/422-E_BS. Finally, the research adhered to the principles outlined in the Declaration of Helsinki.

An average of 27 months had elapsed between the baseline visit and the follow-up visit. At the baseline visit, OCT data from 29 participants in the FH– ApoE ε4– group and 35 subjects in the FH+ ApoE ε4+ group were analyzed. At the follow-up visit, we analyzed 15 subjects with FH– ApoE ε4– and 21 participants with FH+ ApoE ε4+ (Figure 1).

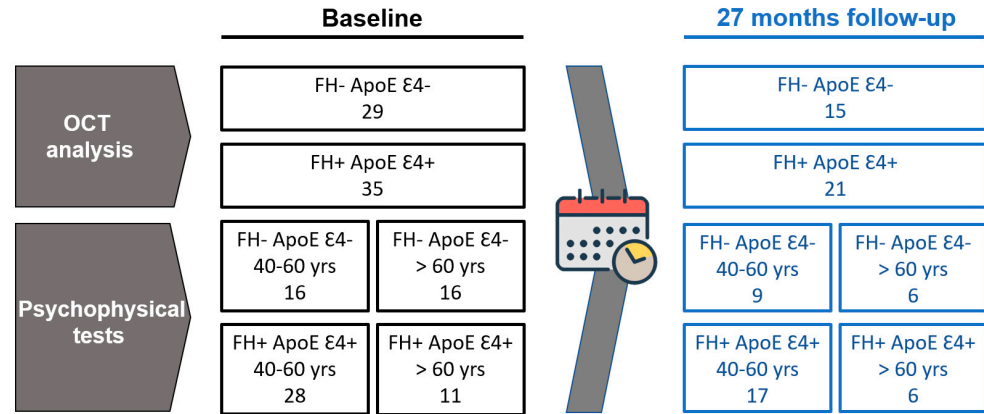


Figure 1. Diagram of the study groups in the baseline and 27-month follow-up examination. In black, participants included in the baseline visit, in blue, participants with follow-up visit. OCT: optical coherence tomography; FH: family history; ApoE: apolipoprotein E; yrs: years old.

To analyze all psychophysical tests, patients were classified into two groups based on their age: a group between 40 and 60 years old, and a group over 60 years old. This classification was made taking into account the deterioration of vision that occurs from the age of 60 onwards. The groups’ distributions are detailed in Figure 1.

Patients were explored since May 2019 to September 2021.

2.2. Optical Coherence Tomography (OCT)

The analysis of macular region thickness was performed using OCT images obtained using the Spectralis OCT system (Heidelberg Engineering, Heidelberg, Germany), following the established OCT protocol [7].

The Heidelberg OCT utilizes segmentation software (Heidelberg, Germany, version 1.10.4.0) that enables estimation of retinal layer thickness in the macular area, including

total retinal thickness. These measurements were evaluated by the same optometrist (IL-C) and manually adjusted in case of segmentation errors made by the software. The following retinal layers were examined: retinal pigment epithelium (RPE), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), and retinal nerve fiber layer (RNFL). The macular area analysis followed the standard of the Early Treatment Diabetic Retinopathy Study (ET-DRS). Peripapillary RNFL (pRNFL) was assessed in six sectors: nasal (N), inferonasal (IN), superonasal (SN), inferotemporal (IT), superotemporal (ST), and temporal (T). Additionally, a global mean of all peripapillary sectors was calculated. Only macular scans meeting the following quality criteria were included: an average of 16 B-scans and a minimum signal-to-noise ratio of 25. pRNFL images were required to have a minimum of 40 B-scans and a minimum signal-to-noise ratio of 20. Thickness measurements were reported in microns, based on the calibration provided by the device manufacturers.

2.3. Psychophysical Test

2.3.1. Visual Acuity

The best monocular visual acuity (VA) was assessed using a standard clinical Snellen eye chart, following the methodology described by Salobar-García et al. [22]. Best corrected VA was measured, and subjects began reading each row from top to bottom. VA was recorded when subjects could no longer recognize at least five out of eight letters (56.25%), which represented the highest point on the psychometric acuity function.

2.3.2. Contrast Sensitivity

With the best corrected VA for far vision, we analyzed the CS using the CSV-1000E system (VectorVision, Greenville, OH, USA). Both the illumination at which the test should be performed, and the distance, followed the manufacturer's recommendations. Four spatial frequencies were analyzed: 3, 6, 12 and 18 cycles per degree.

2.3.3. Color Perception Test

In this study, color perception was assessed in individuals with the best corrected near vision using the Roth 28-Hue Color Test (Luneau technology, Paris, France) [23]. The number of errors was recorded and categorized as deuteranomalous, tritanomalous, or protanomalous (indicating deficiencies in perceiving green, blue, or red, respectively). Deuteranomaly errors were identified when the caps were misplaced between positions 42 and 85, while blue axis errors were determined based on the manufacturer's manual, considering caps 43 to 64 in the incorrect position. The quantity of deutan and tritan errors was quantified. The administration of this test followed the procedure outlined in a previous study [24].

2.3.4. Perception Digital Test

In order to evaluate visual perception impairments in patients with Alzheimer's disease (AD), the PDT (perception digital test) has conventionally been employed [24,25]. This test comprises 15 slides, each displaying an image positioned differently in space and distorted using specific effects, such as geometric effects (mosaic) and the 24/48-frame effect implemented in the program. Consequently, the patient is required to identify the correctly oriented image, and successful completion of the test is achieved when all plates are correctly identified by the subject.

2.3.5. Visual Field

Visual field testing was performed using the Humphrey740i perimeter (Carl Zeiss Meditec, Dublin, CA, USA), using the SITA FAST 30-2 analysis strategy. The trial lens was calculated from the patient's best corrected VA for distance. The test was performed in a darkened room and monocularly, occluding the non-tested eye with a patch.

The parameters analyzed in the study were: number of fixation losses, percentage of false positives and false negatives, mean deviation, deviation from the model, and visual field index.

2.4. Allelic Characterization

The method for detecting the APOE polymorphism has already been described in previous work [7,16]. The genotype was determined using Taqman assay technology. For this purpose, DNA was extracted from a peripheral blood sample collected in an EDTA tube using standard protocols. The C____904973_10 and C__3084793_20 assays were used to analyze the rs7412 and rs429358 SNPs, which conform the haplotype that determines the APOE isoforms. Allelic detection was performed using an Applied Biosystems 7500 Fast Real-Time PCR machine (Applied Biosystems, Foster City, CA, USA), using the appropriate assay quality controls.

2.5. Statistical Analysis

Statistical analysis was conducted using SPSS 27.0 software (SPSS Inc., Chicago, IL, USA). The data were reported as median (interquartile range) and mean \pm standard deviation (SD). The chi-square test was employed to analyze qualitative variables between the study groups (FH– ApoE ϵ 4– and FH+ ApoE ϵ 4+). Within the same group, the Wilcoxon test was used to compare data between the baseline visit and the follow-up visit. The Mann–Whitney U test was utilized to compare follow-up visits between the highest and lowest risk groups. A p -value of <0.05 was considered statistically significant.

2.6. Colorimetric Representation

The color scale function of the Excel program (Microsoft, Redmond, Washington, DC, USA, version 2306) was used for the representation of changes in macular and peripapillary thickness between the baseline and follow-up visits of the study groups. The computer program directly assigns the color tone based on the normalized thickness change. A white color or a value of 1 is used when there is no difference, while a blue color with a value of -0.85 indicates thinning, and a red color with a value of 1.15 represents thickening.

3. Results

3.1. Demographic Study

During the baseline examination, OCT of 64 participants and visual function tests of 71 subjects were analyzed, at the 27-month follow-up examination there was a loss of participants due to the health situation caused by COVID-19 leading to analysis of OCT of 36 participants and visual function tests of 38 subjects.

The mean age of the FH– ApoE ϵ 4– group at the baseline visit was 60.77 ± 7.28 and at the follow-up visit of 61.00 ± 7.56 . In this study group, we found no statistically significant differences in the MMSE score ($p = 0.480$) between the baseline (28.52 ± 0.81) and follow up visits (28.60 ± 0.91).

In the FH+ ApoE ϵ 4+ group, at the baseline visit, the mean age was 56.59 ± 6.58 and at the follow-up visit, the age was 58.63 ± 5.06 . We also found no statistically significant differences in the MMSE score ($p = 0.052$) between the baseline (28.93 ± 0.53) and 27-month follow up visits (29.92 ± 0.70).

3.2. Longitudinal Study in the FH– ApoE ϵ 4– Group

When we compare the macular area in the FH– ApoE ϵ 4– group between the measurements taken at the baseline visit and the measurements taken 27 months later of the total retinal thickness, we found a statistically significant decrease in thickness in the superior sector of the inner ring (344.00 (334.50 – 354.00), baseline vs. 344.00 (328.25 – 355.75) follow-up visit) ($p < 0.05$) (Figure 2).

We also found a statistically significant increase in RNFL thickness in the temporal sector of the outer macular ring (19.00 (18.00–21.00), baseline vs. 19.50 (18.00–20.75), follow-up) ($p < 0.05$) (Figure 2).

The GCL in this group showed a statistically significant reduction in the thickness of the inner macular ring in the superior (54.00 (50.00–56.00) baseline vs. 54.00 (49.25–54.25) follow-up) ($p < 0.05$) and nasal sectors (53.00 (49.50–56.00), baseline vs. 53.00 (50.50–53.00), follow-up) ($p < 0.05$). We also found a statistically significant increase in the overall volume of this layer (1.10 (1.03–1.17), baseline vs. 1.09 (1.00–1.15), follow-up) (Figure 2).

Baseline vs 27months follow-up in FH- ApoE $\epsilon 4$ -

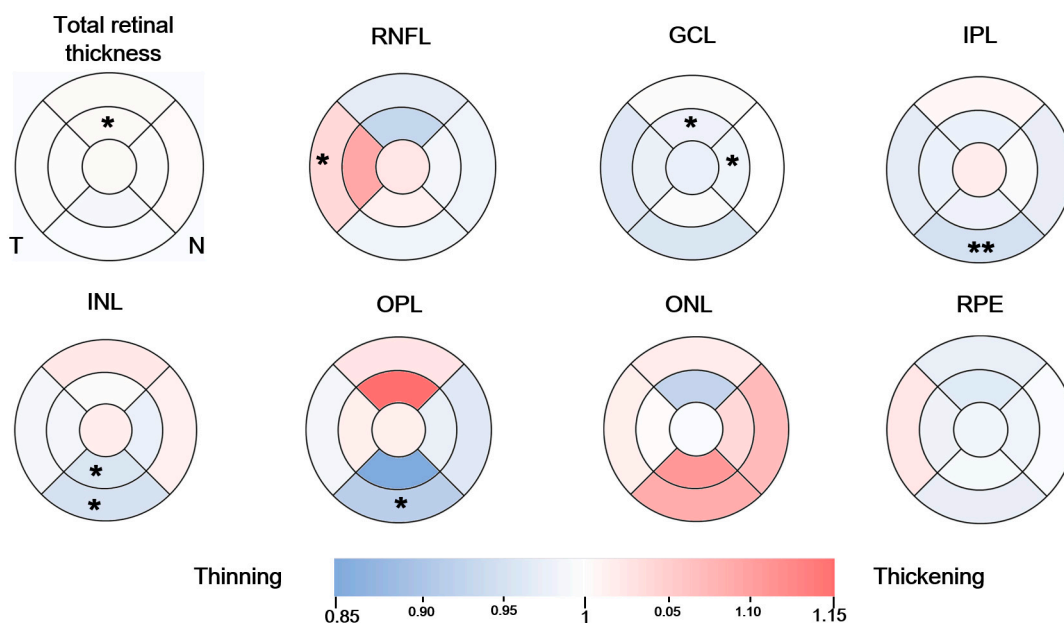


Figure 2. Colorimetric differences in the retinal thickness in each layer between baseline measurements and 27-month follow up in the FH– ApoE $\epsilon 4$ – group in the macular OCT rings. In blue, thinning, in red, thickening. (RPE: retinal pigment epithelium, ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; RNFL: retinal nerve fiber layer). * $p < 0.05$, ** $p < 0.01$. Wilcoxon test.

In addition, in the IPL, a significant thickness reduction was found in the inferior sector of outer macular ring (29.00 (27.00–30.00), baseline vs. 27.00 (24.75–29.00) follow-up) ($p < 0.01$) (Figure 2) and a significant decrease in overall volume (0.93 (0.87–0.98), baseline vs. 0.93 (0.83–0.96) follow-up) ($p < 0.05$).

In the INL, the inferior sectors in both macular rings showed a significant thickness decrease (inner (43.00 (39.50–45.00), baseline vs. 40.50 (38.00–43.00) follow-up)) (outer (32.00 (30.00–34.00), baseline vs. 29.50 (28.75–34.00) follow-up)) ($p < 0.05$ in both cases) (Figure 2).

When comparing between the first measurement and at 27-month follow-up, we found a statistically significant thickness decrease in the inferior sector of the outer macular ring in the OPL (30.00 (27.00–32.50), baseline vs. 27.00 (25.75–28.50), follow-up) ($p < 0.05$) (Figure 2).

We found no statistically significant differences when comparing between baseline and follow-up in either ONL or the RPE (Figure 2).

When analyzing the pRNFL, we found a statistically significant thickness decrease in the IN sector (111.00 (102.50–132.00), baseline vs. 112.00 (99.75–125.25) follow-up) ($p < 0.05$) (Supplementary Figure S1).

3.3. Longitudinal Study in the FH+ ApoE $\epsilon 4$ + Group

In the FH+ ApoE $\epsilon 4$ + group, when compared the macular thickness in the total retinal thickness measurements in the baseline with the 27-month follow-up, we observed sta-

tistically significant differences in: (i) the superior sectors of both the inner and outer rings (344.00 (339.00–352.00), baseline vs. 342 (333.5–347.25), follow-up) and (298.00 (294.00–302.00), baseline vs. 295.00 (290.00–308.00), follow-up), respectively) ($p < 0.01$ in both cases); (ii) in the nasal sector of the inner ring (345.00 (340.00–355.00), baseline vs. 342.00 (334.50–352.50) follow-up) and the outer ring (316.00 (307.00–327.00) baseline vs. (314.00 (307.00–326.00) follow-up) ($p < 0.05$ in both cases); (iii) the inferior sector of the outer ring (289.00 (282.00–297.00), baseline vs. 284.00 (277.00–291.50) follow-up) ($p < 0.01$); (iv) the temporal sector of both the inner and outer ring (329.00 (323.00–337.00), baseline vs. 284.00 (277.00–291.50) follow-up); (282.00 (277.00–290.00), baseline vs. 280.00 (267.00–285.50) follow-up), respectively ($p < 0.01$ and $p < 0.05$) (Figure 3); and in total volume (8.66 (8.48–8.83), baseline vs. 8.52 (8.31–8.77) follow-up) ($p < 0.01$) (Figure 3).

Baseline vs 27months follow-up in FH+ ApoE ε4+

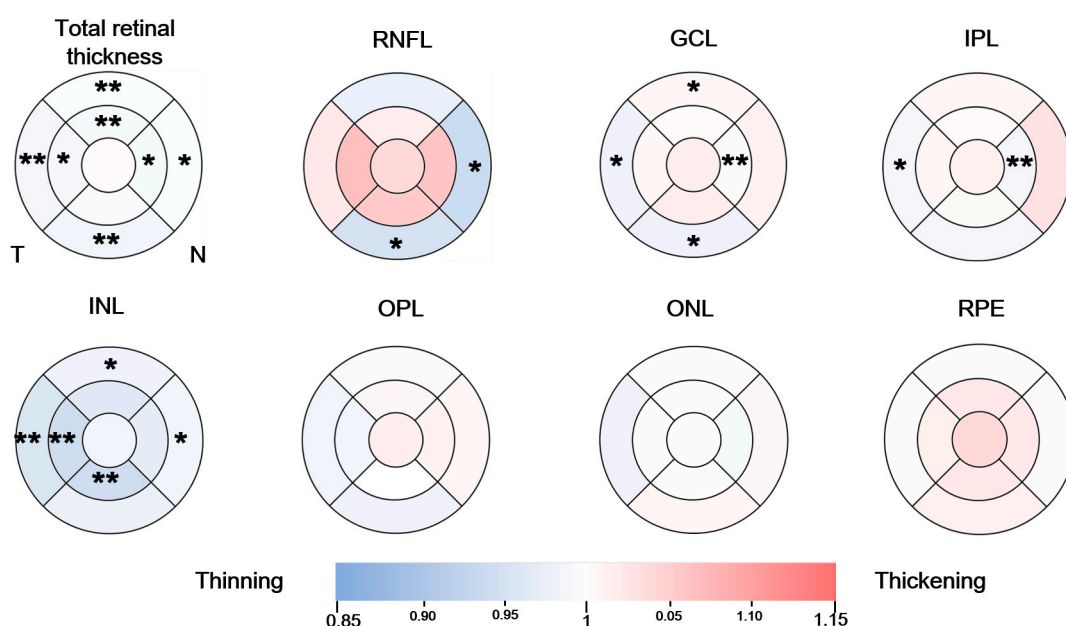


Figure 3. Colorimetric differences in the retinal thickness in each layer between baseline measurements and 27-month follow-up in the FH+ ApoE ε4+ group in the macular OCT rings analysis. In blue, thinning, in red, thickening. (RPE: retinal pigment epithelium, ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; RNFL: retinal nerve fiber layer). * $p < 0.05$, ** $p < 0.01$. Wilcoxon test.

The RNFL showed a statistically significant thinning of the outer macular ring in the nasal sector (51.00 (43.00–54.00) baseline, vs. 47.00 (45.00–47.00) follow-up) ($p < 0.05$) and in the inferior sector of the outer ring (41.00 (37.00–44.00), baseline vs. 38.00 (36.50–38.00), follow-up) ($p < 0.05$) (Figure 3). We also found a statistically significant decrease in the global volume of RNFL (0.94 (0.86–1.02), baseline vs. 0.89 (0.86–0.89) follow-up) ($p < 0.05$) (Figure 3).

Furthermore, when we compared between the baseline and follow-up visits in the GCL, we observed a statistically significant thickness decrease in the nasal sector in the inner macular ring (52.00 (47.00–54.00), baseline vs. 51.00 (49.00–53.50), follow-up) ($p < 0.01$) and in the outer macular ring in the inferior and temporal sectors (33.00 (30.00–35.00), baseline vs. 33.00 (29.50–34.00), follow-up); (34.00 (32.00–37.00), baseline vs. 34.00 (31.00–37.50), follow-up), respectively ($p < 0.05$, in all cases) (Figure 3). We also found a statistically significant increase in the outer macular ring in the superior sector (34.00 (31.00–37.00), baseline vs. 34.00 (33.00–36.50), follow-up), and in the overall volume of this layer (1.08 (1.02–1.13), baseline vs. 1.07 (1.015–1.12), follow-up) ($p < 0.01$) (Figure 3).

In the IPL, we found a statistically significant thickness decrease in the nasal sector of the inner macular ring (42.00 (40.00–44.00), baseline vs. 42.00 (39.50–43.50), follow-up) ($p < 0.01$) and in the temporal sector of the outer macular ring (32.00 (30.00–34.00), baseline vs. 32.00 (29.00–33.50), follow-up) ($p < 0.05$). We also found a statistically significant reduction in the overall volume of this layer (0.90 (0.85–0.94), baseline vs. 0.90 (0.83–0.93), follow-up) ($p < 0.05$) (Figure 3).

In the INL, the thickness analysis between the baseline and follow-up shows statistically significant decrease in the inferior sector of the inner macular ring (41.50 (40.00–45.00), baseline vs. 39.00 (38.00–42.00), follow-up) ($p < 0.01$) and in the temporal sector in both macular rings (inner (38.00 (36.00–40.25), baseline vs. 36.00 (34.50–38.00), follow-up)) and (outer (32.00 (31.00–34.00), baseline vs. 32.00 (29.00–32.50), follow-up)) ($p < 0.01$ in both cases). We also found a statistically significant reduction in outer macular ring thickness in the nasal and superior sectors (34.00 (32.00–36.00), baseline vs. 33.00 (31.50–34.50), follow-up) ($p < 0.05$) and superior (31.00 (29.50–33.00) baseline, vs. 31.00 (29.50–32.00), follow-up, respectively) ($p < 0.05$, in both cases). We also found a significant reduction in the overall volume of this layer (0.96 (0.9–0.98), baseline vs. 0.93 (0.87–0.96), follow-up) ($p < 0.05$) (Figure 3).

We found no statistically significant differences when comparing between baseline and 27-month follow-up measurements in the OPL, ONL, and RPE.

In this study group, in the pRNFL we found a statistically significant thickness decrease in the temporal sector (66.00 (64.00–74.00), baseline vs. 66.00 (60.00–71.50) follow-up) ($p < 0.05$) (Supplementary Figure S1).

3.4. Analysis between the Study Groups at 27-Month Follow-Up

When we compared between the study groups, we only found statistically significant differences in INL. In this layer we observed a statistically significant reduction in thickness in the foveal sector (22.00 (18.75–23.00), FH– ApoE $\epsilon 4-$ vs. 19.00 (16.00–21.00), FH+ ApoE $\epsilon 4+$) and in the temporal sector of the inner macular ring (38.00 (36.75–39.25), FH– ApoE $\epsilon 4-$ vs. 36.00 (34.50–38.00), FH+ ApoE $\epsilon 4+$) ($p < 0.05$, in both cases) (Figure 4).

FH- ApoE $\epsilon 4-$ vs FH+ ApoE $\epsilon 4+$ at 27months follow-up

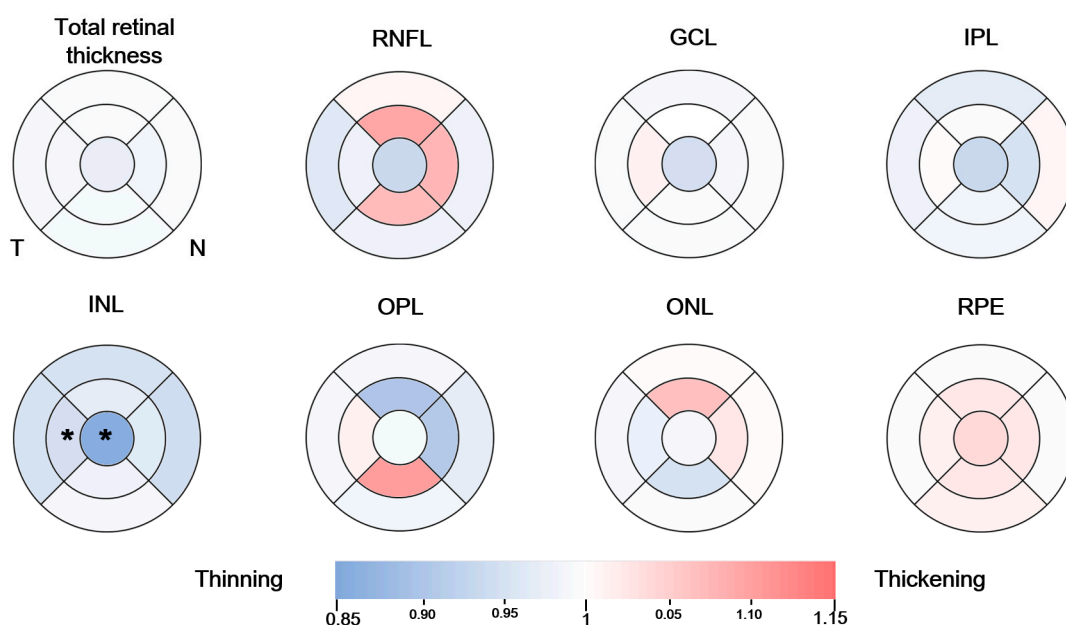


Figure 4. Colorimetric differences in the retinal thickness in each layer between FH– ApoE $\epsilon 4-$ and FH+ ApoE $\epsilon 4+$ at 27-month follow-up. In blue, thinning, in red, thickening. (RPE: retinal pigment epithelium, ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; RNFL: retinal nerve fiber layer). * $p < 0.05$, Mann–Whitney U test.

3.5. Longitudinal Psychophysical Test

3.5.1. Longitudinal Study in the FH– ApoE $\epsilon 4$ – 40–60 yrs and FH– ApoE $\epsilon 4$ – > 60 yrs Groups

When we compared the results obtained at baseline and at 27-month follow-up, we found no statistically significant differences ($p > 0.05$) in the 40–60 age group in the battery of psychophysical tests analyzed (VA, CS, PDT, color perception test, and computerized perimetry) (Figure 5A and Table 1).

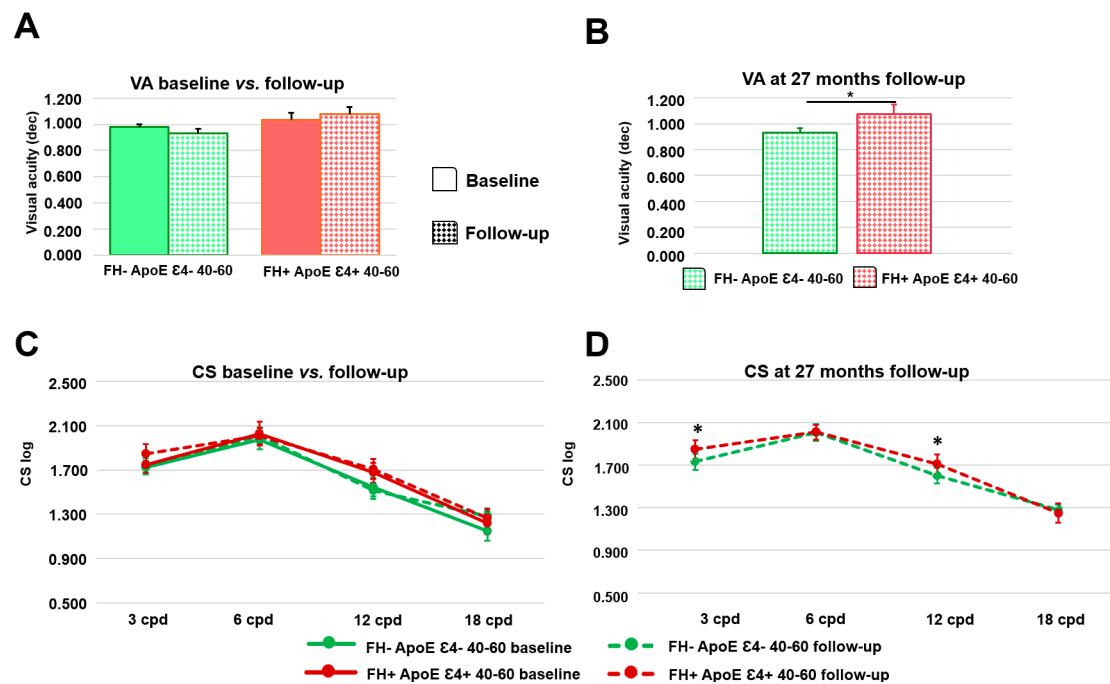


Figure 5. (A) Visual acuity between baseline and follow-up measurements in the 40–60 yrs groups. (B) Visual acuity at 27-month follow-up between FH– ApoE $\epsilon 4$ – 40–60 yrs and FH+ ApoE $\epsilon 4$ + 40–60 yrs. (C) Contrast sensitivity between baseline and follow-up measurements. (D) Contrast sensitivity at 27-month follow-up between FH– ApoE $\epsilon 4$ – 40–60 yrs and FH+ ApoE $\epsilon 4$ + 40–60 yrs. VA: visual acuity; dec: decimal, CS: contrast sensitivity; cpd: cycles per degree. (A,C) * $p < 0.05$, Wilcoxon test. (B,D) * $p < 0.05$, Mann–Whitney U test.

In the groups of participants over 60 years of age, we found no statistically significant differences in the psychophysical test analyzed ($p > 0.05$) (Supplementary Table S1).

3.5.2. Longitudinal Study in the FH– ApoE $\epsilon 4$ – 40–60 yrs and FH– ApoE $\epsilon 4$ – > 60 yrs Groups

We also found no statistically significant differences between baseline and follow-up in the psychophysical tests in any of the age groups of the FH+ ApoE $\epsilon 4$ + participants (Figure 5A,C, Table 1 and Supplementary Table S1).

3.5.3. Analysis of Psychophysical Test between the Study Groups at 27-Month Follow-Up

When we compared the measurements obtained at 27-month follow-up, we found that the FH+ ApoE $\epsilon 4$ + 40–60 years group (1.077 ± 0.148) shows a statistically significant increase in VA compared to the FH– ApoE $\epsilon 4$ – 40–60 yrs group (0.933 ± 0.070) ($p < 0.05$) (Figure 5B and Table 1).

Additionally, at 27 months we found statistically higher contrast sensitivity in the FH+ ApoE $\epsilon 4$ + 40–60 yrs group at the spatial frequencies of 3 and 12 cpd compared to the FH– ApoE $\epsilon 4$ – 40–60 yrs group ($(1.731 \pm 0.148$ FH– ApoE $\epsilon 4$ – 40–60 yrs vs. 1.845 ± 0.176 FH+ ApoE $\epsilon 4$ + 40–60 yrs) ($p < 0.05$) and (1.511 ± 0.142 , FH– ApoE $\epsilon 4$ – 40–60 yrs vs. 1.708 ± 0.181 FH+ ApoE $\epsilon 4$ + 40–60 yrs) ($p < 0.01$), respectively) (Figure 5D and Table 1).

Table 1. Psychophysical test measures in subjects aged 40–60 years.

	FH– ApoE ϵ 4– 40–60 yrs			FH+ ApoE ϵ 4+ 40–60 yrs			<i>p</i> -Value at 27 Months FH– ApoE ϵ 4– 40–60 yrs vs. FH+ ApoE ϵ 4– 40–60 yrs	
	Baseline	Follow-Up	<i>p</i> -Value	Baseline	Follow-Up	<i>p</i> -Value		
Visual acuity	0.981 ± 0.040	0.933 ± 0.070	0.102	1.036 ± 0.095	1.077 ± 0.148	0.285	0.014 *	
Contrast sensitivity	3 cpd	1.724 ± 0.121	1.731 ± 0.148	0.131	1.749 ± 0.148	1.845 ± 0.176	0.100	0.047 *
	6 cpd	1.972 ± 0.171	2.007 ± 0.158	0.943	2.028 ± 0.213	2.008 ± 0.139	0.823	0.932
	12 cpd	1.544 ± 0.170	1.511 ± 0.142	0.311	1.675 ± 0.181	1.708 ± 0.181	0.259	0.008 **
	18 cpd	1.144 ± 0.164	1.283 ± 0.100	0.119	1.219 ± 0.197	1.261 ± 0.176	0.059	0.777
Color perception	Total errors	1.500 (0.000–6.000)	2.000 (0.000–6.000)	0.127	3.000 (0.000–3.000)	2.000 (0.000–4.500)	0.304	0.710
	Tritan errors	1.000 (0.000–2.750)	0.000 (0.000–3.000)	0.303	0.000 (0.000–3.000)	0.000 (0.000–1.500)	0.263	0.620
	Deutan errors	1.000 (0.000–2.750)	0.000 (0.000–3.000)	0.347	0.000 (0.000–3.000)	0.000 (0.000–1.500)	0.205	0.853
PDT	0.000 (0.000–1.000)	0.000 (0.000–0.000)	0.157	0.000 (0.000–0.000)	0.000 (0.000–0.500)	0.157	0.454	
Visual Field	Fixation losses	0.500 (0.000–1.000)	1.000 (0.000–1.000)	0.408	0.000 (0.000–1.000)	0.000 (0.000–1.000)	0.890	0.311
	False positives %	1.000 (0.000–5.000)	2.000 (0.000–2.500)	0.414	3.000 (0.000–5.750)	2.000 (0.000–6.500)	0.552	0.460
	%False negatives	0.000 (0.000–1.000)	0.000 (0.000–3.500)	0.273	0.000 (0.000–4.000)	1.000 (0.000–3.000)	0.959	0.954
	VFI (%)	99.500 (99.000–100.000)	99.000 (98.500–100.000)	0.083	100.000 (99.000–100.000)	99.000 (97.000–100.000)	0.305	0.735
	Mean deviation (MD)	−0.885 (−1.868–2.950)	−0.420 (−1.695–0.115)	0.484	−0.650 (−1.675–0.013)	−0.560 (−2.155–0.165)	0.999	0.936
	Pattern standard deviation (PSD)	1.635 (1.410–1.833)	1.640 (1.445–2.085)	0.575	1.535 (1.318–1.848)	1.660 (1.4650–2.420)	0.064	0.500

Median (interquartile range); * $p < 0.05$; ** $p < 0.01$ Wilcoxon test and Mann–Whitney U test. FH; family history; ApoE: apolipoprotein E; cpd: cycles per degree; PDT: perception digital test; VFI: visual field index.

4. Discussion

Few longitudinal studies have been performed in participants with significant genetic risk for the development AD, and many of them consider preclinical stages of the disease when there are already memory complaints, MCI, or even positive biomarkers such as A β + or pTau+ in (cerebrospinal fluid) CSF and PET. Moreover, in some cases, participants are included over time, so they cannot be said to be purely longitudinal.

Although sporadic AD is a multifactorial disease and there are numerous known and unknown genes that contribute to the increased risk of development, the presence of the ϵ 4 allele for the ApoE gene is currently recognized as the most important [26]. Moreover, despite being cognitively healthy, carriers of this allele (ϵ 4) already show a higher burden of A β than carriers of the other isoforms (ApoE ϵ 2 and ϵ 3) [27]. Therefore, characterizing this population at high genetic risk is important in order to understand the evolution of these subjects over time.

On the other hand, the inclusion of control participants is really complex because it is difficult to create a sample of controls with no risk variables if we consider that as we have mentioned above, AD is a multifactorial disease of which we do not know many factors or their interactions yet [28], therefore we have delimited participants to an age-matched population as control, with no family history of AD or vascular dementia and without any ϵ 4 allele for the ApoE gene.

On the other hand, despite the health situation caused by COVID-19 and the loss of patients between the baseline visit and the 27-month follow-up visit, we monitored 15 subjects without significant genetic risk factors for AD and 21 subjects at risk for AD for longitudinal study.

In our study, in which we performed both structural and functional analysis, when comparing between the baseline visit and the follow-up visit at 27 months, more sectors with statistically significant retinal macular thickness changes were found in the FH+ ApoE

$\epsilon 4+$ group compared with those in the FH– ApoE $\epsilon 4-$ group. These changes were mostly decreases in thickness, although there were also increases, possibly due to compensatory mechanisms [29]. However, in participants who did not have significant genetic risk for AD, the changes found were increases and decreases in thickness with no statistical significance. These alterations could be due to a normal retinal aging process [30,31].

When we compared the FH– ApoE $\epsilon 4-$ and the FH+ ApoE $\epsilon 4+$ groups at the follow-up visit, statistically significant decreases appeared in the INL. These significant thinnings occur in the foveal sector and in the temporal sector of the inner macular ring. At the first visit the changes were mostly in the IPL, which contains the synaptic connections between amacrine, bipolar, and ganglion cells [7]. This could be due to neuroinflammatory processes in which microglia perform stripping phenomena (disconnection of abnormal synapses), producing thinning in this layer. In the follow-up visit, significant thinning was found in the INL, possibly due to the death of some of these cells that began to disconnect 27 months ago [31–33]. Furthermore, it is noteworthy that these changes, although significant, do not become pathological, as these participants are cognitively healthy and we do not know if they will eventually develop AD.

In other longitudinal studies in subjects with $A\beta+$ markers, such as van de Kreeke et al., at 22-month follow-up study, they observed a thickness decrease in all retinal layers except mRNFL, but no change in retinal thickness between $A\beta+$ and $A\beta-$ participants at the beginning of the study [34]. One possible explanation is the relatively brief follow-up period, and given that preclinical phases can last up to 20 years, it is possible that even if there is an increase in neurodegeneration, it is so subtle as to be difficult to detect [35].

In other study with a 27-month follow-up, the $A\beta+$ subjects did show a decrease in the volume of the IPL, ONL, and mRNFL, and an increase in the volume of the OPL. However, the participants of this study, in addition to having a family history of the disease, already had subjective memory complaints, and these subjects were in more advanced stages of the disease [21]. A further study also found structural changes in the retina of subjects with subjective cognitive decline and $A\beta+$ and that this macular thickening of the nasal region was maintained at 24 months. They also found a thinning of the RNFL in $A\beta+$, but without reaching statistical significance when compared to $A\beta-$ [36]; these findings being compatible with some of the changes found in our study. A correlation has also been found between worse subjective cognitive impairment scores and a reduction in RNFL volume in a 27-month longitudinal study among subjects with a first-degree family history of AD, carriers of any $\epsilon 4$ allele for ApoE, and subjective cognitive impairment [37].

Moreover, a more recent investigation revealed that individuals carrying the ApoE $\epsilon 4+$ allele exhibited greater thinning in the macular central subfield thickness compared to the ApoE $\epsilon 4-$ group. However, no statistically significant differences were observed in the percentage change rate of central macular thickness, GCL-IPL complex thickness, and RNFL thickness at the 24-month follow-up when comparing between these groups. In this study, an additional analysis was conducted to compare subjects in the ApoE $\epsilon 4+$ risk group who also had a family history of dementia with those in the $\epsilon 4-$ group who did not have such a family history. No significant differences were found in any OCT parameter, both at the baseline assessment and at the 2-year follow-up. Nevertheless, the ApoE $\epsilon 4+$ group and subjects with a family history demonstrated a higher rate of decrease in GC-IPL thickness compared to the ApoE $\epsilon 4-$ group without a family history [5]. However, when analyzing the demographics of the study groups, we can see that there are different races within the study groups who are subjects with an average age of over 65 years. On the other hand, the study of individual layers of the retina provides more information than the analysis of complexes. For example, when analyzing the GCL-IPL complex, information is probably lost because the ganglion cell layer has other cells such as displaced amacrine cells that can be mistaken for ganglion cells [38–40].

In another study that has looked at retinal changes associated with ApoE genotype and AD, the study patients had MCI and average ages of over 70 years. In addition, in this study the subjects in the control group were patients who were waiting to undergo cataract

surgery. This may cause the OCT images to be of poor quality and they should be excluded from the study [41].

Changes in retinal layer thicknesses have also been detected in cognitively healthy subjects who carry the E280A presenilin 1 mutation (PSEN1). Significant thinning was found in the ONL, OPL, and INL layers [42]. The authors hypothesize that the cell death and consequent thinning of these layers may be due to loss of PSEN1 proteolytic function [43].

In our study, changes also occurred in the peripapillary area over 27 months, but when we performed a comparison at the follow-up visit between the study groups (FH–ApoE ϵ 4– and FH+ ApoE ϵ 4+) we found no statistically significant differences. This again highlights that during AD, changes occur early in the macular area and that changes in the peripapillary area are a sign of disease progression to more advanced stages [44,45].

Longitudinal studies have also been carried out to understand the evolution of brain changes in subjects at significant risk for AD. Shi et al., in healthy subjects, showed a correlation between the measurement of RNFL in the superior and inferior sectors and a decrease in the volume of the cingulate cortex and hippocampus, as well as the decline in episodic memory at 12 months [46]. Additionally, in a previous work, we found that in subjects with significant genetic risk factors for the development of AD there is a correlation between retinal areas where changes occur and brain areas closely related to AD such as the lingual gyrus, the hippocampus, and the entorhinal cortex [16]. Other authors have conducted longitudinal studies that also analyzed both functional brain changes and A β accumulation in cognitively healthy subjects and classify them by ApoE status. They found a relationship between ApoE ϵ 4 and higher rates of change in A β accumulation in cognitively healthy middle-aged subjects, and therefore this accumulation changes regionally with age, being earlier in the precuneus and later in the visual cortex. These subjects show earlier and faster accumulations and higher rates of volume alterations in the structures of the medial temporal lobe. However, in these middle-aged subjects carrying ApoE ϵ 4+, they did not perform any ophthalmological studies [47]. It has been described that these cerebral structural alterations in preclinical AD follow a biphasic pattern, first developing a thickening of the cortex due to an inflammatory process generated by the sum of the pathogenic effects between Tau and A β , followed by a second phase of atrophy where the effect of Tau predominates [48].

To the best of our knowledge, this is one of the first studies to analyze the visual function in subjects who are at high genetic risk for developing AD. In the present work, when we compared the follow-up visit values between the higher and lower risk group, we did find that the FH+ ApoE ϵ 4+ 40–60 yrs group had statistically ($p < 0.05$) higher VA and CS at the spatial frequency of 3 and 12 cpd than the FH– ApoE ϵ 4– 40–60 yrs group. These findings were already observed the first time they were evaluated, so functional changes remain stable at 27 months. The increase in VA and CS in subjects at high genetic risk for the development of AD is contrary to the findings that pointed to a progressive worsening of these visual abilities in the clinical phases [45]. This is consistent with the fact that in stage 0 disease, the pattern of atrophy mainly affects the frontal, parietal, and temporal areas with slight preservation of the primary sensory and visual cortexes [48–50]. In addition, it is known that early accumulation of A β produces alterations in the function of inhibitory neurons, which could be related to an alteration of the dopaminergic system [51,52]. It is possible that in individuals with significant genetic risk factors for the development of AD, A β oligomers affect the dopaminergic modulatory activity of the retina causing early hyperexcitability leading to an initial increase in VA and CS [53]. In addition, different ApoE alleles are known to confer different risks in ocular diseases leading to vision loss. The ϵ 2 and ϵ 3 alleles are known to predispose to an increased risk of age-related macular degeneration and the ϵ 4 allele has also been associated with a decreased risk of developing glaucoma [54]. At the time the participants included in this study were analyzed, all were ophthalmologically healthy and those with ocular pathology were excluded from the study [55,56]. Among the possible limitations of our study is the fact that we have not adjusted the statistics for multiple comparisons. This is because it is a novel longitudinal

exploratory study in a highly characterized study population, so our findings could be the starting point for future research in the identification of parameters of great interest in the study of high-risk populations. Another limitation of our work is the loss of participants in the longitudinal study due to the health situation caused by the COVID-19 pandemic. In addition, in the baseline visit the number of participants who underwent the psychophysical tests is higher than the number of participants who undertook the OCT. This was due to the fact that in three participants with FH− ApoE ϵ 4− and in four participants with FH+ ApoE ϵ 4+ OCT, images could not be included for analysis. Images with artefacts due to motion or flicker were eliminated; although artefacts are common in OCT images, they could interfere with the quantitative data.

Finally, the longitudinal study of these participants was performed at the 27-month follow-up to determine whether the changes found in the baseline visit remained stable or evolved over time. It should be considered that these subjects are healthy and that we do not know whether or not they will develop the disease in the future, so 27 months is a short follow-up time, but at least it gives us an approximation of what is happening both functionally and structurally.

5. Conclusions

In conclusion, in cognitively healthy subjects, but with significant genetic risk factors for the development of AD, retinal structural changes are observed that progress at 27-month follow-up, but the functional changes detected at the baseline visit remain stable, probably because of the maintenance of the hyperexcitability situation that causes the accumulation of A β and impairs the functioning of the dopaminergic system.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biomedicines11072024/s1>, Figure S1: Colorimetric differences in the peripapillary retinal nerve fiber layer thickness between baseline measurements and 27-month follow-up in the FH− ApoE ϵ 4− and FH+ ApoE ϵ 4+ groups and between groups at the 27-month follow-up; Table S1: Measurements of psychophysical tests in subjects over 60 years of age.

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