Glaucoma

Sex-Specific Effect of BDNF Val66Met Genotypes on the Progression of Open-Angle Glaucoma

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PURPOSE. To investigate whether the brain-derived neurotrophic factor (BDNF) Val66Met genotype is associated with the rate of progression of open-angle glaucoma (OAG).

METHODS. In this retrospective cohort study, 148 OAG patients (292 eyes) were enrolled with a median follow-up period of 5.3 (range, 1.1–8.6) years. All participants had undergone regular clinical examinations by using spectral-domain optical coherence tomography (SD-OCT) scans and Humphrey (SITA) visual field tests. BDNF Val66Met polymorphisms were genotyped in all participants. Longitudinal visual field and retinal nerve fiber layer (RNFL) changes were compared between Met carriers ($n = 68$, 135 eyes) and Val homozygotes ($n = 80$, 157 eyes) by using the generalized estimating equations (GEE) model and Kaplan-Meier survival analysis.

RESULTS. There was no significant difference in mean rates of progression for the two genotypes. However, there was a significant association between the Val66Met genotypes and slower OAG progression, as suggested by a higher rate of global RNFL loss in Val/Val homozygotes ($P = 0.008$) in the long-term survival analysis. The effect demonstrated a degree of sex specificity, with the significant difference present only in females ($P = 0.016$) but not males. Similar sexual dimorphism was presented in superior ($P = 0.005$ in females, $P = 0.38$ in males) and inferior ($P = 0.004$ in females, $P = 0.41$ in males) RNFL loss. No significant difference was observed in visual field parameters.

CONCLUSIONS. Our results suggested that carriage of Met allele reduces the rate of long-term OAG progression. However, the fact that this effect is observed only in females indicates BDNF Val66Met influences the progression rate of OAG in a sex-specific manner.

Keywords: open-angle glaucoma, BDNF, Val66Met, glaucoma progression

Glaucoma is a vision-threatening disease characterized by progressive loss of retinal ganglion cells (RGCs), and it is one of the leading causes of irreversible blindness worldwide, influencing more than 64 million people.[1] Glaucoma is thought to be caused by interactions of multiple genetic and environmental factors. Although several genetic loci and genes have been identified and associated with open-angle glaucoma (OAG), the main genes that confer significant susceptibility remain unknown.[2–5]

Recent studies have shown that molecules with neurotrophic actions affect RGCs development and maintenance. Accumulating evidence suggests that brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, provides neuroprotective effects by enhancing the survival and regrowth rate of axotomy-induced RGCs.[6–9] It is also shown that BDNF is transported both anterogradely and retrogradely, and secretion of BDNF is involved in reducing die-back and promoting neuronal regeneration after optic nerve (ON) injury.[10,11] In animal studies, BDNF has been reported to play a critical role in preservation of inner retinal integrity and insufficiency of BDNF exacerbates degenerative changes in glaucoma.[12] Upregulation of BDNF downstream survival pathways was found to be neuroprotective for RGCs in experimental glaucoma model.[13]

A functional polymorphism in the human BDNF gene, specifically, resulting in an amino acid residue substitution from valine (Val) to methionine (Met) at codon 66 (Val66Met, NCBI database dbSNP rs6265) in the pro-region of BDNF has been associated with intracellular processing and altered activity-dependent secretion of BDNF.[14,15] The role and association of BDNF Val66Met polymorphism with various neurodegenerative and psychiatric disorders have been extensively reviewed elsewhere.[16-18] Interestingly, the influence of Val66Met polymorphism in several diseases including multiple sclerosis (MS),[19] Parkinson's disease (PD),[20] and major depressive disorders (MDD)[16] showed sexual dimorphism. With regard to glaucoma, Val66Met was suggested to be significantly associated with progression of primary open-angle glaucoma (POAG) as defined by nerve fiber layer analyzer (GDx) and rim area (RA) in a Polish cohort.[21] OCT was reported to have higher sensitivity compared to GDx metrics in detecting glaucoma[22,23] as well as discerning MS patients from normal controls.[24-27] On the other hand, automated perimetry is a crucial part of the clinical assessment of glaucoma as it provides outcomes directly related to a patient's quality of life.[28,29] The aim of this study was to examine the associations between glaucoma progression (determined by OCT and perimetry) and carriage of BDNF Val66Met in an Australian cohort. We also evaluated

several other parameters to determine whether there was any particular glaucoma phenotype associated with the polymorphism.

METHODS

Participants

Open-angle glaucoma patients ($n = 148$) were consecutively enrolled in the study between September 2017 to June 2018 from two ophthalmology clinics in Sydney, Australia (Macquarie University Hospital and Eye Associates). Participants with a history of other types of ocular or neurologic disease that could affect the testing results, for instance, retinal and optic nerve disorders (other than glaucomatous abnormalities) were excluded. "Glaucoma" was defined on clinical examination by loss of neuroretinal rim +/− corresponding visual field defect; intraocular pressure (IOP) was not used as a criterion. All participants had been reviewed by a glaucoma subspecialist on a regular basis (every 4–6 months) and were undergoing regular ocular examination, OCT scans and visual field tests.

The patients were followed-up over a median of 5.3 years (range, 1.1–8.6). Patient data were sourced retrospectively from the clinical database. Both eyes of participants were used in the analysis and for patients with unilateral glaucoma, only the diseased eye was included (the excluded nonglaucoma eyes [$n = 4$] were not under any treatment).

This study was conducted in accordance with the approval of the Human Research Ethics Committee (Medical Science) at Macquarie University and adhered to the tenets of the Declaration of Helsinki. A written informed consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from peripheral blood using a commercially available DNA extraction kit (Qiagen, Hilden, Germany). Quantification of extracted DNA was performed with a spectrophotometer (Thermo Scientific, Rockford, IL, USA). The G → A nucleotide substitution, identifying the Val → Met amino acid change, was assayed by polymerase chain reaction (PCR, Eppendorf, Hamburg, Germany) using a Master cycler gradient thermal cycler. The primers used for PCR were as follows: forward 5′ ACTCTGGAGAGCGTGAATGG 3′ and reverse 5′ TCCAGGGTGATGCTCAGTAGT 3′. The amplification conditions were initiated at 95°C for 5 minutes, followed by 30 cycles comprising of denaturation at 94°C for 1 minute, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute, with a final extension step of 5 minutes at 72°C. The BDNF Val66Met genotypes were determined by direct sequencing of PCR products (both directions, Australian Genome Research Facility, Sydney, Australia).

OCT Scans

All participants underwent Cirrus (Cirrus HD-OCT; Carl Zeiss Meditec, Germany) or Spectralis (Spectralis HRA+OCT; Heidelberg Engineering, Germany) spectral-domain optical coherence tomography (OCT) scans. For each individual participant, the same device was used during the follow-up. A peripapillary circular scan was performed to obtain the RNFL thickness profile from both OCT machines. The global retinal nerve fiber layer (gRNFL) as well as averages of the superior and inferior quadrant RNFL thicknesses, were used for analysis in this study. For Cirrus, overall (gRNFL), superior and inferior RNFL thicknesses were attained. For Spectralis, the thickness of superior and inferior sectors was determined by averaging the NS/TS and NI/TI sectors. The follow-up function was activated

to ensure the RNFL thicknesses were obtained at the same locations as the baseline scans. The percentage loss of RNFL (RNFL % loss) was defined as the proportion of RNFL loss at each follow-up visit divided by baseline RNFL thickness. Only images with high quality[30] without segmentation failure and involuntary blinking were retained for analysis.

Visual Field (VF) Tests

Visual field testing is performed using the Swedish Interactive Testing Algorithm (SITA Standard)[31] 24-2 protocol on the Humphrey Visual Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA). Only visual fields with fewer than 33% false-negative errors, fewer than 33% false-positive errors, and fewer than 20% fixation losses were considered reliable.[32] Baseline VF parameters including mean deviation (MD), pattern standard deviation (PSD), and VF index (VFI) were collected and analyzed. The rate of progression was defined as the percentage of VFI loss per year and was obtained directly from the Humphrey software. Central vision loss was defined as the presence of scotomas at the most central four points.

Statistics

All statistical analysis was performed using SPSS software version 22.0 (SPSS, Chicago, IL, USA). The participants were stratified into two groups in data analysis (Val homozygotes and Met carriers, Val/Met and Met/Met genotype). Univariate comparisons between two genotype groups were based on GEE method, and on the χ^2 test for categorical variables including sex, family history, glaucoma surgery history, pseudoexfoliative syndrome (PXF) and eyes with central vision loss. Additionally, glaucoma progression across time by genotype group was compared while stratifying according to sex. The age, sex, family history of glaucoma, number of glaucoma medications, sub-type of glaucoma (POAG where IOP recorded over 20 mm Hg at least once occasion, or normal tension glaucoma [NTG] where IOP never recorded >20 mm Hg), as well as glaucoma surgery history, age of onset, PXF and OCT machine types were all included as covariates in the GEE analysis. Kaplan-Meier event rate curves and the log-rank (Mantel-Cox) test were used to compare the cumulative risk ratio of glaucoma progression between groups stratified by Met allele carriage. The first time that more than 5% of RNFL thickness loss was found as compared with baseline was regarded as the endpoint in survival analysis. A P value of less than 0.05 was considered statistically significant.

RESULTS

Demographic and clinical characteristics of the participants included in this study are shown in Table 1. Two hundred and ninety-two eyes of 148 patients with OAG met the inclusion criteria. The mean age of the patients was 70.0 ± 9.6 years (range, 43–96). Median follow-up period was 5.3 years (range, 1.1–8.6). Central corneal thickness (CCT) and best-corrected visual acuity (BCVA) were recorded at the baseline. Met carriers were found to have significantly lower CCT compared to Val homozygotes (536.7 vs. 551.4 μm; $P = 0.03$). No significant difference was found in family history, glaucoma history, multiple drug treatment, PXF or central vision loss between two genotype groups (Table 1).

The genotype frequencies of Val66Met among the cohort were 80 (54.1%) for genotype GG (Val/Val), 65 (43.9%) for GA (Val/Met), and 3 (2.0%) for AA (Met/Met). The allele frequency of the A allele (Met) was 23.9% in the study subjects.

TABLE 1. Demographic and Clinical Characteristics of Participants at Baseline

Demographics	Total (n = 148)	Val/Val (n = 80)	Met Carriers (n = 68)
Eyes, N (%)†	292	157 (55.3)	135 (44.7)
Female, N (%)*	75 (50.7)	39 (48.8)	36 (52.9)
Age, y, Mean (SD)†	70.0 (9.6)	70.0 (8.4)	70.0 (10.5)
Age of onset, y, Mean (SD)†	60.5 (11.3)	60.6 (11.9)	60.3 (10.5)
Follow-up period, y, median (range)†	5.3 (1.1–8.6)	5.1 (1.1–8.4)	5.9 (1.5–8.6)
Glaucoma severity			
gRNFL, μm, Mean (SD)†	76.1 (12.7)	75.3 (12.7)	77.1 (12.7)
VFI, %, Mean (SD)†	90.0 (16.7)	89.4 (17.3)	90.8 (16.0)
MD, dB, Mean (SD)†	−3.9 (5.7)	−4.2 (5.9)	−3.6 (5.5)
PSD, dB, Mean (SD)†	4.2 (3.7)	4.2 (3.5)	4.1 (4.0)
Highest recorded IOP, mm Hg, Mean (SD)†	19.8 (5.0)	19.8 (5.3)	19.5 (4.8)
CCT, μm, Mean (SD)†	544.9 (42.5)	551.4 (39.3)	536.7 (43.9)
BCVA, LogMAR, Mean (SD)†	0.07 (0.2)	0.06 (0.2)	0.07 (0.2)
Family history, n (%)*	81 (54.7)	45 (56.3)	36 (54.5)
Treatment			
SLT/Trabeculectomy, n (%)*	12 (8.1)	4 (5.0)	8 (12.1)
No. of medications*	1.9 (0.9)	2.0 (0.9)	1.9 (0.9)
PXF, n (%)*	6 (4.1)	2 (2.4)	4 (6.1)
Eyes with central vision loss, n (%)*	79 (27.1)	47 (29.9)	32 (23.7)
NTG, n (%)†	50 (33.8)	26 (32.5)	24 (36.4)
Systemic diseases			
CVD other than HTN, n (%)*	18 (12.2)	13 (16.3)	5 (7.4)
HTN, n (%)*	54 (36.5)	32 (40.0)	22 (32.4)
DM, n (%)*	13 (8.8)	8 (10.0)	5 (7.4)
Migraine, n (%)*	16 (10.8)	9 (11.3)	7 (10.3)

No parameter showed significant difference between two genotype groups except for CCT ($P = 0.03$). Four patients had unilateral glaucoma and their nonglaucoma eyes (not under any treatment) were excluded in the analysis. SLT, selective laser trabeculoplasty; CVD, cardiovascular disease; HTN, hypertension; DM, diabetes mellitus.

  * χ^2 test.
  † GEE model.

The OCT and HVF rate of progression was compared between Val66Met genotype groups and are summarized in Table 2. There were borderline associations between the BDNF Val66Met polymorphism genotype and gRNFL percentage loss ($P = 0.08$), superior RNFL percentage loss ($P = 0.09$) and inferior RNFL percentage loss ($P = 0.08$). The differences were not statistically significant for gRNFL thickness, superior RNFL thickness, inferior RNFL thickness, or visual field progression rate.

As there was a borderline trend for higher rate of RNFL loss in Val homozygotes, survival analysis of RNFL loss was further performed in all subjects. This analysis demonstrated significantly higher gRNFL percentage loss in Val/Val genotype groups in overall (log-rank test, $P = 0.008$) and female participants (log-rank test, $P = 0.016$). In the first 4 years of follow-up, the differences between two groups were trivial. After 4 years, Val homozygotes started to exhibit greater levels of gRNFL percentage loss and the differences continued to increase over time (Figs. 1A, 1B). However, there was no significant difference between the two groups in male subjects as shown in Kaplan-Meier analysis (log-rank test, $P = 0.15$; Fig. 1C). A similar pattern was also shown in superior RNFL loss, with significant differences between the two genotype groups in overall (log-rank test, $P = 0.008$) and females (log-rank test, $P = 0.005$) but not in males (Figs. 2A–C). In the inferior sector (Figs. 3A–C), however, only female participants exhibited a statistically significant percentage loss in Val/Val genotype (log-rank test, $P = 0.004$).

DISCUSSION

Our study demonstrated that the carriage of BDNF Val66Met polymorphism was associated with a significantly slower glaucoma progression in females in an Australian cohort. This genotypic effect of BDNF on glaucoma progression remains positive even after adjustment for a full range of possible confounding covariates, including age, family history of glaucoma, type of OAG (POAG or NTG), number of glaucoma medications, glaucoma surgery history, age of onset, PXF and OCT machine types (Spectralis or Cirrus). Furthermore, the effect showed a sex specificity: Stratified survival analysis demonstrated that the influence of BDNF Val66Met seems to be more important in women. Although there were several types of concomitant systemic diseases—including cardiovascular diseases, diabetes mellitus as well as migraine—that might have an influence on the progression of OAG, our results remained unchanged even after adding the carriage of these diseases as covariates in an additional GEE analysis. To our knowledge, this is the first study to report the association between BDNF genotype and glaucoma progression in an Australian population. While the absolute rates of RNFL loss

TABLE 2. Association Between BDNF Val66Met Polymorphism and Glaucoma Progression

Progression	Val/Val	Met Carriers	Difference Value	Difference 95% CI	P Values
OCT					
Global RNFL (μm p.a.)	−0.87	−0.57	−0.3	−0.89 to 0.29	n.s.
Global RNFL % loss (% p.a.)	−1.35	−0.24	−1.11	−2.34 to 0.12	**0.08**
Superior RNFL (μm p.a.)	−1.15	−0.55	−0.6	−1.38 to 0.18	n.s.
Superior RNFL % loss (% p.a.)	−1.42	−1.07	−0.35	−0.76 to 0.06	**0.09**
Inferior RNFL (μm p.a.)	−1.33	−1.14	−0.19	−1.26 to 0.88	n.s.
Inferior RNFL % loss (% p.a.)	−1.77	−0.08	−1.69	−3.57 to 0.2	**0.08**
HVF					
Progression rate of VFI (% p.a.)	−0.6	−0.68	0.08	−0.37 to 0.52	n.s.

GEE models were applied assuming additive effect for the minor allele variant (Met carriers), adjusted for age, sex, family history of glaucoma, number of glaucoma medications, sub-type of glaucoma (POAG or NTG), glaucoma surgery history, age of onset, PXF and OCT machine types. Borderline significance ($0.1 < P < 0.05$) is shown in bold. After including CCT as a covariate, the P values remained unchanged except for superior RNFL ($P = 0.08$) and superior RNFL % loss ($P = 0.04$). n.s., not significant.
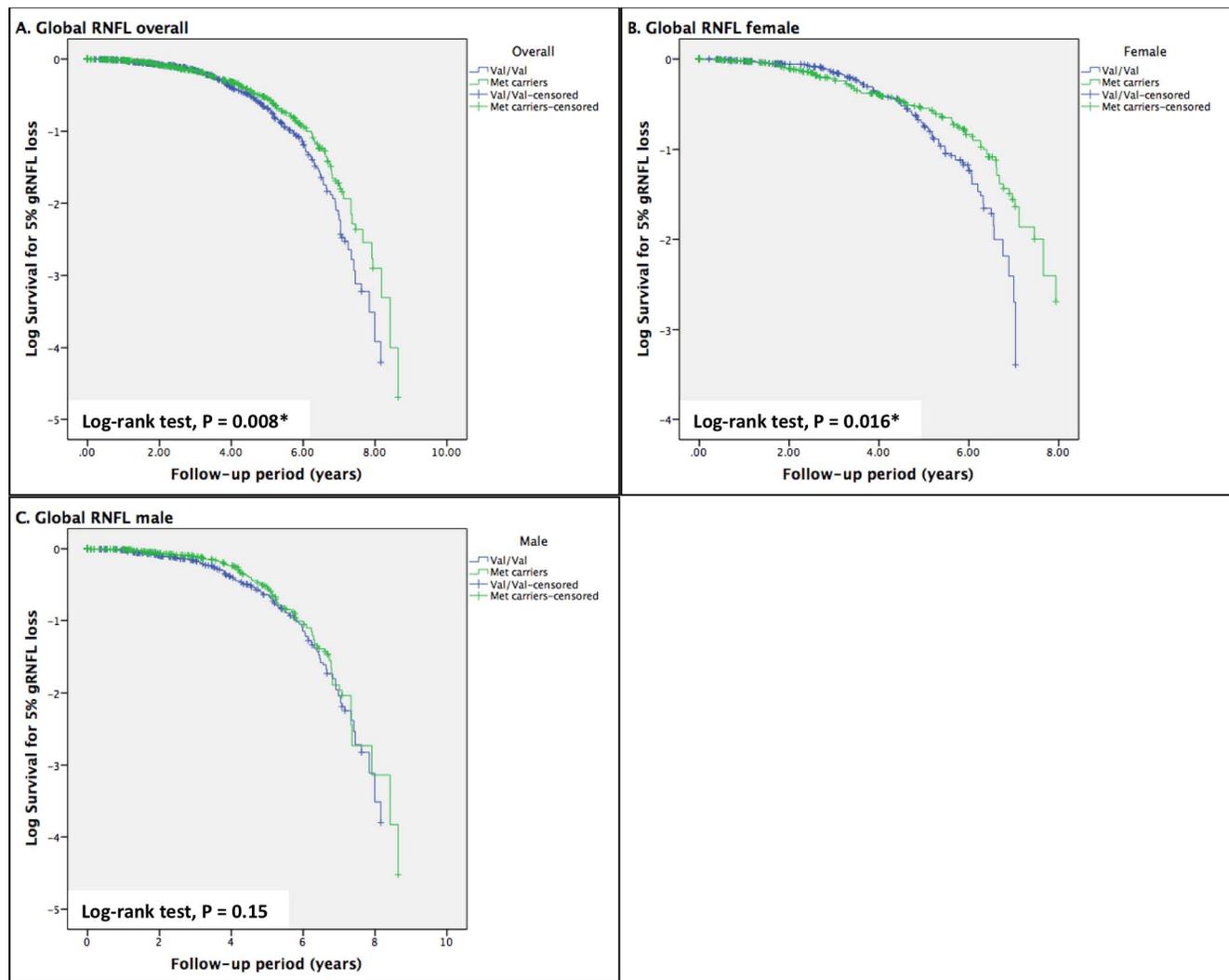
FIGURE 1. Kaplan-Meier survival analysis of global RNFL percentage loss in different genotype groups in (A) all participants, (B) female participants, and (C) male participants.

throughout the study were not significantly different, the survival analysis showed a faster loss after several years follow-up in females.

It has been reported that thinner CCT is a risk factor for the faster rate of progression in glaucoma.[33] Since the Met carriers showed a slower rate of progression in our study, the thinner CCT could even be reducing our ability in identifying the difference between the polymorphism groups. In addition, CCT was included as a covariate in further sub-analysis; it showed no significant effects on the current findings, and the P values were still borderline (0.08 for global RNFL % loss, 0.08 for superior RNFL, and 0.08 for inferior RNFL % loss) except for superior % loss ($P = 0.04$).

The frequency of the BDNF Met allele varies greatly among ethnic groups. In our Australian Caucasian population, the frequency of Met allele is 23.9%, which is close to the frequency of 24% in the Italian Caucasian panel[34] but much lower than that in people of Asian ancestry (range, 39.0%–46.3%).[17] However, the lower frequency is not necessarily responsible for the observed ethnic disparity in the RNFL thickness as the individuals of European descent were reported to have thinner RNFL measurements compared to Asians, Hispanics, and people of African ancestry.[35,36]

During the early stage of the entire follow-up period, two genotype groups showed similar rates of progression. However, after approximately 4 years, Val homozygotes started to exhibit faster progression, and this might be suggestive that the Met allele could have a neuroprotective effected on glaucoma progression in OAG patients. It has been reported that any obstruction in BDNF synthesis or retrograde transport can result in RGC death and, consequently, glaucoma.[37,38] In pathological condition, Met allele may regulate neuronal BDNF secretion thus affecting the functional activity of RGCs. The protective effect of BDNF Met allele in the present study was also found in several studies with respect to neurodegenerative and psychological disorders including MS,[39] PD,[20] and depression.[40]

Our results suggested that there was no difference in the prevalence of BDNF Val66Met polymorphism among the glaucoma subjects compared to the Polish study.[21] However, the opposite effects of BDNF Val66Met reported by that study could be resulted from different study design and clinical parameters evaluated. The Polish study appears to be a cross-sectional study, while ours involved, on average, 5 years of longitudinal monitoring.

The reason this polymorphism should specifically influence progression rate among glaucoma patients—and especially in
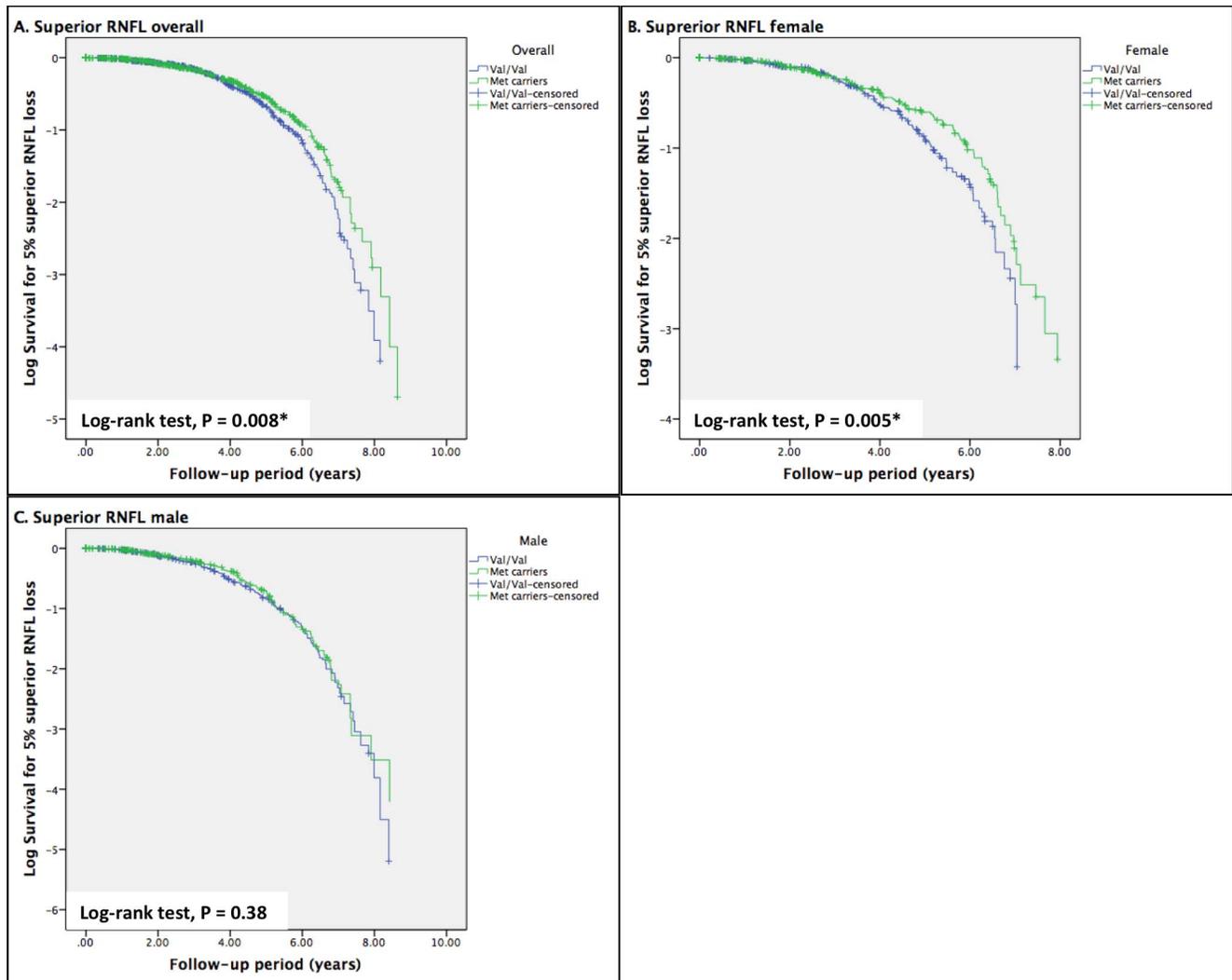
FIGURE 2. Kaplan-Meier survival analysis of superior RNFL percentage loss in different genotype groups in (**A**) all participants, (**B**) female participants, and (**C**) male participants.

women in the present study—is not known. Studies have revealed that the BDNF Val66Met polymorphism has a sex specific influence on planning ability in PD[20] and susceptibility in MS,[19] both showing the effect is most apparent in women. While in contrast, another meta-analysis study reported a significant genotypic effect of BDNF Val66Met on MDD only in men.[16] These results indicate that many genes or genetic variants, such as BDNF polymorphism, can act differently in males and females. Many of these sex differences may be caused by hormonal influences on expression and regulation of specific genes, or other nongenetic factors that are associated with sex. It has been reported that estrogen interacts largely with BDNF expression during embryonic development in animal models,[41,42] with estrogens having a positive effect on levels of BDNF in all cases. Hormonal variation, especially estrogen modulation, can have a great impact on the expression of BDNF, potentially through transcription. However, this modulation has the potential to be neurotrophic or neurotoxic with respect to different diseases and is cell type-, region-, and age-dependent.[43] The interactions between estrogen and BDNF has been extensively reviewed elsewhere,[43] providing a possible cellular milieu for the observed sexual dimorphism in the present study.

A potential limitation is that our sample size ($n = 148$) was relatively small; however, the genotype and sex distributions in the cohort were favorable. Secondly, the gene-dosage effect was not examined due to the limited Met/Met (A/A) homozygous cases in our study population. Also, the allele frequency (23.9% in the present cohort, which is close to an Italian Caucasian cohort of 24%[34]) of patients was not compared with local healthy controls. There were 9 patients with less than 2 years follow-up, which might not be long enough to detect glaucoma progression. However, after removing those 9 cases in an addition analysis, the difference between two genotype groups remained significant. In terms of determining rates of RNFL progression, two different types of OCT device were used, so the rate of RNFL loss can be expected to be slightly different. However, we found comparable rates and ratio of Val/Val: Met carriers between the two devices at two sites and by using percentage rate of loss to compensate for level of severity, we believe this also helped minimize differences between devices.

In conclusion, the results of this study supported our hypothesis that BDNF Val66Met polymorphism is associated with differences in progression rate in glaucoma patients. While the carriage of Met alleles seems to have a protective effect on RGCs, the influence is only significant in female
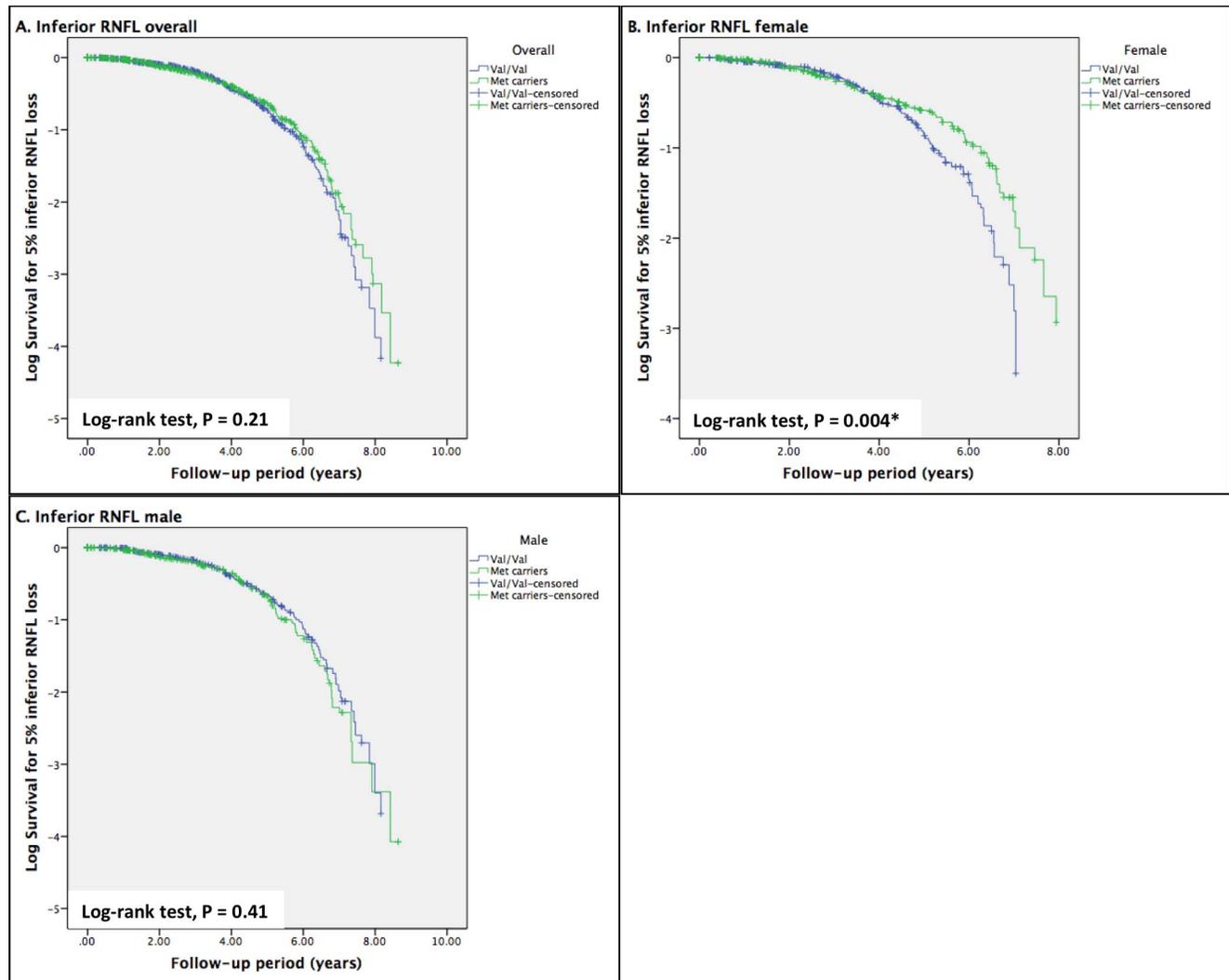
FIGURE 3. Kaplan-Meier survival analysis of inferior RNFL percentage loss in different genotype groups in (**A**) all participants, (**B**) female participants, and (**C**) male participants.

subjects. Futures studies are needed to explain the underlying biological mechanisms of our observation, especially the potential effects of other glaucoma-susceptibility genes and sex specificity.

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