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ORIGINAL PAPER

Mutations in *MERTK* are not associated with age-related macular degeneration

Hasenin Al-khersan · Alan Kwong · Michael A. Grassi

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Abstract

Purpose To assess whether mutations in *Mer tyrosine kinase* (*MERTK*) are associated with age-related macular degeneration (AMD).

Methods An association study using whole-genome sequencing was performed to determine whether rare variants in *MERTK* are associated with AMD. The data set included 4787 propensity score-matched case–control samples: 2394 AMD cases and 2393 controls. Whole-genome sequencing was performed and variants in *MERTK* were identified. Combined annotation-dependent depletion (CADD) scores and allele frequencies were calculated for each variant identified in *MERTK*. Student's *t*-test was used to assess the mean number of *MERTK* variants per subject between case and control cohorts (Bonferroni adjusted $\alpha = 0.0125$). The number of subjects carrying at least one high CADD score loss-of-function or nonsynonymous mutation in each cohort was compared using Fisher's exact test ($p < 0.05$).

Results No significant difference was found in the mean number of *MERTK* variants in AMD versus control subjects ($p = 0.0502$). Additionally, there was no significant difference between cohorts in the number of subjects with at least one high CADD score loss-of-function or nonsynonymous variant ($p = 0.15$ at CADD > 10 and $p = 0.91$ at CADD > 20).

Conclusions The present study provides a meaningfully negative result demonstrating that rare variants in *MERTK* are not associated with AMD. The study also demonstrates the role of large sample size genetic studies utilizing whole-genome sequencing as a powerful tool that can resolve clinically relevant questions regarding the genetic basis of ophthalmic disease.

Keywords Retina · Genetics · Age-related macular degeneration

Hasenin Al-khersan and Alan Kwong have contributed equally to this work.

H. Al-khersan
Pritzker School of Medicine, The University of Chicago, Chicago, IL, USA

A. Kwong
Department of Biostatistics and the Center for Statistical Genetics, University of Michigan, Ann Arbor, MI, USA

M. A. Grassi (✉)
Grassi Retina, 1012 95th St., Suite 9, Naperville, IL 60564, USA
e-mail: mag@grassiretina.com

M. A. Grassi
Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, USA

Introduction

Whole-exome sequencing (WES) and genome-wide association studies (GWAS) have begun to elucidate

the genetic basis of age-related macular degeneration (AMD) [1]. Identification of genetic associations in AMD can both advance understanding of disease pathology and reveal therapeutic targets. One potential AMD gene is *Mer tyrosine kinase* (*MERTK*—OMIM#604705), a receptor tyrosine kinase of the Tyro3/Axl/Mer family of tyrosine kinases [2]. *MERTK* is involved in the retinal pigment epithelium's (RPE) role in recycling photoreceptor outer segments. As photoreceptors shed light-sensitive disks in their outer segments, they are phagocytized by the RPE [3, 4]. Mutations in *MERTK* have been shown to cause accumulation of debris in the subretinal space and are implicated in autosomal recessive retinitis pigmentosa (RP) [4].

In a previous study using WES in a family with RP, we identified compound heterozygous mutations in *MERTK,* one a novel nonsense *MERTK* mutation (NM_006343.2:p.Arg727*/c.2179C > T) that was previously unreported and the other a previously characterized missense mutation [5, 6]. The proband's mother was a confirmed heterozygous carrier of the novel nonsense *MERTK* mutation whose examination interestingly revealed intermediate AMD (Fig. 1). Given the physiological role of *MERTK* in clearing retinal debris and the finding of AMD, the present study aimed to determine whether rare mutations in *MERTK* are associated with AMD. While similar studies have previously utilized common variants to map genetic associations in AMD, the present work employed whole-genome sequencing instead to enable the identification of highly penetrant rare variants that may be sufficient for its development.

Methods

Research was performed in concordance with the Declaration of Helsinki and with institutional IRB approval and informed consent. All work was conducted in a HIPAA compliant manner. A database of the whole-genome sequencing data of AMD subjects and matched controls was assessed to determine whether *MERTK* mutations were associated with AMD. The database consisted of whole-genome sequencing data from the AREDS and AREDS2 studies from the University of Michigan Kellogg Eye Center, the National Eye Institute, and the University of Pennsylvania [7]. Population control samples were obtained from the Michigan Biobank for unmatched cases. A greedy algorithm was used to match cases to controls according to propensity scores, which were calculated using age and sex as covariates. Case–control pairs were sequenced together at the Michigan Sequencing Core to minimize batch effects. The final data set contained 4787 subjects: 2394 with

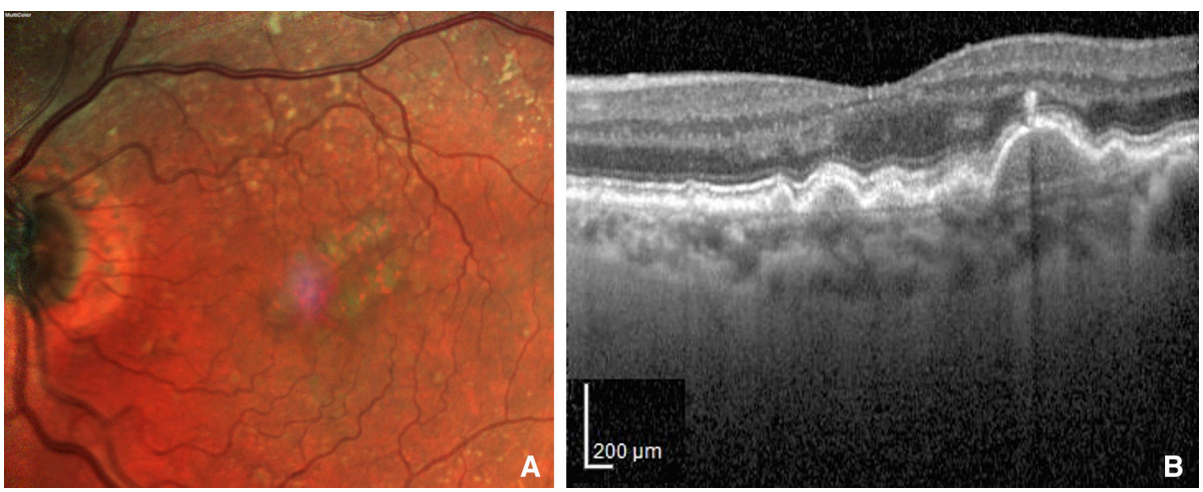

Fig. 1 Multicolor 30° retinal photograph and spectral-domain optical coherence tomography of the left eye. **a** Multicolor 30° retinal photograph centered on the macula of the left eye shows extensive intermediate drusen forming soft, confluent druse in the parafovea. Associated pigment migration into the retina is evident as are focal areas of evolving retina pigment epithelial atrophy. **b** Spectral-domain optical coherence tomograph of the left eye revealing numerous drusen, pigment migration into the retina, and preservation of the outer retinal laminar structures

large drusen, geographic atrophy, choroidal neovascularization, or mixed GA/CNV cases, and 2393 controls. Single nucleotide polymorphisms (SNPs) and indels were called with GotCloud (University of Michigan) in the region of *MERTK* (2:112656056-112787138–GRCh37 coordinates). Variants were annotated using Variant Effect Predictor build 84 (Ensembl). Predicted pathogenicity of called variants was determined using ClinVar (NCBI).

Variants were classified as loss-of-function (LOF), nonsynonymous, synonymous, or noncoding mutations. LOF mutations were defined as splice donors and acceptors, frameshifts, and stop gains. The Student's *t*-test was used to assess the difference in the mean number of *MERTK* variants per subject between case and control cohorts. Bonferroni adjustment was performed for multiple comparisons ($n = 4$, adjusted $\alpha = 0.0125$). Combined annotation-dependent depletion (CADD) scores, a scoring tool predicting the pathogenicity of SNPs, and allele frequencies (AF) were calculated for each variant [8]. Fisher's exact test was used to compare the number of subjects carrying at least one LOF or nonsynonymous mutation at a threshold of CADD > 10, CADD > 20, and AF $< 1\%$. The Gene-Tissue Expression (GTEx) portal (NIH Common Fund) was searched to determine whether noncoding mutations occurred in expression quantitative loci (eQTLs).

Results

The average age of the control population was 74.9 years while the average age of the cases was 75.1 years ($p = 0.49$). The controls were 45.2% male compared to 44.9% male in the cases ($p = 0.86$). All samples (cases and controls) were of European ancestry.

Sequencing was performed at an average depth of 6X across samples. In total, 2062 *MERTK* variants were called: 446 variants exclusively in controls, 468 exclusively in cases, and 1158 variants within both cases and controls. Of the variants called, 2014 were noncoding, 14 synonymous, 33 nonsynonymous, and 1 splice donor (rs371956016/c.2189 + 1G > T). The splice donor variant was the lone ClinVar-predicted pathogenic variant and was found in a control. No LOF variants were called in the AMD cohort. None of the missense mutations in the AMD cohort were ClinVar pathogenic or likely pathogenic variants.

There was no statistically significant difference in the mean number of variants carried by subjects in the control and case cohorts (311 and 318 variants, respectively, $p = 0.0502$). In subanalyses of each variant class, the mean number of LOF, nonsynonymous, and synonymous variants per subject was not significantly different between case and control cohorts (Table 1). The increase in the mean number of noncoding variants in cases relative to controls was insignificant following Bonferroni adjustment for multiple comparisons ($p = 0.0492$, adjusted $\alpha = 0.0125$). None of the noncoding variants were found in expression quantitative trait loci (eQTLs) based on a search of the GTEx portal. Lastly, no statistically significant difference was detected in the number of cases versus controls carrying at least one LOF or nonsynonymous mutation with CADD score cutoffs of > 10 ($p = 0.15$) and > 20 ($p = 0.91$) or AF $< 1\%$ ($p = 0.84$) (Table 2).

Discussion

Allelic variation is responsible for a broad spectrum of retinal diseases. For example, different mutations in the *Retinal Degeneration Slow* (*RDS*) gene cause RP and pattern dystrophy [9]. Heterozygous SNPs in the *TIMP Metallopeptidase Inhibitor 3* (*TIMP3*) gene increase the risk of developing AMD while more severe mutations in this same gene cause Sorsby fundus dystrophy [10, 11]. We aimed to explore if mutations in *MERTK,* a known recessive RP gene, could also in the heterozygous state cause AMD.

The present study did not demonstrate an association between rare *MERTK* variants and AMD. From the genetic data of 4787 individuals, no ClinVar-predicted pathogenic *MERTK* variants were called in the AMD cohort. Additionally, there were no significant differences between cases and controls in the number of subjects carrying variants with CADD scores > 10 or > 20 or with an AF $< 1\%$. The mean number of LOF, nonsynonymous, synonymous, and noncoding variants was not significantly different between cohorts after Bonferroni correction, suggesting *MERTK* variant density was not associated with AMD. Additionally, while there were more noncoding variants in the case cohort, none of the variants were

Table 1 Average number of variants per control and AMD subjects

Variant class	Mean variants per control subject	Mean variants per AMD subject	*p* value	Control 95% CI	AMD 95% CI
Loss-of-function	0.0004	0	0.32	− 0.0004–0.001	N/A
Nonsynonymous	3.2	3.3	0.11	3.2–3.3	3.25–3.39
Synonymous	1.9	1.9	0.25	1.8–1.9	1.86–1.95
Noncoding	305.8	312.2	0.049	301.2–310.4	307.7–316.8
Total variants	310.9	317.5	0.0502	306.2–315.6	312.8–322.1

The mean number of variants per subject for each class was calculated for all individuals in the database. The Student's *t*-test was performed to analyze the difference in the number of variants per subject for each variant class as well as the total variants per subject between the two cohorts. Bonferroni adjusted $\alpha = 0.0125$

Table 2 Carriers of loss-of-function (LOF) and nonsynonymous variants

	Carriers (one or more LOF or nonsynonymous variant)		
	CADD > 20	CADD > 10	AF < 1%
Control cohort	8	39	49
AMD cohort	3	41	52
p value	0.15	0.91	0.84

Variants were assigned combined annotation-dependent depletion (CADD) scores and allele frequencies (AF). Carriers were defined as subjects with at least one LOF or nonsynonymous variant meeting CADD score cutoffs of > 10 or > 20 or AF < 1%. Fisher exact test was used to determine whether there was a significant difference in the number of carriers between the control and AMD cohorts for each scoring threshold

predicted to be pathogenic by ClinVar or to influence gene expression as eQTLs. The large sample size also increased the power to identify small statistical differences that may be clinically irrelevant, which is reflected in the crossing confidence intervals (Table 1). Taken together, these results suggest that mutations in *MERTK* are not associated with AMD.

While a negative result, this is an important and novel finding. No prior genetic association study for AMD, even large meta-analyses with more than 77,000 subjects from the AMD Gene Consortium study, can provide comparable resolution to the present work to address the study objective [12]. This is because prior GWAS employed SNPs that are markers of common variation throughout the genome. Only approximately 50 variants in *MERTK* were assessed with the sole use of a GWAS genotyping array in the AMD Gene Consortium study. In our study, 2062 variants were called in *MERTK* using whole-genome sequencing.

While imputation increases variant identification in GWAS studies, imputation relies on reference panels of normal subjects. Such panels typically cannot identify rare, highly penetrant variants like the nonsense allele (p.Arg727*) that set the hypothesis for this study. Additionally, sequencing-based case–control studies use samples enriched for AMD cases compared to the general population, increasing the likelihood of discovering disease-specific rare variants. Many have argued that in fact rare variants, given their higher penetrance, provide more biologically meaningful insights relative to common variation [13, 14].

The main limitation of the present study is the depth of sequencing coverage. Sequencing depth of coverage is a key consideration in sequencing-based case–control studies because greater sequencing depth of coverage leads to a greater ability to accurately identify variants. An additional limitation is the possibility that *MERTK* variants and other unknown function-altering variants in separate genes could act in concert to increase the risk of developing AMD. The present study was not designed to identify such polygenic interactions.

Conclusions

Ultimately, the present study did not demonstrate an association between rare *MERTK* variants and AMD. Though our AMD patient was a heterozygous carrier of a novel nonsense *MERTK* mutation, this was likely an incidental finding related to the high prevalence of AMD. Nevertheless, the study demonstrates the significance of whole-genome sequencing rather than SNP markers in large sample size cohorts as a powerful tool that can definitively resolve clinically relevant questions in genetic association studies.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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