Exome sequencing to reveal presymptomatic genetic markers for primary open angle glaucoma

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KEY MESSAGES

1. *MEGF11* was identified as a new putative disease-causing gene for primary open angle glaucoma (POAG) in a Chinese population.
2. Mutations in *MEGF11* contributed to about 1.1% of Chinese patients with POAG.
3. The association of variant rs4236601 in the *CAV1/CAV2* gene locus with POAG in Chinese patients is confirmed.
4. A common variant in this locus, rs3801994, is suggested as a new genetic biomarker for POAG in Chinese patients.
5. Both *MEGF11* and *CAV1/CAV2* gene loci are excellent genetic biomarkers that can be incorporated into future genetic diagnostic platforms for POAG.

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Introduction

Glaucoma is the leading cause of irreversible blindness worldwide. The majority of glaucoma cases are primary open-angle glaucoma (POAG), which is caused by interactions of multiple environmental and genetic risk factors. In the complex form of POAG, many genes can be involved, each having a small-to-moderate effect. By contrast, in the Mendelian form of POAG, mutations are usually in a single gene.

Different genetic strategies have been applied to identify POAG genes. With the advent of the next-generation sequencing, most or all types of genomic variation in all exons (ie, the exome) can be detected. The exome constitutes approximately 1% of the whole genome, and approximately 85% of causal mutations for human diseases are located within its coding region and splice sites.[1]

In a previous study, *TCF4* rs613872 was found to be strongly associated with Fuchs corneal dystrophy in Caucasians but not in Chinese, whereas single-nucleotide polymorphisms (SNPs) in *PTPRG* were not associated with Fuchs corneal dystrophy in Caucasians or Chinese populations.[2] Therefore, we did not involve these two genes in the present study. In this study, we used exome sequencing, Sanger sequencing, and SNP genotyping to identify new causative and susceptible gene variants for POAG.

Methods

Study subjects

Five Chinese pedigrees with POAG were recruited for exome sequencing, as were >500 unrelated Chinese POAG patients with variable age of onset, highest intraocular pressure before treatment, and disease severity, as well as >500 unrelated control subjects who had normal visual acuity, no major ocular disorders except for mild senile cataract or refractive errors if any, intraocular pressure <21 mmHg. All patients, family members, and control subjects underwent detailed ophthalmic examination.

Exome sequencing and mutation analysis

For exome sequencing, all available affected and unaffected family members in the POAG pedigrees were included. Total DNA was extracted from peripheral blood using standard protocols for exome sequencing. A pipeline data analysis was used to determine the disease-causing mutations. Segregation analysis was performed after filtering of variants. The variants that are completely segregated with the disease (ie, those occur in patients only but not in controls) were considered as candidate disease-causing mutations. In order to determine the mutation frequency of the newly identified gene (*MEGF11*) in POAG in the Chinese population, all exons of the gene were sequenced in the >500 unrelated patients with POAG using Sanger sequencing. Subsequently, the mutations detected were excluded in the >500 controls to determine the causality of the mutations.

Evaluation of the CAV1/2 gene associated with POAG

By using the sample set and the genetic information, we also evaluated the role of *CAV1/2* gene locus in POAG in three Chinese cohorts from Hong Kong, Shantou (southern Chinese) and Beijing (northern Chinese). We investigated seven SNPs, including rs4236601 that was reported in the previous GWAS[3]

and six haplotype-tagging SNPs covering the *CAV1/CAV2* locus based on the Chinese Han Beijing population in HapMap project (namely rs6466579, rs7801950, rs3779512, rs3807989, rs3801994, and rs1049337).

Data processing and analysis

Bioinformatics analysis was performed for all candidate mutations. Online computer programmes (eg, PolyPhen2, SIFT, and the ASSA) were used to predict the potentially functional impacts of each missense or nonsense variant to the protein or to alternative splicing. Genetic association and genotype-phenotype correlation analyses were performed using chi-square test and logistic regression.

Results

In the five Chinese POAG pedigrees, the exome sequencing data included all annotated variants such as single nucleotide variants, small deletions and insertions (indels), and splice site variants in the exon-intron junctions. After the pipeline filtering procedure and segregation analysis, we found no single variant that was completely cosegregated with POAG in any of the three POAG pedigrees. Therefore, exome sequencing alone did not identify disease-causing gene for POAG.

We then reviewed the data in one of the pedigrees, which is the GLC1N-linked POAG family.[4] We narrowed the targeted variants to the GLC1N region (15q 22-24). In this family, we identified one deletion mutation c.1090delT in the *MEGF11* gene to be segregated with POAG in the pedigree except one married-in subject (II:15) [Fig 1). This mutation is predicted to cause a large C-terminal protein truncation of MEGF11 (p.Cys364ValfsX12). It is therefore likely glaucoma related. Sequencing analysis of this deletion in the rest of the family members who had no identifiable sign of glaucoma at the time of recruitment, revealed its presence in another four kindred members (III:4, III:6, III:16, and III:17), of whom III:16 was a homozygote (Fig 2). With the unexpected founding of the mutation in the married-in husband II:15, we recruited the kindred members of II:15 (ie, II:15M and II:15S) and found the deletion in subject II:15S and not II:15M (Fig 1). So both II:15 and II:15S, aged 46 and 47 years, respectively, at the time of recruitment, were heterozygous for *MEGF11* c.1090delT but they did not have glaucoma. These two families are unrelated. Thus, the *MEGF11* c.1090delT mutation seems necessary, but not sufficient, for the pathogenesis of glaucoma in the *GLC1N*-linked family.

We then screened the *MEGF11* coding sequence in another 453 unrelated POAG patients and identified the c.1090delT deletion in a male Chinese POAG patient from the Beijing cohort. This deletion was absent in 529 Chinese. We also identified three splice site mutations (IVS17+2insT, IVS17-4C>G and c.2472A>C) in patients but not in controls (Table).

Association of CAV1/CAV2 with POAG

SNP rs4236601, which was identified in the

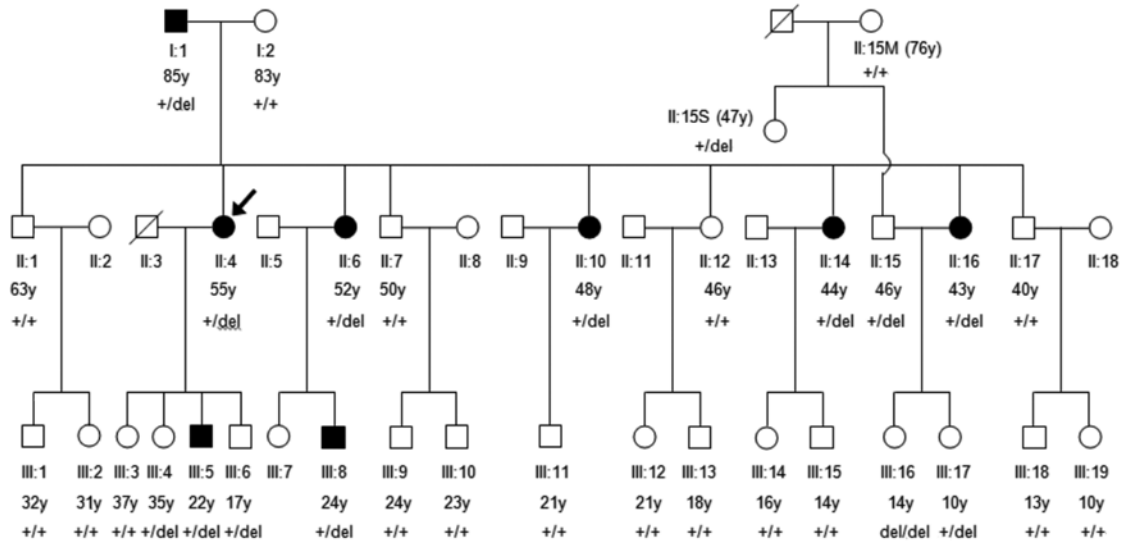

FIG 1. Segregation of *MEGF11* c.1090delT in the *GLC1N*-lined pedigree with primary open angle glaucoma. The genotype of the c.1090delT mutation is shown, where +/+ represents the wild-type, +/del represents heterozygote, and del/del represents homozygote

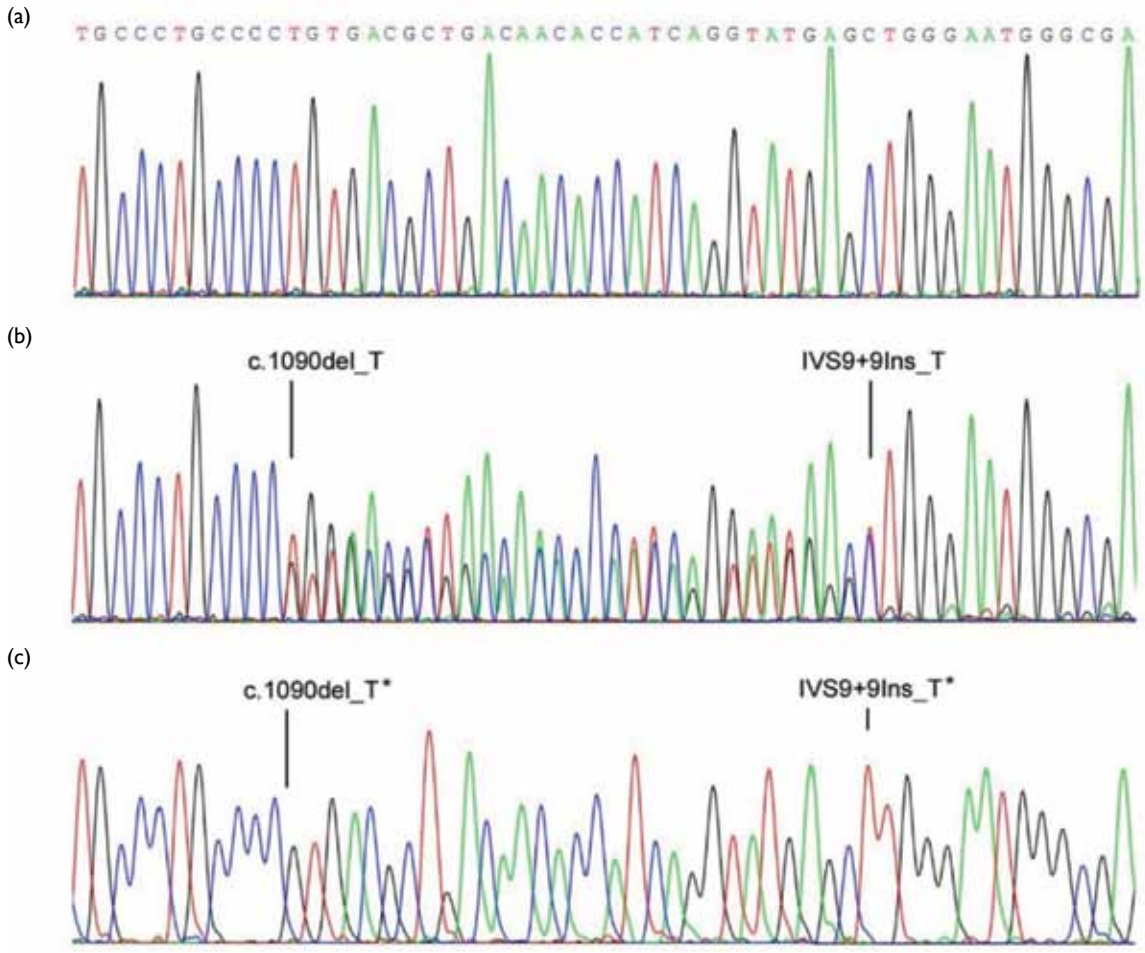

FIG 2. Chromatograms of the *MEGF11* gene sequence flanking the c.1090delT mutation. (a) Reference sequence, (b) c.1090delT mutation detected in an affected subject III:5 of the Hong Kong pedigree with juvenile open-angle glaucoma who also carries the IVS9+9insT variant, and (c) homozygous for c.1090delT mutation detected in subject III:16 in the Hong Kong pedigree with juvenile open-angle glaucoma who was also homozygous for IVS9+9insT. No detectable glaucomatous change was found in this subject at the last visit when she was 14 years old.
* Homozygous for the substitution

GWAS,[3] conferred an increased risk of POAG (A allele, P=0.0072, odds ratio [OR]=4.72), with a population attributable risk of 1.47%. A common SNP, rs3801994, showed a borderline association with POAG (A allele, P=0.036, OR=1.31). This SNP conferred a population attributable risk of 4.33%. Based on the findings in the Hong Kong cohort, we genotyped the SNPs rs4236601 and rs3801994 in the cohorts from Shantou, Beijing, and Osaka. SNP rs4236601 was associated with POAG in the Shantou cohort (P=0.0079, OR=4.23). Also, rs4236601 showed a significant association with POAG in the Beijing cohort after adjusting for age and gender (P=0.030, OR=3.92). In contrast, rs3801994 was not significantly associated with POAG in the Shantou or Beijing cohort, but their ORs were toward the same direction with that in the Hong Kong cohort. By pooling the data of rs4236601 and rs3801994 from the 3 Chinese cohorts, the SNP rs4236601 was strongly associated with POAG prior to ($P=1.1\times10^{-4}$, OR=3.80), with no inter-cohort heterogeneity ($I^2=0$). SNP rs3801994 showed a borderline association with POAG (P=0.022, OR=1.23, $I^2=0$).[5]

Discussion

Although exome sequencing alone did not lead to the identification of a disease-causing gene for POAG in the five POAG pedigrees, the combination of exome sequencing data and our previous linkage study in a Hong Kong Chinese POAG pedigree identified a novel deletion mutation c.1090delT in the *MEGF11* gene to be implicated in POAG. The mutation was deemed necessary for glaucoma pathogenesis in the POAG pedigree. This mutation was also detected in a Chinese POAG patient from the Beijing cohorts but was absent in 529 unrelated Chinese. Therefore, this *MEGF11* deletion is likely to be pathogenic

TABLE. Distribution of rare variants of *MEGF11* in patients with primary open angle glaucoma (POAG) and controls*

Variation category	Hong Kong cohort		Beijing cohort		Shantou cohort		Pooled Chinese subjects	
	POAG (n=181)	Control (n=182)	POAG (n=177)	Control (n=200)	POAG (n=95)	Control (n=147)	POAG (n=453)	Control (n=382)*
Coding variants								
Any coding variants (variants present in both cases and controls inclusive)	14 (7.7)	13 (7.1)	9 (5.1)	11 (5.5)	4 (4.2)	ND	27 (6.0)	24 (6.3)
Missense variants	10 (5.5)	10 (5.5)	6 (3.4)	5 (2.5)	2 (2.1)	ND	18 (4.0)	15 (3.9)
Variants predicted damaging (PolyPhen)	2 (1.1)	5 (2.8)	1 (0.6)	4 (2.0)	1 (1.1)	ND	4 (0.9)	9 (2.4)
Variants predicted benign (PolyPhen)	8 (4.4)	5 (2.8)	5 (2.8)	1 (0.5)	1 (1.1)	ND	14 (3.1)	6 (1.6)
Variants in EGF-like domains	2 (1.1)	1 (0.6)	2 (1.1)	1 (0.5)	1 (1.1)	ND	5 (1.1)	2 (0.5)
Variants in EGF-like domains and predicted damaging	0	1 (0.6)	0	1 (0.5)	1 (1.1)	ND	1 (0.2)	2 (0.5)
Variants result in premature truncation	1 (0.6)	0	1 (0.6)	0	0	ND	2 (0.4)	0/529
Coding variants exclusively present in cases/controls	9 (5.0)	9 (5.0)	7 (4.0)	6 (3.0)	2 (2.1)	ND	18 (4.0)	15 (3.9)
Missense variants exclusively present in cases/controls	7 (3.9)	6 (3.3)	4 (2.3)	2 (1.0)	2 (2.1)	ND	13 (2.9)	8 (2.1)
Damaging variants exclusively present in cases/controls	3 (1.7)	5 (2.8)	1 (0.6)	2 (1.0)	1 (1.1)	ND	5 (1.1)	7 (1.8)
Intronic variants								
Any intronic variants	8 (4.4)	7 (3.9)	10 (5.6)	14 (7.0)	2 (2.1)	ND	20 (4.4)	21 (5.5)
Variants at invariant AG/GT splice acceptor/donor site	0	0	0	0	1 (1.0)	ND	1 (0.2)	0

* No significant difference detected for each comparison of the proportion

† Included only controls from the Hong Kong and Beijing cohorts. For the truncation mutation, controls were pooled from the three cohorts (n=529)

for POAG. However, four mutation carriers in the pedigree (III:4, III:6, III:16, and III:17) were found without glaucomatous features at the time of study enrolment. Moreover, subject III:16 was homozygous for the deletion while her father (II:15) and aunt (II:15S), from a different pedigree, were unaffected mutation carriers. The *MEGF11* c.1090delT is thus likely to be the causal mutation for POAG in this *GLC1N*-linked family with incomplete penetrance.

Additionally, we identified three splice junction mutations, which are likely to result in leaky splice sites, in three patients. These four mutations are likely to result in MEGF11 proteins lacking one of the 15th, 16th, and 17th EGF-like domains, suggesting that absence of either one of these domains could be pathogenic for glaucoma. If these four putative mutations, namely c.1090delT, IVS17+2insT, IVS17-4C>G, and c.2472A>C, are in fact pathogenic, the *MEGF11* mutations may contribute to approximately 1.1% (5/453) of index POAG patients in Chinese.

We also confirmed the association between SNP rs4236601 at the *CAV1/CAV2* locus and POAG in Chinese. The minor allele A increased the risk of POAG by over fourfold in southern Chinese and nearly threefold in northern Chinese. Moreover, we identified a common SNP rs3801994 for POAG in Chinese, with an OR of 1.23. Thus, our data highlighted an important role of the *CAV1/CAV2* gene in the genetic susceptibility of POAG.

The mutation frequency of *MEGF11* (1.1%) and the population attributable risk of *CAV1/CAV2* (4.33%) are low, therefore to design a diagnostic gene chip based on the current findings provides limited practical value. However, they are excellent candidate genes that should be incorporated into future gene chips for the genetic diagnosis of POAG, especially for Hong Kong Chinese population.

Conclusions

MEGF11 is likely a causative gene of POAG, contributing to 1.1% of POAG patients in the Chinese population. rs4236601 is associated with POAG in the southern and northern Chinese. A common SNP at the *CAV1/CAV2* locus, rs3801994, is suggested as a new genetic biomarker for POAG in Chinese. Further genetic studies are needed to identify more genetic biomarkers for POAG.

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References

1. Choi M, Scholl UI, Ji W, et al. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci U S A 2009;106:19096-101.
2. Lau LC, Ma L, Young AL, et al. Association of common variants in TCF4 and PTPRG with Fuchs' corneal dystrophy: a systematic review and meta-analysis. PLoS One 2014;9:e109142.
3. Thorleifsson G, Walters GB, Hewitt AW, et al. Common variants near CAV1 and CAV2 are associated with primary open-angle glaucoma. Nat Genet 2010;42:906-9.
4. Wang DY, Fan BJ, Chua JK, et al. A genome-wide scan maps a novel juvenile-onset primary open-angle glaucoma locus to 15q. Invest Ophthalmol Vis Sci 2006;47:5315-21.
5. Rong SS, Chen LJ, Leung CK, et al. Ethnic specific association of the CAV1/CAV2 locus with primary open-angle glaucoma. Sci Rep 2016;6:27837.