



Genome-wide association study in primary angle closure glaucoma

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Comment on: Khor CC, Do T, Jia H, *et al.* Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. *Nat Genet* 2016;48:556-62.

Received: 19 December 2016; Accepted: 30 December 2016; Published: 31 January 2017.

doi: 10.21037/aes.2017.01.04

View this article at: <http://dx.doi.org/10.21037/aes.2017.01.04>

Before the Human Genome Project, family-based mapping, positional cloning and mutation screening of candidate genes were largely used to ascertain the etiology of a given genetic disease. While this method proved to be very effective for most Mendelian single-gene disorders, it was not efficient for multigenic disorders with unknown genetic etiology. Subsequent advancement in molecular genetics techniques provided an opportunity to screen millions of biological markers in thousands of patients in a relatively short period of time. One rapid technique that is already used in a large number of clinical disorders is genome-wide association study (GWAS). Other screening methods include next-generation exome sequencing, whole-genome sequencing and targeted regional sequencing.

One of the major ocular disorders that involve millions of people around the world is glaucoma (1). While certain types of primary congenital and juvenile glaucoma are caused by known genes [i.e., *CYP1B1* (2), *MYOC* (3)], for most of other primary (open angle) and secondary (angle closure, exfoliation, pigmentary) glaucoma subtypes, either limited (4-8) or no definitive causative genes have been identified. Moreover, this has been more prominent for primary angle closure glaucoma (PACG) that is one of the principal causes of irreversible blindness in the Asian population (9).

The molecular genetics studies of PACG have been hindered by a few impediments. The classical genetic linkage studies and positional mapping of this condition have been mainly unsuccessful due to lack of suitable large families with multiply affected subjects. So far, one

PACG locus (*GLC2A* on 10q) has been identified for a Singaporean family (10) and another mapped onto the 3q27.1 region (11). However, no causative genes have been reported for PACG as yet.

PACG also has common clinical presentations with microphthalmia and nanophthalmia including, short axial length, shallow anterior chamber depth, small eyes with larger lenses, and elevated intraocular pressure. Due to these clinical similarities, a number of microphthalmia- and nanophthalmia-causing genes [*CHX10* (12), *MFRP* (13) and *PRSS56* (14,15)] have been screened in PACG subjects and no causative mutations are identified. As yet, no single causative PACG gene has been identified. However, in one Chinese family, a compound heterozygote mutation in *MYOC* (Arg46Stop) and *CYP1B1* (Leu432Val) has been reported (16).

Successive GWAS investigations led to the discovery of numerous candidate susceptibility genes for PACG. However, most of these were either not reproduced in other population, or their concluding results were controversial. The most significant GWAS in the Asian population (17) identified three single-nucleotide polymorphisms (SNPs) of rs11024102 (*PLEKHA7*; 11p15.1), rs3753841 (*COL11A1*; 1p21.1), and rs1015213 (8q11.23) that were all highly associated with the PACG phenotype (Table 1).

In a more recent publication (18), the original GWAS investigation in the Asian population (17) has been significantly expanded and now includes over 40,000 subjects (including 10,503 PACG cases) from 24 countries including, individuals from Asia, Australia, Europe, North

Table 1 PACG-associated SNPs (18), neighboring genes and their homologous map locations in mouse

Gene/region	PACG associated SNP	dbSNP 147 position (bp)	Location of SNP in the gene	SNP/gene map position	Mouse homologue gene location
<i>EPDR1</i>	rs3816415	37,948,709	Intron 1	7p14.1	chr13: 19,591,708-19,619,952
<i>GLIS3</i>	rs736893	4,217,028	Intron 2	9p24.2	chr19: 28,258,851-28,680,077
<i>DPM2-FAM102A</i>	rs3739821	127,940,198	Intergenic	9q34.11	chr2: 32,570,858-32,573,579; chr2: 32,535,332-32,569,756
<i>CHAT (C10orf53)</i>	rs1258267	49,687,724	Intron 1	10q11.23	chr14: 32,408,203-32,465,909
<i>FERMT2 (PLEKHC1)</i>	rs7494379	52,944,673	Intron 2	14q22.1	chr14: 45,458,792-45,530,118
<i>COL11A1</i>	rs3753841	102,914,362	Exon 50–53	1p21.1	chr3: 114,030,479-114,220,718
<i>PCMTD1-ST18</i>	rs1015213	51,974,981	Intergenic	8q11.23	chr1: 7,088,891-7,173,628; chr1: 6,487,231-6,860,940
<i>PLEKHA7</i>	rs11024102	16,987,058	Intron 2	11p15.1	chr7: 116,123,485-116,308,381

America, and South America. This is one of the largest GWAS study for any subtypes of glaucoma published so far. The results of this study are very interesting as firstly, all of the original three SNPs have been replicated in this much larger group of cases and controls from other population. Furthermore, this study was able to identify five new genomic locations (*Table 1*) that are highly associated with the PACG phenotype in different populations. Of the eight reported PACG-associated SNPs, five are intronic, two intergenic and only one is located within an exon of *COL11A1* gene. The eight PACG-associated SNPs are located on seven different chromosomes. Interestingly, the homologous of these genes are located on different mouse genome and only *CHAT* and *FERMT2* are within 13-Mb of each other on mouse chromosome 14 (*Table 1*). Though the SNP-associated alleles are either missing or different in mouse, these are conserved in macaque, orangutan and chimp and, therefore, may have evolutionary relevance to PACG. Two of the associated SNPs map to the same chromosome (rs736893 on 9p24.2 and rs3739821 on 9q34.11), however, there is considerable genomic distance between them. This in turn may indicate that each of such associated-SNP is probably independent and each has taken a different evolutionary course. However, eventually their biological influence must have emerged into a functionally similar pathway and thus, contributed to the development of PACG. Furthermore, if the reported neighboring genes of PACG-associated SNPs have a direct biological participation in etiology of this ocular condition then, one could only assume that whilst their role may have

been independent from each other, they potentially and collectively had a multi-emerging effect on evolution of PACG. Also, as such SNPs only account for a fraction of total disease variance in PACG (1.8%), one could anticipate that there are a very large number of such associated SNPs in the genome that influence a group of genes in a multi-collaborative manner and thus, lead to the development of PACG. Therefore, even a much larger number of cases and normal subjects than reported here (18) must be evaluated before other SNPs with much smaller effect can be detected through GWAS. The cost of such studies may be overwhelming and, therefore, other methods of molecular investigations may be more practical. Perhaps, the cost of whole-genome sequencing and its subsequent pipeline data and bioinformatics analyses will be more economical in a near future for PACG and other clinical disorders.

The GWAS investigative studies by *Nature* only discover regions of genome that are associated with a given phenotype and, therefore, such studies rarely lead to actual defective genes involved in the etiology of a phenotype. Most GWAS investigations lead to the identification of a group of SNP markers that are highly associated with the clinical phenotype. More often, such SNPs are located either outside of all known genes or map intergenic to other genes. Others may be located within the intronic regions of certain genes and, to the lesser degree, fewer SNPs may well map and even alter the coding regions of known genes. Therefore, it is imperative that biological significance of such associated SNPs to the clinical entity is well investigated. Although at the first glance, most of

the identified SNPs may not show any clear involvement in etiology of the clinical phenotype, there is a good chance that their proximity to a given gene influences the expression or function of that otherwise normally-coded gene. Once GWAS identifies such a neighboring gene, its mRNA and protein products can be investigated in the affected tissues of the disorder. If significant deviations in expression between normal and diseased tissues are identified, then the likelihood of that gene's products being involved in etiology of that disorder is increased. However, if the gene's products are only expressed within the affected tissues of the diseased organ, that is not by itself a conclusive evidence for the involvement of that gene in that disorder. Many gene products can be locally produced or otherwise expressed within the normal and affected tissues of a given disorder. Moreover, such gene expressions may only have a transient role with no significant involvement in the disease process. As it is clearly shown in this newly published PACG-GWAS investigation (18), both mRNA and protein of the identified neighboring genes are expressed in various ocular tissues. However, this is not indicative that any of these genes have explicit roles in the development of PACG. Nevertheless, this study highlighted potential importance of cell-cell adhesion (*EPDR1*, *FERMT2/PLEKHC1*, *PLEKHA7*) and collagen metabolism (*COL11A1*) in PACG pathogenesis. The study has further implicated possible participation of acetylcholine metabolism (*CHAT*), an unknown metabolic pathway mediated through zinc-finger activation (*GLIS3*) and changes in glycosylation (*DPM2-FAM102A*) in the development of PACG phenotype.

Though this newly published GWAS (18) has led to identification of eight highly PACG-associated SNP markers, there is still no direct biological and functional evidence that the neighboring genes to such SNPs are involved in the etiology of PACG. Currently, due to our lack of understanding of functional and biological significance of such millions of randomly distributed SNPs in our genome, it is always and equally likely that such associated-SNPs may influence the function of other genes on different parts of the genome or have no biological significance at all. Even the evidence that the neighboring genes to these SNP markers are expressed in various normal ocular tissues is not sufficient evidence for their participation in the etiology of PACG. Though one may always speculate the involvement of such genes in PACG, but before a comprehensive molecular, biological, biochemical, functional and animal models are thoroughly investigated, one has to treat the involvement of these neighboring genes in PACG only as

probable. Therefore, the notion that PACG may also be the result of evolutionary defects in the same neighboring genes' pathways is only exploratory at this time and must be treated with extreme care.

The most noteworthy of this GWAS discovery (18) is in potential clinical usefulness of these SNPs in determination and identification of unaffected and indecisive cases that are at high risk of developing PACG. The next important observation is that, if indeed, these genes are directly involved in the etiology of PACG then, for the first time, their participations in various biological processes will be an important guiding principle for better understanding of pathways involved in PACG mechanism. This could potentially lead to future targeted studies, better understanding and perhaps, one day, development of appropriate drug treatments for this blinding condition.

Although identification of these eight susceptibility loci (18) accounts for only a small fraction of the total expected disease variance in PACG, the potential clinical and impetus that it provides for future study of potential biological processes and knowledge of biochemical and biological pathways that are integrate part of PACG maturity cannot be overemphasized.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by Section Editor Fang Fan, MD (Department of Ophthalmology, Hebei General Hospital, Shijiazhuang, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/aes.2017.01.04>). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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doi: 10.21037/aes.2017.01.04

Cite this article as: Sarfarazi M. Genome-wide association study in primary angle closure glaucoma. *Ann Eye Sci* 2017;2:4.