Editorial

Targeted Screening for Predominant CYP1B1 Mutations in Primary Congenital Glaucoma

Mansoor Sarfarazi, PhD, FARVO

Department of Surgery, Molecular Ophthalmic Genetics Laboratory, UConn Health, Farmington, CT, USA

J Ophthalmic Vis Res 2018; 13 (4): 373-375

Advances in molecular genetic techniques and their application to various clinical entities have provided major improvements in prenatal, pre-symptomatic and preclinical diagnosis of human medical conditions. Further investigations are underway to develop faster, better and less costly diagnostic techniques that can be easily implemented in everyday clinical settings. However, all such clinical studies and technical improvements require extensive initial investigation of the clinical entities for a given targeted population and development of a simple molecular test that can easily be implemented in laboratories with limited access to modern and expensive testing equipment.

Analogous to other human clinical conditions, numerous forms of ocular disorders have been subjected to such clinical and molecular studies. More specifically, over the past 25 years, all types of glaucoma have been molecularly investigated by researchers around the world. Initial studies involved genetic linkage mapping of various familial forms of glaucoma and subsequent identification of their specific gene mutations. However, due to the clinical complexity of different glaucoma sub-types, variable and often late age of onset, small family size and limited availability of affected individuals for inclusion in a-genome-wide positional mapping, the pace of glaucoma gene discoveries has been slower than other clinical disorders. Consequently, numerous genome-wide association studies, and more recently exome and whole genome sequencing of a group of familial and unrelated glaucoma subjects, have been undertaken by different researchers. Although such studies reported various associations between glaucoma subtypes and certain DNA polymorphisms, final conclusions on exome and whole genome sequencing of familial and sporadic glaucoma subjects are still eagerly awaited.

As noted here, prior gene identification for a given clinical condition is a prerequisite for further population-based screening of that disorder. For different glaucoma subtypes only a handful of genes have been identified. One of the genes that our group initially identified for primary congenital glaucoma (PCG) is Cytochrome P450-1B1 (CYP1B1). In 1995, we studied a group of Turkish PCG families and mapped the first PCG locus (GLC3A) onto the 2p21 region.[1] Subsequent screening of candidate genes from this region identified three truncating CYP1B1 mutations in five GLC3A-linked families.[2] We further screened a total of 22 PCG families from six diverse populations and increased the total number of CYP1B1 mutations to 16.[3] Successively, our findings were confirmed in a larger group of PCG families. By now, CYP1B1 has been screened in many diverse groups of populations and a large number of PCG-causative mutations, and many other normal sequence variations have been reported.[4] At minimum, a total of 130 different CYP1B1 mutations have been identified in PCG subjects from distinct worldwide populations.[4] The spectrum of CYP1B1 mutations and their frequency varies in different populations. However, certain CYP1B1 mutations are more prevalent than others and are mainly identified in Middle Eastern and Asian populations in which consanguineous marriages are more frequent. CYP1B1 mutations have also been reported in other clinical conditions including Peters' Anomaly, anterior segment dysgenesis and primary open angle glaucoma.[5] A number of CYP1B1 polymorphisms have also been shown to be associated with different forms of cancer.

One of the databases (http://databases.lovd.nl/shared/genes/CYP1B1) curating for CYP1B1 mutations currently lists a total of 287-pathogenic variants of which only 157 are listed as unique alterations. Most CYP1B1 mutations are substitutions (72%) followed by deletions (17%), duplications (6%), Indels (3%) and insertions (2%). Furthermore, the current Genome Aggregation Database (gnomAD)[6] enlists 372 variants within the two coding exons of the CYP1B1 gene, the majority of which (n = 329) are missense DNA alterations in exon-2 (n = 195) and exon-3 (n = 134) of the gene. As of this writing, the "gnomAD" database is compiled of at least eight different populations and contains a total of

138,632 allelic data (123,136 exomes and 15,496 genomes) from randomly sequenced normal individuals.[6]

Although various CYP1B1 mutations have been reported in specific regions of the world,[4] it is unlikely that a common founder PCG-causing mutation can be attributed to a given Asian or Caucasian population. The only exception is probably the presence of the E387K mutation in the Gypsy population of Slovakia Roma that is believed to have a founder effect with 100% frequency in PCG subjects of this specific sub-group.[7] While certain PCG-causing mutations (i.e., V364M, L385F and R390H) are more commonly reported in Asian populations, other CYP1B1 mutations (i.e., G61E, R368H, R390H, E387K, E229K, R390C, duplications and deletions) are more recurrently involved in Caucasian PCG patients.

The article by Qashqai et al[8] in this issue of *Journal of Ophthalmic and Vision Research* investigated four known CYP1B1 mutations that have previously been reported to be prevalent in Iranian PCG subjects.[9] These four mutations include p.Gly61Glu (G61E), p.Arg368His (R368H), p.Arg390His (R390H) and p.Arg469Trp (R469W) which have been reported in Middle Eastern, Asian and Caucasian PCG populations. Their frequencies in the current "gnomAD" database are 0.031% (69/225,784), 0.538% (1,466/272,586), 0.010% (28/275,226), and 0.005% (12/246,264), respectively.[6]

The G61E mutation is prevalent in Saudi Arabia and has also been reported in PCG subjects from Iran,[9] India, Morocco, Oman, Turkey,[3] Tunisia, Lebanon and Egypt.[4] In the current article by Qashqai et al,[8] the G61E mutation was observed in 5/700 (0.71%) normal subjects in Gilan province and in 4/137 (2.92%) normal subjects within the sub-region of Talesh. The observed frequency of the G61E mutation in the normal population of Gilan province is twenty-three-times of that reported in the "gnomAD" database.[6] According to Wikipedia, the population of Gilan and Talesh region is estimated to be around 2.5 million and 200,000, respectively. Therefore, the total expected number of subjects carrying the G61E mutation within this province and its sub-region would be 17,857 and 5,839 individuals, respectively. However, one has to consider age and expected number of child-bearing adults in any given population; the actual ratio of males and females carrying the G61E mutation; the overall likelihood of two gene carriers producing any offspring; and the fact that typically only one-fourth of newborn babies will become affected; therefore the ultimate number of PCG-born subjects may be significantly smaller. Even though the total number of PCG-affected newborn subjects with homozygote G61E mutations in this population may be less remarkable (4,464 and 1,460 respectively), prenatal or newborn screening for the G61E mutation within the province of Gilan, and/or more specifically within the Talesh region, may be beneficial and warranted.

Similarly, the R368H mutation has been reported in PCG individuals from Iran,[9] Pakistan, India, Morocco, Oman, and Gypsies of Bulgaria. The Qashqai et al[8] study identified this mutation in a total of 7/700 (1%) of normal subjects in Gilan province. However, six of the R368H mutations were concentrated within the eastern region of Gilan province with an observed frequency of 2.33% (6/258). This frequency of the R368H mutation in the normal population of the eastern region is four-times of that reported in the "gnomAD" database.[6] For the province of Gilan with an estimated population of over 2.5 million, the total expected number of subjects carrying the R368H mutation (7/700 = 1%) will be around 25,000. If the eastern region of Gilan province has a population of approximately 1.5 million, then the total number of expected R368H gene carriers (6/258 = 2.33%) within this region will be around 34,884 individuals. Therefore, prenatal or newborn screening for the R368H mutation within the eastern region of Gilan may be even more advantageous and merited.

The R390H is a generally common PCG causing mutation in Asian populations and has been reported in China, Pakistan, Iran,[9] Saudi Arabia and UK.[3] Likewise, the R469W mutation is reported from Iran,[9] Pakistan, UK,[3] China, Saudi Arabia, Lebanon, Turkey[3] and Morocco. However, none of these two mutations were observed in the study by Qashqai et al.[8]

Screening of a total of 700 normal subjects from the Gilan province led to detection of five G61E and seven R368H mutations within the two different coding exons of the CYP1B1 gene. Taken together, these twelve mutations were present in 1.71% of the 700 normal subjects who were screened. Therefore, if one wants to expand this study to include the estimated population of Gilan province (i.e., Over 2,500,000 individuals) then, a total of 42,857 people would be expected to be gene carriers for these two PCG-causing mutations within this specific region of Iran. However, due to the considerations stated above, the actual number of people carrying these two CYP1B1 mutations are expected to be far less than anticipated. As the combined observed "allelic-frequency" of G61E and R368H mutations in Gilan province is estimated to be 0.86%, the corresponding number of people expecting to yield the same "allelic-frequency" in a population of over 2.5 million is projected to be around 21,429 people.

The actual incidence of PCG is largely unknown in Iran. However, a large proportion of mutations in the Iranian population are routinely inherited through identical by descent (IBD) and more commonly described within the regionally inbred population. Therefore, as the PCG incidence rate in other similarly structured population-based studies from India and Saudi Arabia has been estimated to range from 1 in 1,200-1,300 cases,[8] one may adopt a middle range figure of 1 in 2,500 as the presumptive Iranian PCG incidence

rate. If this assumption is fairly correct, for the province of Gilan with a population of approximately 2.5 million studied by Qashqai et al,[8] one may expect a total of 1,000 PCG affected cases and further 4,000 people with PCG-carrying gene mutations.

As noted by Qashqai et al,[8] 70% of Iranian PCG subjects are shown to be caused by CYP1B1 mutations. Furthermore, only four CYP1B1 mutations (i.e., G61E, R368H, R390H and R469W) are responsible for 77.3% of all PCG cases in Iran. Therefore, population-based screening for these four mutations in the Northwest region of Iran would be a reasonable undertaking for identification of newborn babies at risk of developing PCG. At most, of the 4,000 anticipated PCG-gene carriers in Gilan, 70% (2,800 people) are expected to have different mutations in CYP1B1 and 77.3% (or 2,164 subjects) are expected to carry one of the four common mutations of G61E, R368H, R390H or R469W.

In summary, the study by Qashqai et al[8] undertook the challenge of screening for four of the common CYP1B1 mutations in a total of 700 normal subjects from Gilan province in northwestern Iran with an estimated population of 2.5 million. Their study only identified two of the four studied CYP1B1 mutations within this population. Although the overall study population was relatively small (i.e., 700 of 2.5 million or, 0.03% of Gilan population), and therefore the observed percentages of G61E and R368H mutations may be far from their actual frequencies, screening for these two particular mutations may still identify a significant number of adult CYP1B1 gene carriers that could potentially give birth to babies with the PCG phenotype. From an economical aspect, testing for these two mutations using the two simple and efficient protocols developed by these investigators[8] will definitely overshadow potential costs of lifelong management of such newborns who are certainly at much higher risk of PCG.

REFERENCES

1. Sarfarazi M, Akarsu AN, Hossain A, Turacli ME, Aktan SG, Barsoum-Homsy M, et al. Assignment of a locus (GLC3A) for primary congenital glaucoma (Buphthalmos) to 2p21 and evidence for genetic heterogeneity. *Genomics* 1995;30:171-177.
2. Stoilov I, Akarsu AN, Sarfarazi M. Identification of three different truncating mutations in cytochrome P4501B1 (CYP1B1) as the principal cause of primary congenital glaucoma (Buphthalmos) in families linked to the GLC3A locus on chromosome 2p21. *Human Molecular Genetics* 1997;6:641-647.
3. Stoilov I, Akarsu AN, Alozie I, Child A, Barsoum-Homsy M, Turacli ME. et al. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome P4501B1. *American Journal of Human Genetics* 1998;62:573-584.
4. Sharafieh S, Child AH, Sarfarazi M. Molecular Genetics of Primary Congenial Glaucoma. 2012;295-310. In: Genetic Diseases of the Eye. Edited by: E.I. Traboulsi. Oxford University Press.
5. Sarfarazi M, Monemi S, Choudhary D, Rezaie T, Schenkman JB. Roles of CYP1B1, Optineurin, and WDR36 gene mutations in Glaucoma. 2008;233-273. In: Ophthalmology Research: Mechanisms of the Glaucomas; Disease Processes and Therapeutic Modalities. Edited by: J. Tombran-Tink, C.J. Barnstable and M.B. Shields. Humana Press.
6. Lek M, Konrad J. Karczewski and Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016;536:285-291 (See: http://gnomad.broadinstitute.org/about).
7. Plasilova M, Stoilov I, Sarfarazi M, Kadasi L, Ferakova E, Ferak V. Identification of a single ancestral CYP1B1 mutation in Slovak Gypsies (Roms) affected with primary congenital glaucoma. *Journal of Medical Genetics* 1999;36:290-294.
8. Qashqai M, Suri F, Yaseri M, Elahi E. p.Gly61Glu and p.Arg368His Mutations in *CYP1B1* That Cause Congenital Glaucoma May Be Relatively Frequent in Certain Regions of Gilan Province, Iran. *JOVR* 2018.
9. Chitsazian F, Tusi BK, Elahi E, Saroei HA, Sanati MH, Yazdani S, et al. CYP1B1 mutation profile of Iranian primary congenital glaucoma patients and associated haplotypes. The Journal of molecular diagnostics. *JMD* 2007;9:382-393.

Access this article online	
Quick Response Code:	Website: www.jovr.org
	DOI: 10.4103/jovr.jovr_232_18

How to cite this article: Sarfarazi M. Targeted screening for predominant CYP1B1 mutations in primary congenital glaucoma. J Ophthalmic Vis Res 2018;13:373-5.