

## DNA Sequence Variants in *LOXL1* and Pseudoexfoliation Glaucoma

a report by

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Glaucoma is a group of diseases resulting in an irreversible degeneration of the optic nerve. It is one of the leading causes of blindness worldwide, estimated to affect more than 60 million people by 2010.<sup>1</sup> Pseudoexfoliation syndrome (PXFS) results in the deposition of microfibrillar material throughout the eye, with over 50% of cases developing glaucoma. Primary open-angle glaucoma (POAG) and pseudoexfoliation glaucoma (PXFG) are the most common forms of glaucoma. Genetic factors play an important role in the development of these disorders.<sup>2-5</sup> Fourteen chromosomal loci have been designated *GLC1A* to *GLC1N* for POAG.<sup>2,6</sup> From these loci, three genes have been identified as causative factors for POAG. Mutations in the *MYOC* gene at *GLC1A* primarily cause high-tension glaucoma (HTG).<sup>7,8</sup> The *OPTN* gene at *GLC1E* appears to contribute to normal-tension glaucoma (NTG).<sup>9,10</sup> The *WDR36* gene at *GLC1G* is considered to be a modifier gene affecting both HTG<sup>11-13</sup> and NTG patients.<sup>11,13</sup> However, mutations in these genes together only account for fewer than 10% of all POAG cases.<sup>2</sup> Collectively, these results underscore the genetic complexity of POAG and the need for the identification of genes that confer significant susceptibility.

### Genetics of Pseudoexfoliation Glaucoma

PXFS and associated glaucoma also appear to be a genetically complex disease. A genome-wide scan with 1,000 microsatellite markers in a Finnish family with PXFS suggested linkage to 18q12.1–21.33 and regions of chromosomes 2q, 17q, and 19q.<sup>4</sup> Loss of heterozygosity has been reported on chromosomes 13q12.11, 7p13, 7q21.3, and 7q21.11 in patients with PXFS.<sup>14,15</sup> Recently, a genome-wide association study identified a strong association of the *LOXL1* gene with PXFG in patients from Iceland and Sweden.<sup>5</sup> This association has been replicated in our study of a US clinic-based population with broad ethnic diversity<sup>16</sup> and in other studies using Caucasian,<sup>17-23</sup> Indian,<sup>24</sup> and Japanese<sup>25-29</sup> ethnic populations. These studies indicate that *LOXL1* is a major gene associated with PXFG, accounting for up to 99% of PXFG cases in most populations.



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### Initial Association Studies of *LOXL1* with Pseudoexfoliation Glaucoma

The initial genome-wide association study using 304,250 single nucleotide polymorphisms (SNPs) identified a strong association of SNP rs2165241 in the first intron of *LOXL1* with PXFG in Icelandic patients.<sup>5</sup> This strong association was replicated in Swedish patients with PXFG.<sup>5</sup> Further genotyping identified a strong association of two non-synonymous SNPs (rs1048661 and rs3825942) in the first exon of *LOXL1* with PXFG in both Icelandic and Swedish patients (see *Table 1*).<sup>5</sup> The intronic SNP rs2165241 was no longer significant after adjusting for both non-synonymous SNPs. SNP rs1048661 (R141L) changes an arginine to a leucine and rs3825942 (G153D) changes a glycine to an asparagine in the *LOXL1* protein. Compared with allele T, allele G of rs1048661 has a 2.56- and 2.39-fold increased risk of developing PXFG in Icelandic and Swedish populations, respectively (see *Table 1*). For rs3825942, relative to allele A, allele G confers a 13.23- and 27.28-fold increased risk of developing PXFG in Icelandic and Swedish populations, respectively (see *Table 1*). Jointly, these two SNPs accounted for more than 99% of all PXFG cases in these populations.<sup>5</sup>

### Follow-up Association Studies of *LOXL1* with Pseudoexfoliation Glaucoma

Replication of these initial results in other major populations was important because the Nordic population has a relatively limited gene pool and a high prevalence of PXFS and PXFG.<sup>30</sup> *Table 1* summarizes all of the association studies of *LOXL1* with PXFG in different populations published to date.<sup>5,16-29</sup> We have replicated the association of *LOXL1* with PXFG in a US clinic-based population with broad ethnic diversity.<sup>16</sup> Our studied population was predominantly Caucasian patients of European ancestry, but the sample also included 6% African-Americans. Intriguingly, unlike the highly significant association found in the previous study,<sup>5</sup> rs1048661 ( $p=0.0031$ ) was much less significant than rs3825942 ( $p=1.3 \times 10^{-13}$ ) in our study.<sup>16</sup> Haplotype analysis suggested that the effect of rs3825942 on PXFG was independent of rs1048661, whereas rs1048661 was no longer associated with PXFG after controlling for rs3825942.<sup>16</sup> Most importantly, the risk allele of rs1048661 associated with PXFG in Japanese studies (allele T)<sup>25-29</sup> is different from that in other populations (allele G),<sup>5,16-24</sup> which argues that SNP rs1048661 itself does not contribute to the disease. Taken together, it appears that rs3825942 is the only non-synonymous SNP in the *LOXL1* gene that independently contributes to PXFG, and the contribution of rs1048661 appears to be less significant. The apparent association of rs1048661 with PXFG in some populations is likely to be the result of linkage disequilibrium with rs3825942.

The risk allele frequency of rs3825942 is extremely high in patients with PXFG in most of the populations studied (92–99%) (see *Table 1*). However, the risk

**Table 1: Distribution of *LOXL1* Coding Variants in Patients with Pseudoexfoliation Glaucoma Among Different Populations**

Population	rs1048661 (R141L) Allele G				rs3825942 (G153D) Allele G		
	n (PXFG/Controls)	Frequency (PXFG/Controls)	p	OR (95%CI)	Frequency (PXFG/Controls)	p	OR (95%CI)
Icelandic <sup>5</sup>	75/14,474	0.827/0.651	1.8x10 <sup>-6</sup>	2.56 (1.74–3.77)	0.987/0.847	4.1x10 <sup>-9</sup>	13.23 (5.59–31.3)
Swedish <sup>5</sup>	199/198	0.834/0.682	2.7x10 <sup>-7</sup>	2.39 (1.72–3.34)	0.995/0.879	9.1x10 <sup>-14</sup>	27.28 (11.44–65.1)
Caucasian/ African-American <sup>16</sup>	146/88	0.840/0.719	0.0031	2.06 (1.29–3.30)	0.990/0.795	1.3x10 <sup>-13</sup>	24.77 (7.50–81.8)
Caucasian (Midwest US) <sup>17</sup>	72*/75	0.819/0.600	3.6x10 <sup>-5</sup>	3.03 (1.77–5.17)**	0.986/0.880	3x10 <sup>-4</sup>	9.68 (2.20–42.5)**
Caucasian (Durham, US) <sup>18</sup>	50/235	0.787/0.665	0.022	1.86 (1.10–3.15)	0.939/0.844	0.019	3.05 (1.20–7.76)
Caucasian (Utah, US) <sup>19</sup>	49/170	NA	NA	NA	1.00/0.85	4.5x10 <sup>-5</sup>	35.04 (2.14–573)**
Caucasian (American/European) <sup>20</sup>	133/333	0.852/0.703	2.5x10 <sup>-6</sup>	2.44 (1.67–3.56)**	0.985/0.798	5.6x10 <sup>-13</sup>	16.43 (6.01–44.9)**
Australian <sup>21</sup>	86*/2,087	0.78/0.66	8.5x10 <sup>-4</sup>	1.86 (1.27–2.76)	0.95/0.84	7.8x10 <sup>-5</sup>	3.81 (1.88–9.02)
German <sup>22</sup>	311/348	0.839/0.644	1.4x10 <sup>-15</sup>	2.89 (2.21–3.77)	0.953/0.857	4.8x10 <sup>-9</sup>	3.41 (2.22–5.24)
Italian <sup>22</sup>	133/70	0.815/0.693	0.0053	1.96 (1.22–3.15)	1.000/0.821	2x10 <sup>-12</sup>	117.7 (7.10–1,949)**
Austrian <sup>23</sup>	167/170	0.841/0.671	2.6x10 <sup>-7</sup>	2.69 (1.59–4.54)	0.994/0.817	5.8x10 <sup>-15</sup>	37.29 (6.35–218)
Indian <sup>24</sup>	52*/97	0.72/0.63	0.16	1.49 (0.89–2.51)	0.92/0.74	1x10 <sup>-4</sup>	4.17 (1.89–9.18)
Japanese <sup>25</sup>	27/189	0.00/0.46	1.1x10 <sup>-10</sup>	0.01 (0.001–0.18)**	1.000/0.857	0.003	18.31 (1.11–300)**
Japanese <sup>26</sup>	106/172	0.038/0.497	1.4x10 <sup>-34</sup>	0.04 (0.02–0.08)**	0.986/0.863	1.4x10 <sup>-7</sup>	11.02 (3.39–35.9)
Japanese <sup>27</sup>	95/190	0.005/0.474	6.8x10 <sup>-39</sup>	0.006 (0.001–0.042)**	0.995/0.850	5.8x10 <sup>-10</sup>	33.35 (4.58–243)**
Japanese <sup>28</sup>	89*/191	0.006/0.450	1.5x10 <sup>-34</sup>	0.007 (0.001–0.049)**	0.994/0.853	4.6x10 <sup>-9</sup>	30.40 (4.17–221)**
Japanese <sup>29</sup>	36/138	0.042/0.493	1.7x10 <sup>-12</sup>	0.045 (0.014–0.15)**	1.000/0.877	0.0052	20.63 (1.25–341)**

\* These numbers indicate pseudoexfoliation syndrome (PXFS) because pseudoexfoliation glaucoma (PXFG) was not analyzed independently in these studies.

\*\* The odds ratios (ORs) and 95% confidence intervals (CIs) are calculated according to the genotype data presented in these studies.

allele of rs3825942 is also prevalent in control samples, with a frequency of over 85% in most populations, indicating that additional genetic and/or environmental factors could be involved in the development of PXFG. In some populations, such as the Australian population, the frequency of the rs3825942 risk allele is much higher than the disease prevalence, suggesting a reduced penetrance in these populations.<sup>21</sup> This result further suggests that additional factors, both genetic and environmental, and potentially additive and protective, could influence the development of this complex disorder. It has been reported that homocysteine levels are elevated in the aqueous humor, tear fluid, and plasma of patients with PXFG.<sup>31–33</sup> These studies indicate that hyperhomocysteinemia may be associated with pseudoexfoliation and may contribute to the vascular impairment and the alteration of extracellular matrix observed in patients with PXFG. The genes that affect homocysteine levels may be secondary factors that could contribute to PXFG.

### Functional Studies of *LOXL1*

*LOXL1* is a member of the lysyl oxidase family of proteins that catalyze the polymerization of tropoelastin to form the mature elastin polymer.<sup>34</sup> Elastin fibers are a major component of many structures in the eye, including those that could be involved in PXFG, such as the extracellular matrix of the trabecular meshwork and the lamina cribrosa of the optic nerve.<sup>35,36</sup> *LOXL1* is also involved in elastin homeostasis and renewal, and participates in spatially organizing elastogenesis at sites of elastin deposition. Binding of *LOXL1* to the elastin scaffold is required for this function.<sup>37</sup> The LOX family has five members, including the LOX protein and the LOX-like proteins (*LOXL1* to *LOXL4*). All of these LOX family members have seven exons with a similar structure. Exons 2 to 6 show strong homology and encode the C-terminal catalytic domain of these proteins. Both rs1048661 (R141L) and rs3825942 (G153D) are located

in the N-terminal pro-peptide, which may have a role in directing the *LOXL1* protein to sites of elastogenesis, but is unlikely to affect the catalytic activity of the protein.<sup>37</sup> However, the biological effect of these missense alterations on the expression of *LOXL1* in ocular tissues has not been determined. The exact role of *LOXL1* in the eye is still unclear, and further investigations are ongoing. As the catalytic domains are located in the highly conserved C-terminal of the *LOXL1* protein, it is important to further investigate the association of this portion of *LOXL1* with PXFG.

### Conclusions and Future Prospects

In summary, a strong association of *LOXL1* with PXFG has been identified and replicated in several major populations. These studies indicate that *LOXL1* is a major gene associated with PXFG, accounting for 99% of PXFG cases in most populations. The difference in the risk allele of rs1048661 associated with PXFG between Japanese and other populations suggests that SNP rs3825942 is most likely to be responsible for disease development. However, the biological effect of this missense alteration (G153D) on the expression of *LOXL1* in ocular tissues remains to be determined, and the precise role of *LOXL1* in the eye is still under investigation. It is not yet known whether the G153D missense change creates a gain of function or loss of function of the protein, or whether this SNP is in linkage disequilibrium with other DNA sequence variants that affect the expression of the gene. The high prevalence of the rs3825942 risk allele in control populations and the apparently variable penetrance of the condition in some populations suggest that additional genetic factors and/or environmental exposures that may function as additive or protective factors could be involved in the development of this complex disease. Other proteins that are involved in the maintenance of elastin fibers and the

alteration of extracellular matrix, such as the genes that determine homocysteine levels, are good candidates for secondary genetic factors that could contribute to this common blinding disease. ■

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## National Human Gene Research Institute Provides \$20 Million for DNA Sequencing Technologies

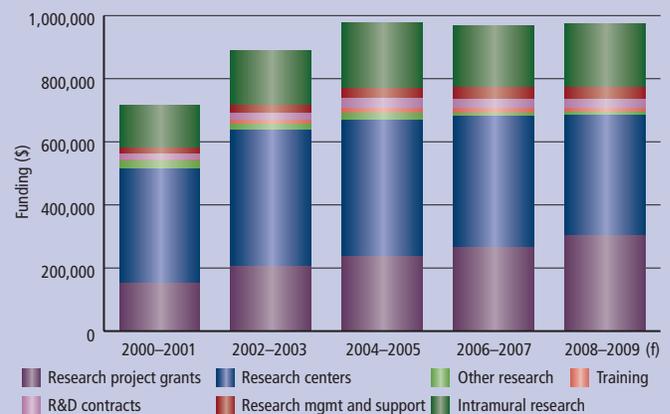
The National Institutes of Health (NIH) awarded over \$20 million in grants for innovative DNA sequencing technologies this summer, channelled through the National Human Gene Research Institute (NHGRI). Building on the foundations laid down by the Human Genome Project, the grants are intended to catalyze the development of sequencing technologies that could cut the cost of whole-genome sequencing to \$1,000, allowing the process to become a routine part of medical care.

“The ability to sequence any individual’s genome inexpensively and accurately is the quantum leap needed to usher in an age of personalized medicine in which healthcare providers will routinely use an individual’s genetic code to prevent, diagnose, and treat diseases,” said Alan E Guttmacher, MD, Acting Director of NHGRI. Nanopore technologies were particularly prominent among grantees. Daniel Branton, PhD, and Jene A Golovchenko, PhD, at Harvard University received \$6.5 million for a four-year project to optimize nanopore technology, culminating in a nanopore detector chip capable of sequencing a mammalian gene in a single day.

At the University of Pennsylvania, Marija Drndic, PhD, successfully obtained \$820,000 for a three-year investigation into the use of nanoelectrodes to sense and manipulate molecules passing through the nanopore. Jiali Li, PhD, and team at the University of Arkansas received \$830,000 for a three-year project to develop a nanopore sensing system that labels nucleotides to better differentiate the electrical signal difference among DNA bases.

Sources: National Human Gene Research Institute, National Institutes of Health.

### National Human Genome Research Institute Funding (2000–2009)



### ‘\$100,000 Genome’ Grants

NHGRI provided grants for research that could reduce the cost of human-sized genomes by 100-fold. Recipients include Steven A Benner, PhD, and his laboratory at the Foundation for Applied Molecular Evolution, which received \$1.1 million for his three-year project entitled ‘Near-Term Development of Reagents and Enzymes for Genome Sequencing.’ Jingyue Ju, PhD, at Columbia University, New York, successfully obtained \$950,000 for a two-year project entitled ‘DNA Sequencing with Reversible dNTP and Cleavable Fluorescent ddNTP Terminators.’ ■