Association of **LOXL1** Polymorphisms With Pseudoexfoliation, Glaucoma, Intraocular Pressure, and Systemic Diseases in a Greek Population. The Thessaloniki Eye Study

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**PURPOSE.** To investigate the association of the two single-nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 (**LOXL1**) gene with pseudoexfoliation syndrome (**PEX**), pseudoexfoliative glaucoma (**PEXG**), and primary open-angle glaucoma (**POAG**) in a Greek population-based setting, from the Thessaloniki Eye study.

**METHODS.** A total of 233 subjects with successful DNA extraction, PCR amplification, and genotyping were included in the genetic analysis of **G153D** and **R141L** SNPs of **LOXL1** gene and classified into four groups: controls (**n = 93**); subjects with **PEX** (**n = 40**); **POAG** (**n = 66**); and **PEXG** (**n = 34**). Multinomial logistic regression was used to test their association with **LOXL1** SNPs with adjustment for covariates. The association of **LOXL1** with IOP (in untreated subjects) and with systemic diseases was explored.

**RESULTS.** Both **LOXL1** SNPs were present in high frequencies in controls and cases. The **G153D** was strongly associated with both **PEX** (odds ratio [OR] = 23.2, **P = 0.003** for allele **G**) and **PEXG** (OR = 24.75, **P = 0.003** for allele **G**) and was not associated with **POAG** (**P = 0.451**). In contrast, the **R141L** was not associated with **PEX** (**P = 0.81**), **PEXG** (**P = 0.063**), or **POAG** (**P = 0.113**). No association of the **G153D** with either intraocular pressure (IOP) or systemic diseases was found.

**CONCLUSIONS.** In the Thessaloniki Eye Study, the **G153D** SNP of **LOXL1** gene was strongly associated with both **PEX** and **PEXG**, whereas the **R141L** was not associated. No association of the **LOXL1** with IOP or with systemic diseases was found. These findings further support the hypothesis that the **LOXL1** gene contributes to onset of **PEXG** through **PEX**. Gene variants of **LOXL1** do not help to identify those with **PEX** at increased risk for glaucoma development.

Keywords: **LOXL1**, pseudoexfoliation, pseudoexfoliative glaucoma

**Pseudoexfoliation syndrome (PEX)** is an age-related disorder of the extracellular matrix characterized by production and progressive accumulation of small, white deposits of a fibrillar material in various intraocular and extraocular tissues. This syndrome occurs worldwide; however, the reported prevalence estimates vary considerably.1

Pseudoexfoliation syndrome is the most common identifiable cause of secondary glaucoma, named pseudoexfoliative glaucoma (**PEXG**).2 Usually, **PEXG** presents with higher IOP than the primary open-angle glaucoma (**POAG**) and its prevalence is more IOP-dependent than **POAG**.3

Only a few large population-based studies have specifically investigated **PEX** and **PEXG** prevalence or incidence.4–6 The Thessaloniki Eye Study was designed to address the prevalence of the major eye diseases and disorders, including **PEX**, in the Greek population of Thessaloniki. The reported prevalence of **PEX** in subjects aged older than 60 years was 11.9%.4 Among those with pseudoexfoliation, only 15% presented with glaucoma.4 In the absence of glaucoma, subjects with **PEX** compared with those without **PEX**, presented with similar clinical characteristics except that they have higher intraocular pressure (IOP), only by 0.6 mm Hg.7 The reasons why some subjects develop **PEX** currently remain unclear. In addition, it remains unclear why a proportion of subjects with **PEX** develop glaucoma while the majority of those with **PEX** do not get glaucoma.

Further pseudoexfoliative material has been also identified by electron microscopy in visceral organs such as lung, heart,
liver, kidney, gallbladder, and meninges. This widespread distribution led to the hypothesis of possible association of PEX with systemic cardiovascular or cerebrovascular diseases. Although there is some evidence in favor of this hypothesis, other studies did not confirm this association and thus, this hypothesis remains controversial.17,18

The lysyl oxidase-like 1 (LOXL1) gene has been identified as a potential genetic risk factor for both PEX and PEXG since 2007, when a genome-wide association study on pseudoexfoliative individuals from Iceland and Sweden was performed.11 This study demonstrated a strong association (>99% population attributable risk) of PEX and PEXG conferred by three single-nucleotide polymorphisms (SNPs) in the LOXL1 gene on chromosome 15q24.1. Two SNPs were identified in the first coding exon (rs3825942 or G153D and rs1048661 or R141L) and one within the first intron of this gene (rs2165241). Although the rs2165241 was more significantly associated with PEXG than the two exonic SNPs, this association was not statistically significant after adjusting the results for the two other SNPs.11 Several studies of different ethnic populations have reported associations of LOXL1 with PEX and PEXG. Many of them did not differentiate between PEX and PEXG. Recently, two clinical-based studies in Greece examined the association of LOXL1 SNPs with PEX and PEXG and reported conflicting results.12,13

The discovery of common sequence variants in the LOXL1 gene that confer susceptibility to PEXG, primarily through PEX syndrome, has shed some light in the pathogenesis of the disease.14 Lysyl oxidase-like 1 is one of five enzymes in the family of lysyl oxidases, which are copper-dependent monooxygenases secreted by fibrogenic cells including fibroblasts and smooth muscle cells. These enzymes are involved in the covalent cross-linking of collagen and elastin polymers in extracellular matrix formation. Lysyl oxidase-like 1 is necessary for tropoelastin cross-linking and elastic fiber formation, maintenance, and remodeling.14

The purpose of the present study is to explore the association of two nonsynonymous LOXL1 SNPs (G153D and R141L) with PEX, PEXG, and primary open-angle glaucoma (POAG) in a population-based setting using a well-defined, ethnically homogenous population in Thessaloniki, Greece. In addition, in previous studies no information is provided on the potential association of LOXL1 with IOP or with systemic diseases. This study aims to identify whether the two LOXL1 SNPs were associated with differences in IOP or with the self-reported history of systemic diseases (diabetes mellitus, cardiovascular disease or cardiovascular surgery) in subjects with PEX, PEXG, POAG, and controls.

Methods

The Thessaloniki Eye Study is a cross-sectional, population-based study of chronic eye diseases in the Greek population of Thessaloniki. Thessaloniki is the major urban center in Northern Greece and the second largest city in Greece, after Athens. This urban area has a stable and homogenous population with 97.7% of the population identified as being of Greek ethnicity. The study was approved by the Aristotle University Medical School, Ethics Committee and the Institutional Review Board of the University of California, Los Angeles. All study procedures adhered to the principles outlined in the Declaration of Helsinki for research involving human subjects and all participants gave written informed consent prior to their participation. Details about the recruitment process in the Thessaloniki Eye Study have been previously described.4 In brief, 5000 subjects aged 60 years or older were randomly selected in February 1999 from approximately 321,000 persons registered in the municipality registers of the city of Thessaloniki. Subjects who agreed to participate were invited to the Thessaloniki Eye Study center (Laboratory of Research and Clinical Applications in Ophthalmology at the Aristotle University of Thessaloniki) for an extensive ophthalmologic examination. Clinic-visit examination included measurement of visual acuity using Early Treatment Diabetic Retinopathy Study (ETDRS) charts; Humphrey automated perimetry; Goldmann applanation tonometry (three readings from each eye); gonioscopy; slit-lamp examination (before and after pupil dilation); and fundus biomicroscopy. Details of observation procedures were described elsewhere.4

A home-visit eye examination was organized, proposed, and arranged for persons unable to visit the study examination center due to major disability or illness. Home-visit examination included ETDRS visual acuity measurement, Perkins applanation tonometry, slit-lamp examination, and dilated fundus examination.

Prior to clinical examination, a questionnaire was administered identifying demographic data, ophthalmologic, and medical history. In particular, subjects were queried whether they had ever been diagnosed with hypertension, diabetes mellitus (treated or not with insulin), any cardiovascular disease, heart attack, or they had undergone coronary artery bypass or vascular surgery.

Definitions

Particular attention was given to identify the presence of PEX, which was defined by the presence of pseudoexfoliative material either at the pupil margin or on the lens capsule. Prior to pupil dilation, a detailed high-magnification slit-lamp assessment of the pupil margin was performed. After pupil dilation, the anterior lens surface from each eye was scanned from left to right using a narrow slit-lamp beam and then was examined using a vertical broad slit-lamp beam, looking specifically for early signs of PEX, including pregranular radial lines, as well as established granular deposits. The location of PEX either on the iris, the lens, or both was recorded. The PEX detection and the exact location required consensus agreement between at least two of the three ophthalmologist graders. Dilation was conducted in all patients and even in those with narrow-occludable angle, laser peripheral iridotomy was performed and they were examined later under dilation.

Glaucoma was defined according to specific criteria.4 Subjects were classified as having POAG if they had glaucoma and open, normal-appearing anterior chamber angle and presence of pseudoexfoliation in either eye. Three ophthalmologists were responsible for the ophthalmic examination. At least two of the three examined each patient and all diagnoses were in consensus agreement.

Among the 3617 subjects who were deemed eligible, 2554 participated in the study (participation rate: 71%). Of those participating, 2261 (89%) had the clinic-visit examination and 293 (11%) had the home-visit examination.

A blood sample was taken from all subjects with POAG and PEXG and in a consecutive subset of participants without glaucoma. Written consent was obtained before the blood draw. A total of 235 subjects who gave blood samples were included in this analysis. Among them, 93 had neither PEX nor glaucoma in both eyes (controls), 40 had pseudoexfoliation and no glaucoma in either eye (PEX), 66 were diagnosed with POAG, and 34 with PEXG.
Peripheral blood samples were collected from each subject and DNA was extracted using a DNA extraction kit (QiAmp DNA Mini Kit; Qiagen, Venlo, Limburg, Netherlands). Samples of DNA were amplified by PCR as previously described using the sense: 5’ GCAGGTGTACAGCTTGCTCA 3’ and antisense 5’ ACACGAAACCCTGGTCGTAG 3’ primers.15 Polymerase chain reactions (PCRs) were performed in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Inc., Foster City, CA, USA). The PCR products were sequenced using the same primers as in PCR amplification in a genetic analyzer (ABI PRISM 310 Genetic Analyzer; Applied Biosystems, Inc.), using manufacturer’s protocol. Analysis of individual sequences was carried out using the AB DNA sequencing analysis software (version 5.2).

**Statistical Analysis**

All statistical analyses were performed with a statistical software package (Stata version 10.0; StataCorp LP, College Station, TX, USA). Fisher’s exact test was used for the genotypic comparison and subjects were grouped as follows: (1) control: subjects without PEX, PEXG, POAG; (2) case 1: subjects with POAG; (3) case 2: subjects with PEX; and (4) case 3: subjects with PEXG. Multinomial logistic regression analysis was conducted in order to examine the association between the two \textit{LOXL1} polymorphisms and POAG, PEX or PEXG, adjusted for the following factors: age, sex, history of diabetes, history of cardiovascular disease, history of cardiovascular surgery, and the higher IOP for each strength of the allele’s association with POAG, PEX, and PEXG compared with controls using either the log-additive model (G versus A allele, as identified in a previous report of Thessaloniki Eye study).16

**Table 1.** Descriptive Statistics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>POAG</th>
<th>PEX</th>
<th>PEXG</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (% sample)</td>
<td>92* (39.9)</td>
<td>65* (28.1)</td>
<td>40 (17.3)</td>
<td>54 (14.7)</td>
</tr>
<tr>
<td>Age, y (SD)</td>
<td>68.8 (4.7)</td>
<td>72.6 (6.3)</td>
<td>75.4 (5.6)</td>
<td>74.4 (5.1)</td>
</tr>
<tr>
<td>Male, %</td>
<td>41.3</td>
<td>47.7</td>
<td>50.0</td>
<td>47.1</td>
</tr>
<tr>
<td>Diabetic, %</td>
<td>17.4</td>
<td>27.7</td>
<td>15.0</td>
<td>11.8</td>
</tr>
<tr>
<td>Insulin use, %</td>
<td>3.3</td>
<td>10.8</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Cardiovascular disease, %</td>
<td>38.0</td>
<td>32.3</td>
<td>32.5</td>
<td>41.2</td>
</tr>
<tr>
<td>Cardiovascular surgery, %</td>
<td>9.8</td>
<td>13.8</td>
<td>7.5</td>
<td>8.8</td>
</tr>
<tr>
<td>Highest IOP between two eyes, n (SD)</td>
<td>15.4 (3.2)</td>
<td>18.4 (5.4)</td>
<td>15.6 (3.8)</td>
<td>18.7 (6.0)</td>
</tr>
</tbody>
</table>

For those individuals (stratified in controls, POAG, PEX, and PEXG groups) who have been genotyped for \textit{LOXL1} SNPs and also have age, sex, diabetes history, diabetes treated with insulin, cardiovascular disease, coronary surgery, and IOP data recorded (n = 251).

* One control subject and one subject with POAGG have missing data on covariates and thus were excluded from logistic regression analyses.

Logistic regression analysis adjusted for age was also conducted in order to examine the association of the two \textit{LOXL1} SNPs with IOP in controls and cases not receiving IOP-lowering treatment. The association of the \textit{LOXL1} SNPs with the history of the aforementioned systemic diseases was also examined with regression analysis adjusted for age and sex. Hardy-Weinberg equilibrium testing was performed using Mendel version 13.0. Linkage disequilibrium (LD) and haplotype analysis was also performed using Mendel version 13.0.17 Odds ratios with 95% confidence intervals (CIs) were calculated as measures of each strength of the allele’s association with POAG, PEX, and PEXG. The P values were not adjusted for multiple testing.

**RESULTS**

Successful DNA extraction, PCR amplification, and genotyping were carried out for all 233 enrolled subjects. The general characteristics of the participants (mean age, sex, IOP history of diabetes, insulin use, cardiovascular disease, or cardiovascular surgery) in the four groups are presented in Table 1. The allelic and genotypic distribution of the G153D (rs3825942) and R141L (rs1048661) polymorphisms in controls, POAG, PEX, and PEXG groups are presented in Table 2. All frequencies were in Hardy-Weinberg equilibrium using a heterozygote-homozygote test (P = 0.486 for R141L, P = 0.603 for G143D).18 With regard to the G153D polymorphism, there was a statistically significant difference in subjects with PEX and PEXG compared with controls using either the log-additive allelic model (G versus A allele, P < 0.0001 for PEX, P = 0.0003 for PEXG) or the recessive model (GG versus AG+AA, odds

**Table 2.** Distribution of Alleles and Genotypes of \textit{LOXL1} SNPs

For a detailed understanding of the statistical methods employed and their implications, refer to the original publication. The table herein provides a summary of the observed distributions, with emphasis on the comparison between different groups and the statistical significance of these differences.
Table 3. Association of R141L and G153D Polymorphisms With PEX, PEXG, and POAG

<table>
<thead>
<tr>
<th></th>
<th>Log Additive (allelic)</th>
<th>Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>PEX vs. controls</td>
<td>R141L</td>
<td>0.601</td>
</tr>
<tr>
<td></td>
<td>G153D</td>
<td>0.752</td>
</tr>
<tr>
<td>PEXG vs. controls</td>
<td>R141L</td>
<td>1.347</td>
</tr>
<tr>
<td></td>
<td>G153D</td>
<td>23.203</td>
</tr>
<tr>
<td>PEX vs. controls</td>
<td>R141L</td>
<td>2.268</td>
</tr>
<tr>
<td></td>
<td>G153D</td>
<td>24.784</td>
</tr>
</tbody>
</table>

After adjusting for age, sex, diabetes, cardiovascular disease, cardiovascular surgery, and IOP using multinomial linear regression.

Discussion

In our well-established cohort of subjects aged older than 60 years and living in the city of Thessaloniki (Northern Greece), after adjusting for known risk factors for glaucoma, the G allele of SNP G153D was associated with an odds ratio of approximately 25 with both PEX and PEXG, while the R141L SNP did not present any association. Susceptibility to POAG was not associated with either of the two SNPs. No association of the G153D LOXL1 SNP was found with either IOP differences or with self-reported systemic diseases. To the best of our knowledge, this is the first study on the association of PEX and PEXG with LOXL1 coming from a general population-based cohort (not clinical based) and performing adjustment of the results for other covariates. Also this is the first study reporting lack of association of LOXL1 with the IOP and with systemic diseases.

A meta-analysis was performed in 2010, which aimed to summarize the existing data and also to identify and analyze the consistency and discrepancies of individual studies or population subgroups by stratifying them into four different ethnic groups (Caucasian, Japanese, Chinese, and Indian). According to this meta-analysis, there was no statistical difference in any population group or the overall study for any of the three SNPs between PEX and PEXG, suggesting that the LOXL1 gene may contribute to PEX onset rather than to glaucoma development. The G allele of rs3825942 (G153D) was the common at risk allele for PEX/PEXG, which was consistent in all populations with a total OR of 10.89, and when stratified by ethnic group, the OR was 9.30 in Caucasians, 18.72 in Japanese, 10.97 in Chinese, and 4.17 in Indian populations. In contrast, the T allele of rs2165241 (intronic SNP) and the G allele of rs1048661 (R141L) were risk alleles only in Caucasians and 10.97 in Chinese, and 4.17 in Indian populations. Between any of the SNPs between PEX and PEXG, suggesting that the LOXL1 gene may contribute to PEX onset rather than to glaucoma development. The G allele of rs3825942 (G153D) was the common at risk allele for PEX/PEXG, which was consistent in all populations with a total OR of 10.89, and when stratified by ethnic group, the OR was 9.30 in Caucasians, 18.72 in Japanese, 10.97 in Chinese, and 4.17 in Indian populations. In contrast, the T allele of rs2165241 (intronic SNP) and the G allele of rs1048661 (R141L) were risk alleles only in Caucasians and 10.97 in Chinese, and 4.17 in Indian populations. Associations of opposite alleles at the same biallelic locus with the same disease has been described in genetic studies and it is called the flip-flop phenomenon. When the flip-flop phenomenon occurred across different ethnic groups, it could be explained by the population differences. The meta-analysis findings suggest that the rs3825942 is the common disease-associated polymorphisms across different populations and may have functional impacts on the LOXL1 protein. No association was found between any of the LOXL1 SNPs with POAG or other types of glaucoma (although the available data on the other types of glaucoma such as pigmentary or angle-closure glaucoma was limited). These results are in accordance with the results of our study, where no difference in the association of the two extronic SNPs between PEX and PEXG was reported. However, in our study only the G allele of rs3825942 was significantly associated with both PEX/PEXG (OR = 23.2 and 24.8, respectively). No association of any SNPs with POAG was found in our study as in the other studies. The lack of association between LOXL1 and POAG further supports the...
hypothesis that \textit{LOXL1} is linked to the pathogenesis of exfoliation but not directly to glaucoma development.

More recently in 2013, two studies on the \textit{LOXL1} association with PEX/PEXG in Greek populations have reported intriguing results.\textsuperscript{13,14} Both of them used clinical-based cohort of subjects with PEX, PEXG, and controls. According to the results of the first study from Athens, only G153D was associated with PEX and PEXG, and R141L was not associated with either PEX or PEXG.\textsuperscript{14} The results of this study were in line with the results of our study (coming from the urban area of Thessaloniki, Northern Greece). However, differences in the reported odds ratio can be noticed, since they reported for the allele G of G153D association an OR of 3.52 for PEX and an OR of 3.74 for PEXG, which is smaller than ours (approximately an OR of 23 for PEX and an OR of 25 for PEXG). Partially different were the results from the second study from the region of Epirus.\textsuperscript{13} This study reported an association of G153D SNP with an OR of 2.162 for PEX and an OR of 2.794 for PEXG (much lower than that in our study). According to the results of the adjusted analysis, both \textit{LOXL1} SNPs (G153D and R141L) were associated with PEX and PEXG, while in our study only G153D was associated. Differences among the three studies coming from rather homogenous Greek populations could be explained by the methodological differences in the studies (one population-based, the others clinical-based, the rigorousness of the classification used for cases and controls, the method of genotyping) but also from true differences in geographic distribution patterns (due to either regional gene pools or environmental influences).

In our study, the high-risk G allele of G153D SNP was quite frequent in all groups (approximately in 100% of both PEX and PEXG and 80% in controls and POAG subjects). Although the G allele of G153D confers a very high population attributable risk, its high frequency in controls makes a genetic test for \textit{LOXL1} of limited clinical use. This test will have a very high sensitivity but a very low specificity, meaning that almost all subjects who will develop PEX can be identified by genetic testing, the test will result in a very high number of false positives though. Furthermore, since the frequencies of \textit{LOXL1} SNPs are similar in PEX and PEXG, this genetic screening would not be able to identify those PEX individuals who are at increased risk of glaucoma. Despite the high prevalence of the \textit{LOXL1} variants in the general population, a much lower proportion of the population develops PEX and an even lower proportion of subjects with PEX develop glaucoma, suggesting that other, environmental, genetic or epigenetic factors, that are yet to be identified, may delay, inhibit, or contribute to the development of PEX and PEXG.

This is the first study that tested the hypothesis whether the two alleles of the high risk for PEX/PEXG G153D common sequence variant of \textit{LOXL1} may be associated with differences in IOP. Considering only untreated subjects and after adjusting for age, both the G and A alleles of G153D were not associated with differences in IOP measurements in both controls and POAG subjects. In this context, it is very plausible to assume that \textit{LOXL1} SNPs cannot attribute to the occurrence of high IOP in subjects with PEX and to subsequently development of glaucoma. On the other hand, in our previous report from the Thessaloniki Eye Study, subjects with PEX were three times more likely to have glaucoma than subjects without PEX, notably for the same level of presenting IOP when it was higher than 20 mm Hg.\textsuperscript{3} Based on this finding, we may hypothesize that, in addition to IOP, some other factors may contribute to glaucoma development in subjects with PEX. Whether these factors are genetic (in addition to \textit{LOXL1}), environmental, or epigenetic and how they contribute to the development of glaucoma in subjects with PEX remain to be identified.

The widespread systemic distribution of PEX material in visceral organs like lung, liver, kidney, gallbladder, and cerebral meninges led to the hypothesis that PEX may be associated with systemic comorbidities and comorbidity. Cardio- and cerebrovascular disease like angina, aortic aneurysm, and dementia have been linked to PEX in the first papers that addressed this issue.\textsuperscript{8,9,22} In contrast, there is compelling evidence from epidemiological population-based and case-controls studies that argue against this association.\textsuperscript{7,11,23–26} In the population-based cohort of Thessaloniki Eye Study, we were unable to confirm any association of PEX with diabetes, hypertension, cardiovascular or cerebrovascular disease, and coronary surgery.\textsuperscript{7} This is the first study that examined and didn’t find any association of the high risk for PEX/PEXG G153D SNP of \textit{LOXL1} with any of the aforementioned comorbidities, further supporting the hypothesis that PEX may not be associated with systemic cardiovascular or cerebrovascular diseases.

In the Thessaloniki Eye Study, as a population-based study, the participants were randomly selected from the general population, and therefore selection bias was diminished. Thessaloniki is considered to have a stable and ethnically homogenous population of Greek origin. Genetic homogeneity is of great importance when genetic studies are conducted. One other strength of the present study was the use of a standardized and thorough examination protocol for all the participants in the Thessaloniki Eye Study. Particular attention was given to identify the presence of PEX and the diagnosis of PEXG, and POAG was based on rigorous criteria. By these means, any classification bias was minimized. Age is a strong surrogate for the presence of PEX and one can argue that some of the controls may develop PEX in the future. Although this classification bias exists in all studies involving PEX subjects and controls, it seems unlikely to exist in our study due to the relatively high age of both controls (mean age: 68.8 years) and PEX subjects (mean age: 75.4 years). In addition, we were able to adjust all the results for other known confounders including age, sex, IOP, diabetes, coronary surgery, and cardiovascular disease by using multinomial logistic regression analysis. Most of genetic studies on \textit{LOXL1} have not adjusted their results for potential confounders. Furthermore, this is the first study that ran logistic regression analysis to explore the potential association of the allele G of the G153D \textit{LOXL1} variant with IOP differences in untreated subjects or with systemic diseases among the groups. We were able to perform this analysis given our population-based setting providing a representative random sample and a fair number of previously undiagnosed and untreated glaucoma subjects.

According to the results of our analysis, we confirmed the strong association of G allele of G153D with both PEX and PEXG, as has been widely described in the relevant literature. We did not find a significantly different association of the R141L common variant with PEX/PEXG. One may suggests that this may be due to the limited sample size in our study. However, our study (with 34 PEXG cases and 93 controls with a 0.758 frequency of G allele of R141L in controls) had 79.8% power to detect an association of 3.592 under the recessive model and 93.7% power to detect an association of 2.98 under the log additive model with the significance level of 0.05. Thus, although it is still possible, it is somewhat unlikely that our inability to detect the association is because our sample study is inadequate. In order to further increase the study sample and since we did not find different association between PEX and PEXG with \textit{LOXL1} SNPs, both PEX and PEXG cases were combined in one group (n = 74) and controls and POAG cases were combined in another (n = 159). When the two groups were compared, the results did not change significance although a trend was noticed for the R141L SNP (OR: 1.69, 1.68, 1.67, and 1.66 for the log additive, recessive, and dominant model, respectively).
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95% CI: 0.92–3.13, \( P = 0.0886 \) for the recessive model GG versus TT/TG). Another limitation of the analysis of association of LOXL1 with systemic diseases may be the use of the self-reported history, which may be subject to recall bias. However, there are studies that have shown that the self-reported history on selected chronic diseases is fairly accurate, especially for diabetes and cardiovascular disease.27–29

According to our study findings, G153D and R141L variants of LOXL1 gene were not heterogenic in PEX and PEXG. Only G153D was strongly associated with PEX/PEXG. No association was found of the high risk G153D allele with increased IOP or with systemic diseases. These findings further support the notion that the LOXL1 gene contributes to onset of PEX, but its role in PEXG development remains unclear. Under which genetic, environmental, or epigenetic conditions subjects with PEX progress to increased IOP and/or glaucoma development remains unanswered. The detection of yet unidentified genetic and nongenetic factors contributing to this complex disorder called pseudoexfoliation may lead to the better understanding of PEXG pathogenesis and toward the development of more precise screening tools for individuals at risk for pseudoexfoliative glaucoma.

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