Experimental Eye Research 168 (2018) 138–148

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Research article

GWAS links variants in neuronal development and actin remodeling related loci with pseudoexfoliation syndrome without glaucoma

Katarzyna Zagajewska[a,1], Magdalena Piątkowska[b,1], Krzysztof Goryca[b], Aneta Bałabas[b], Anna Kluska[b], Agnieszka Paziewska[c], Ewelina Pośpiech[d], Iwona Grabska-Liberek[a], Ewa E. Hennig[b,c,*]

[a] Department of Ophthalmology, Centre of Postgraduate Medical Education, Warsaw, Poland
[b] Department of Genetics, Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
[c] Department of Gastroenterology, Hepatology and Clinical Oncology, Centre of Postgraduate Medical Education, Warsaw, Poland
[d] Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

ARTICLE INFO

Keywords:
Pseudoexfoliation syndrome
Glaucoma
Genome-wide association study
Prediction modeling
Genotyping
Neuronal development

ABSTRACT

Pseudoexfoliation syndrome (PEXS) is an age-related elastosis, strongly associated with the development of secondary glaucoma. It is clearly suggested that PEXS has a genetic component, but this has not been extensively studied. Here, a genome-wide association study (GWAS) using a DNA-pooling approach was conducted to explore the potential association of genetic variants with PEXS in a Polish population, including 103 PEXS patients without glaucoma and 106 perfectly (age- and gender-) matched controls. Individual sample TaqMan genotyping was used to validate GWAS-selected single-nucleotide polymorphism (SNP) associations. Multivariate binary logistic regression analysis was applied to develop a prediction model for PEXS. In total, 15 SNPs representing independent PEXS susceptibility loci were selected for further validation in individual samples. For 14 of these variants, significant differences in the allele and genotype frequencies between cases and controls were identified, of which 12 remained significant after Benjamini–Hochberg adjustment. The minor allele of five SNPs was associated with an increased risk of PEXS development, while for nine SNPs, it showed a protective effect. Beyond the known *LOXL1* variant rs2165241, nine other SNPs were located within gene regions, including in *OR11L1*, *CD80*, *TNIK*, *CADM2*, *SORBS2*, *RNF180*, *FGF14*, *FMN1*, and *RBFOX1* genes. None of these associations with PEXS has previously been reported. Selected SNPs were found to explain nearly 69% of the total risk of PEXS development. The overall risk prediction accuracy for PEXS, expressed by the area under the ROC curve (AUC) value, increased by 0.218, from 0.672 for *LOXL1* rs2165241 alone to 0.89 when seven additional SNPs were included in the proposed 8-SNP prediction model. In conclusion, several new susceptibility loci for PEXS without glaucoma suggested that neuronal development and actin remodeling are potentially involved in either PEXS onset or inhibition or delay of its conversion to glaucoma.

1. Introduction

Pseudoexfoliation syndrome (PEXS) is an age-related disorder in which fibrillogranular aggregates (PEX material) are progressively deposited at abnormally high concentrations in the extracellular matrix (ECM) (Ovodenko et al., 2007). PEX material was primarily recognized in the intraocular tissues, where it is commonly visible in the form of concentric circles at the pupillary margin and anterior lens capsule, but may also be observed at the iridocorneal angle, zonules, ciliary processes, and other parts of the eye (Ritch and Schlötzer-Schrehardt, 2001). PEXS is now considered to be a systemic disease, in which the eye is merely one of the affected organs (Streeten et al., 1992). PEX

Abbreviations: AUC, area under the ROC curve; CI, confidence interval; ECM, extracellular matrix; GWAS, genome-wide association study; IOP, intraocular pressure; LD, linkage disequilibrium; MA, minor allele; NPV, negative prediction value; OAG, open-angle glaucoma; OCT, optical coherence tomography; OlfR, olfactory receptor; OR, odds ratio; PEXG, pseudoexfoliation glaucoma; PEXS, pseudoexfoliation syndrome; PPV, positive prediction value; PCA, principal component analysis; RAF, relative allele frequency; RAS, relative allele signal; SNP, single nucleotide polymorphism

* Corresponding author. Cancer Center-Institute, Roentgena 5, 02-781 Warsaw, Poland.
E-mail addresses: katarzyna.zagajewska@gmail.com (K. Zagajewska), mpiatkowska@coi.waw.pl (M. Piątkowska), krzysztof.goryca@coi.waw.pl (K. Goryca), abalabas@coi.waw.pl (A. Bałabas), akluska@coi.waw.pl (A. Kluska), agapaziewska@poczta.onet.pl (A. Paziewska), ewelina.pospiech@uj.edu.pl (E. Pośpiech), iliberek@gmail.com (I. Grabska-Liberek), hennige@coi.waw.pl (E.E. Hennig).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.exer.2017.12.006
Received 7 June 2017; Received in revised form 5 December 2017; Accepted 20 December 2017
Available online 24 December 2017

material may also be produced in the skin, lungs, liver, and other visceral organs and its presence was shown to be associated with various diseases outside the eyes, such as coronary artery disease, hearing loss, and Alzheimer's disease.

PEXS is the most common identifiable cause of secondary open-angle glaucoma (OAG), accounting for about 25% of all OAG cases worldwide (Ritch and Schlötzer-Schrehardt, 2001). The estimated 15-year risk of PEXS conversion to pseudoexfoliation glaucoma (PEXG) is up to 60% (Benitez-del-Castillo Sanchez et al., 2015; Jeng et al., 2007). Compared with primary OAG, PEXG is characterized by rapid progression of glaucomatous optic nerve damage, usually more advanced visual field loss at diagnosis, and overall worse prognosis, as well as poorer medical therapy outcomes, both conservative and surgical (Ritch, 2001). The deposition of PEX material in the trabecular meshwork causes resistance to the outflow of aqueous humor, which correlates with higher levels of intraocular pressure (IOP) and increased severity of optic neuropathy (Gottanka et al., 1997; Schlötzer-Schrehardt and Naumann, 1995), partly explaining the more aggressive clinical course of PEXG.

A higher risk of mature and nuclear cataract and serious intra- and postoperative complications of cataract surgery is another important clinical consequence of PEXS (Conway et al., 2004; Ritch, 2001). Early cataract surgery is especially advisable in PEXS patients, since apart from the improvement in visual acuity, the lens is removed, which is one of the possible sources of PEX material (Merkur et al., 2001). In addition, the risk of therapy complications is lower in early stages of PEXS, when no severe zonulopathy or nuclear sclerosis has yet developed. Unfortunately, the current diagnosis of PEXS is based on a slit lamp examination, by which the typical deposits are detectable only at advanced stages of the disease, while complications of the syndrome and irreversible damage to some part of the optic nerve may already have occurred at the preclinical stage.

PEXS is a late-onset disorder affecting about 10–20% of the general population over the age of 60 (Forsius et al., 2002; Ringvold, 1999). It is more common in women than in men (Jonasson, 2009). Although PEXS occurs worldwide, the incidence of the syndrome varies widely among different ethnic groups, which is suggested mainly to be related to the variation in their genetic backgrounds (Challa, 2009; Schlötzer-Schrehardt, 2011). The etiology of PEXS remains poorly understood, but the higher risk of the disease in certain ethnic groups, along with strong familial disease aggregation, clearly indicates that genetic factors have a strong influence on its development. Nonetheless, a simple inheritance pattern of the disease is not evident, implying that an interplay of several genes and/or environmental factors lies behind this condition (Damji et al., 1998).

The lysyl oxidase-like 1 (*LOXL1*) gene is the most important genetic risk factor for both PEXS and PEXG identified to date; there is a strong biochemical rationale for its involvement in the development of these diseases. The protein that it encodes is an extracellular copper-dependent enzyme that catalyzes the oxidative deamination of lysine residues of tropoelastin, the first step in the formation of elastic fibers, which are considered a major constituent of PEX material (Thomassin et al., 2005; Wagenseil and Mecham, 2007). Impaired tissue expression or function of LOXL1 contributes to the abnormal elastic fiber production (Lee, 2008). In addition, LOXL1 itself was found to be present in PEX material and to colocalize with elastic fiber components such as elastin, fibrillin-1, and fibulin-4, the expression of which in anterior eye tissues is regulated in accordance with the expression of *LOXL1* (Zenkel and Schlötzer-Schrehardt, 2014).

The strong association between PEXS/PEXG and *LOXL1* gene variants was first discovered by a genome-wide association study (GWAS) in Swedish and Icelandic individuals (Thorleifsson et al., 2007). This involved three single-nucleotide polymorphisms (SNPs): rs2165241, located within the first intron, and two nonsynonymous SNPs, rs1048661 (R141L) and rs3825942 (G153D), in the first exon. It remains unclear whether these two coding variants can efficiently affect *LOXL1* expression (Schlötzer-Schrehardt et al., 2012; Wiggs and Pasquale, 2014), but some recent findings suggest they can be critical for catalytic activation of the enzyme and substrate binding (Sharma et al., 2016; Thomassin et al., 2005). A link between PEXS and the *LOXL1* locus was further confirmed in many other populations worldwide, but the reported results are somewhat controversial and inconclusive (Álvarez et al., 2015; Aung et al., 2015; Challa, 2009; Jonasson, 2009; Malukiewicz et al., 2011). Surprisingly, the particular allele of some SNPs associated with increased disease risk was frequently reversed depending on the ethnic group studied (Founti et al., 2015). Additionally, the risk-associated *LOXL1* variants are usually present at a high frequency in the general population, showing that most people who carry a high-risk allele do not develop PEXS. Recently, a protective rare variant rs201011613 (Y407F) of *LOXL1* was identified by deep sequencing of the entire gene (Aung et al., 2017). This variant was predicted to affect LOXL1 function, however, it was found exclusively in the Japanese population. These findings strongly suggest that there are additional genes, environmental factors, or epigenetic events that modify the susceptibility to PEXS.

Some evidence for the presence of genetic risk factors other than *LOXL1* variants was provided by a genome-wide scan carried out on a large Finnish family, indicating an association of PEXS with loci at 18q12.1–21.33 (Lemmelä et al., 2007). Two more recent GWASs identified additional PEXS susceptibility loci at the contactin-associated protein-like 2 (*CNTNAP2*) gene (Krumbiegel et al., 2011) and the voltage-gated Ca^{2+} channel subunit α1A (*CACNA1A*) gene (Aung et al., 2015). In addition, several candidate genes, such as those encoding clusterin (CLU), apolipoprotein E (APOE), tumor necrosis factor (TNF)-α, matrix metalloproteinases (MMPs), and latent transforming growth factor (TGF)-β binding protein 2 (LTBP2), were suggested to be linked to PEXS/PEXG; however, besides the *LOXL1* locus, to date only polymorphisms in *CLU* and *CNTNAP2* have been associated with the disease in different populations (Aboobakar and Allingham, 2014; Krumbiegel et al., 2009).

In the DNA-pooling GWAS described here, we particularly focused on the appropriate establishment of patient and control groups, limiting the possibility of misclassification. Moreover, to increase the homogeneity of the case cohort, only patients with PEXS without glaucoma were included. Our methodological approach enabled the detection of several new variants associated with PEXS, in addition to *LOXL1*. Based on them, a prediction model for PEXS was proposed. The identified associations suggest that processes such as neuronal development, synapse organization, and synaptic transmission, as well as actin cytoskeleton remodeling, contribute to PEXS.

2. Materials and methods

2.1. Ethics statement

All enrolled patients and control subjects were Polish Caucasians recruited at the Department of Ophthalmology, Centre of Postgraduate Medical Education in Warsaw, Poland, between mid-2012 and mid-2014. The local ethics committee approved the study (No. 48/PW/2011) and all participants provided written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Patients

The study included 209 individuals (149 females and 60 males): 103 with PEXS and 106 perfectly age- and sex-matched healthy controls. Demographic characteristics of the patients and controls are shown in Table 1. All subjects underwent a detailed ophthalmologic examination, including ocular and medical history, distance and near visual acuity (Snellen charts), slit lamp biomicroscopy, Goldmann applanation tonometry, gonioscopy, and dilated fundoscopy. Additionally, automated

Table 1
Demographic characteristics of patients and controls.

	N	Mean Age ± SD	Median Age	Min. – Max.
PEXS	103	77.4 ± 8.1	78	54–96
Female	73	77.3 ± 8.3	78	56–96
Male	30	77.7 ± 7.9	78.5	54–88
Control	106	77.9 ± 7.7	78	53–94
Female	76	77.8 ± 7.7	78	57–94
Male	30	78.1 ± 7.9	79.5	53–88

PEXS; pseudoexfoliation syndrome, *N;* number of subjects, SD; standard deviation.

perimetry was performed with the Humphrey Field Analyzer (Carl Zeiss Meditec AG, Germany) using the SITA Standard 30-2 test. Measurement of retinal nerve fiber layer thickness was performed by spectral domain optical coherence tomography (OCT) using 3D OCT-1000 (Topcon Medical Systems, USA). All assessments were performed by a single ophthalmologist (KZ). Patients with any evidence of glaucomatous optic neuropathy in either eye were excluded from both the control and the PEXS groups. All patients had IOP < 21 mmHg in both eyes. PEXS was proven by the presence of typical PEX material at the papillary margin and/or on the anterior lens capsule in at least one eye, during slit lamp examination after pupil dilation. The presence of corneal or posterior segment pathologies was an exclusion criterion.

2.3. Genome-wide microarray allelotyping

A pooled-DNA sample-based GWAS was performed as described previously (Gaj et al., 2012). Genomic DNA was extracted from whole blood treated with EDTA using a QIAamp DNA Mini Kit (Qiagen, Germany), quantified using a Quant-iT™ PicoGreen dsDNA Kit (Invitrogen, UK), and checked on 1% agarose gel for integrity. DNA samples that passed quality control tests were combined according to diagnosis and gender at equimolar concentrations to obtain 10-DNA sample pools. A total of 10 DNA pools were prepared for each of the two groups (control and PEXS), seven for women and three for men. Pooled-DNA samples were adjusted to a final concentration of 50 ng/μl in Tris-EDTA buffer (pH = 8) and analyzed individually on Illumina Human Omni2.5-Exome BeadChips by a commercial organization (AROS Appl. Biotech., Aarhus N, Denmark). GWAS datasets from this study are available at the GEO database under GSE93205.

2.4. Individual genotyping

For the validation of GWAS findings, the loci represented by blocks of SNPs associated with disease at the $p < 5 \times 10^{-3}$, for which the interval between all pairs of adjacent SNPs was less than 30 kb, were taken into consideration. One index SNP from each of the identified loci, associating at $p < 10^{-4}$, was selected for further validation with individual PEXS and control samples, using the TaqMan SNP Genotyping Assays (Thermo Fisher, USA), SensiMix™ II Probe Kit (Bioline Ltd., UK), and a 7900HT Real-Time PCR system (Thermo Fisher, USA) in a 384-well format.

2.5. Statistical analyses

2.5.1. Clinical data

For the comparison of clinical data between patients with PEXS and control subjects, the Mann–Whitney–Wilcoxon test was used. The *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg algorithm (Benjamini and Hochberg, 1995) and adjusted *p*-values of less than 0.05 were considered significant.

2.5.2. Genome-wide allelotyping

The intensity of each SNP was calculated as the relative allele signal (RAS) for each microarray, as described previously (Gaj et al., 2012). The RAS was used as an approximation of the allele ratio. Student's *t*-test (Welch variant) was used to compare allele ratios between groups. Owing to a lack of the call-rate statistic for pooled samples, quality was assessed by visual inspection of the first two principal components. Principal component analysis (PCA) revealed no outliers (Fig. S1). Population heterogeneity was further assessed with quantile-guantile (Q-Q) plot (Fig. S2). To calculate lambda, *p*-values from Student's *t*-test were converted to chi-square values. Lambda value was of 1.06 and together with Q-Q plot raised no concerns regarding population homogeneity. Probes with missing signal reads were removed. Calculations were performed using the statistical software package R version 3.1.1 (R: The R Project for Statistical Computing).

2.5.3. Individual genotyping

Based on microarray analysis, SNPs potentially associated with PEXS were selected for validation using the TaqMan SNP Genotyping Assay and individual DNA samples. The Hardy–Weinberg equilibrium and associations were examined using the chi-square test implemented in the R package. The odds ratio (OR) and 95% confidence interval (CI) were estimated by normal approximation implemented in the EpiTools R package (CRAN - Package epitools). The Benjamini–Hochberg algorithm was used to adjust the *p*-value significance threshold for multiple comparisons (Benjamini and Hochberg, 1995). The study power calculations were performed with the "pwr" package (CRAN - Package pwr), assuming equal groups of 100 samples and baseline allele frequencies of 0.05, 0.1, 0.2, and 0.5 (Table S1). Calculations were performed for a significance threshold equal to 0.05.

2.5.4. Prediction modeling

Prediction analysis was performed using the binary logistic regression method and PS IMAGO 4 software (IBM SPSS Statistics 24). The prediction model was constructed using the full set of samples minus those with missing data, giving a final number of 193. To evaluate the performance of the model, a 10-fold cross-validation procedure was applied, as described previously (Hastie et al., 2009; Pośpiech et al., 2015). For this purpose, the entire set of samples was split randomly into 10 equinumerous parts indexed by k ($k = 1, 2, \ldots, 10$). For each k, the kth part was excluded and the model was developed using samples from the other $k-1$ parts. Then, the prediction parameters for every k were calculated on the excluded kth part of the samples. The final prediction parameters were estimated based on 10 models built in the cross-validation procedure (Hastie et al., 2009).

At first, multivariate binary logistic regression enabling simultaneous testing of all of the variables was conducted to adjust the association results for all other predictors and finally to select SNPs with independent effects. The selected SNPs were then ranked according to their *p*-values and sequentially introduced into the model to test their impact on prediction accuracy. The probability of disease development for every tested sample was assigned and then used to calculate prediction parameters, including the area under the curve (AUC), sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) (Pośpiech et al., 2012). DNA variants that increased AUC value by at least 0.01 were included in the final prediction model. The remaining parameters describing accuracy of prediction were then calculated using two probability threshold levels: 50% and 70%. A probability threshold of 50% means that PEXS was predicted if the prediction probability was ≥50%. If the probability was calculated to be < 50% then a no-PEXS stage was predicted. A probability threshold of 70% means that PEXS was predicted only if the prediction probability was ≥70%. Prediction results that did not reach the 70% probability threshold were considered to be inconclusive (30%–70%) or no-PEXS (< 30%) (Pośpiech et al., 2015; Walsh et al., 2011). The proportion of overall risk of PEXS development explained by the tested variants was estimated using the Nagelkerke pseudo-R^2 statistic.

3. Results

In search of SNPs associated with PEXS in a Polish population, a cost-effective GWAS using a pooled-DNA sample was conducted, using 10-sample pools of DNA obtained from 100 patients with PEXS without glaucoma (70 females and 30 males; median age 78; mean age 77.0 ± 7.7) and 100 perfectly sex- and age-matched control subjects (70 females and 30 males; median age 78; mean age 77.2 ± 7.2). There were no statistically significant differences between the two groups regarding visual acuity, IOP, iridocorneal angle width, cup-disk ratio, quantitative perimetry parameters, and quantitative OCT parameters for the optic nerve head (Table S2).

3.1. Genotyping

The susceptibility loci for PEXS revealed by pooled-DNA GWAS were selected for further validation in individual samples. Our previous observations (Ostrowski et al., 2016; Paziewska et al., 2017) and other reports (Krumbiegel et al., 2011) indicated that, in the case of relatively small patient cohorts, when the statistical power to detect SNPs at standard genome-wide significance level ($p < 5 \times 10^{-8}$) is insufficient, a focus on the loci forming blocks of associated SNPs that remained in a close distance (less than 30 kb between adjacent SNPs) is a successful approach for SNP selection, even though the association occurs at lower than standard significance level. Based on such an approach, 15 independent loci were identified and, from each of them, one index SNP, associated with PEXS at $p < 10^{-4}$, was selected for further validation. Among the selected loci, eight were represented by blocks of at least 10 SNPs associating at $p < 5 \times 10^{-3}$. The remaining loci were represented by fewer than 10 SNPs in a block, but at least two of them associated at $p < 10^{-4}$.

All selected SNPs were subsequently examined by TaqMan genotyping using individual DNA samples from both PEXS ($N = 103$) and control ($N = 106$) groups (Table 1). As shown in Table 2, 14 of the analyzed SNPs exhibited differences ($p < .05$) in allele frequency between the PEXS and control groups and 12 of them remained significant after adjustment for multiple testing ($p < 3.33 \times 10^{-3}$). In addition, the genotype frequencies of all but two of these SNPs (rs7085835 and rs72769818) were significantly associated with PEXS (Table 2). The strongest association was observed for a previously identified (Thorleifsson et al., 2007) SNP, rs2165241 in *LOXL1* (*p*-values of 2.77×10^{-10} and 1.04×10^{-10} for allele and genotype frequencies, respectively). The other associations have not previously been reported. Among them, the finding with the strongest significance was for rs1852211 in *CD80* ($p = 6.95 \times 10^{-5}$). The next seven SNPs revealed associations at $p \leq 9.15 \times 10^{-4}$.

The minor allele (MA) of five SNPs was associated with an increased risk of PEXS, while that of the remaining nine SNPs showed a protective effect (Table 2). The effect size of all identified susceptibility loci was relatively substantial (OR ≥ 2 or ≤ 0.5), which can be explained by the limited statistical power of our GWAS due to the rather small number of patients in the cohort. Considering the most favorable SNP population frequency of 0.5, our GWAS reached a power ranging from 65% to 86% to detect an effect size of OR from 2.0 to 2.5, respectively (Table S1). For more typical MA frequency of 0.3 and OR equal 1.4 study power was 18%, making it significantly underpowered for such detection. The strongest effect was observed for the SNP rs10888255, a nonsynonymous variant in exon 1 of the *OR11L1* gene (OR = 0.21, 95% CI 0.08–0.051, $p = 3.97 \times 10^{-4}$). Nine other SNPs were also located within gene regions: one in an exon (rs16855789 in *TNIK*), one in a 3′-untranslated region (rs12442768 in *FMN1*), and seven in introns (Table 2). The remaining four SNPs were at intergenic locations (4q26, 5q34, 10p13, and 13q21).

3.2. Prediction modeling

To evaluate the diagnostic power of the selected SNPs for PEXS, a prediction model was developed and parameters describing the accuracy of prediction were calculated, in accordance with a previously described approach (Pośpiech et al., 2015, 2012; Walsh et al., 2011). Multivariate binary logistic regression analysis enabled the selection of 10 significant SNPs ($p < .05$) after controlling for the effects of all of the tested DNA variants. The selected SNPs were found to explain nearly 69% of the total risk of PEXS development, as assessed using the Nagelkerke pseudo-R^2 statistic (Table 3). Among the remaining five SNPs, the associations of three variants were also insignificant after correcting for multiple testing, in the allele and/or genotype frequency analyses (Table 2).

SNPs significant in the multivariate logistic regression analysis were ranked according to their *p*-value and sequentially introduced into the prediction model. The genetic variants that improved the overall accuracy of prediction by increasing the AUC parameter by at least 0.01 were chosen for the final model, which consisted of eight SNPs: rs2165241 (*LOXL1*), rs13117637 (*SORBS2*), rs2058527 (*RBFOX1*), rs1537443 (13q21), rs72769818 (*RNF180*), rs1852211 (*CD80*), rs12442768 (*FMN1*), and rs1375559 (*CADM2*) (Table 3). As expected, the SNP rs2165241 in *LOXL1* showed the strongest independent effect on the risk of PEXS development (OR = 0.03, 95% CI 0.01–0.11, $p = 1.87 \times 10^{-8}$), although the nonrisk allele C was the MA in our study (46% of controls). A strong protective effect was also exhibited by four other variants: rs2058527-T (*RBFOX1*), rs1537443-T (13q21), rs72769818-A (*RNF180*), and rs1852211-A (*CD80*). According to the significance of the effect size, rs13117637-A (*SORBS2*) had the strongest influence, after the *LOXL1* variant, on the predictive accuracy of PEXS (AUC change equal to 0.074). The direction of the effects of all variants was in agreement with those observed in the allelic association analysis.

Next, the accuracy prediction parameters calculated for the 8-SNP model were compared with those obtained for the model consisting of only the well-known PEXS risk variant rs2165241 (*LOXL1*) (Table 4). The overall accuracy expressed by the AUC value increased from 0.672 for rs2165241 alone to 0.890 when the 8-SNP prediction model was applied. Similarly, sensitivity of PEXS prediction increased from 68.7% to 78.8% and specificity increased slightly from 79.8% to 80.9%, when comparing the 1-SNP and 8-SNP models, respectively. When a 70% probability threshold was applied to the 8-SNP prediction model, the prediction accuracy of the model improved, increasing the sensitivity of PEXS prediction to 88% and the specificity to 81.8% (Table 4). However, inconclusive results were obtained at the level of 21.2%, which means that, in 41 of the 193 surveyed individuals, the result of the predictive analysis could not be resolved.

4. Discussion

Considering that PEXG has a more rapid and severe clinical course, as well as more frequent need for surgery, compared with other forms of OAG (Ritch, 2001), an early diagnosis of PEXS with subsequent enhanced surveillance would be beneficial for patients suffering from this disease. An early diagnosis of PEXS is also crucial for reducing the number of complications related to different ophthalmic surgical interventions, including cataract surgery, which occur more frequently in patients with this syndrome (Conway et al., 2004; Merkur et al., 2001). The knowledge of genetic variants associated with PEXS susceptibility could help in diagnosing this disease in its early stages. However, the genetic background of PEXS is still poorly understood. To explore new associations of genetic variants across the entire genome among Polish patients with PEXS, we conducted pooling-DNA-based GWAS with subsequent genotyping of selected SNPs in individual DNA samples.

Besides the obvious great economical advantage of pooling-DNA approach (allelotyping), it has also several limitations. First of all, it

Table 2
The validation analysis of GWAS-selected SNPs allelic and genotypic association with PEXS.

dbSNP ID[a]	Region[b]	Allele frequency (%)				OR (95% CI)	*p*-value	Genotype frequency (%)			OR (95% CI)	*p*-value
		MA	MAF[c]	Control	PEXS			Genotype	Control	PEXS		
rs10888255	1q44	C	0.130	27 (12.7)	6 (2.9)	0.21 (0.08-0.51)	**3.97E-04**	CC	1 (0.9)	0	–	**8.10E-04**
	OR11L1							CG	25 (23.6)	6 (5.8)	0.20 (0.08-0.51)	
	exon 1 (R171P)							GG	80 (75.5)	97 (94.2)	5.25 (2.06-13.39)	
rs1852211	3q13.33	A	0.161	47 (22.2)	16 (7.8)	0.30 (0,16–0.54)	**6.95E-05**	AA	7 (6.6)	2 (1.9)	0.28 (0.06-1.38)	**3.44E-04**
	CD80							AG	33 (31.1)	12 (11.7)	0.29 (0.14-0.60)	
	intron 2							GG	66 (62.3)	89 (86.4)	3.85 (1.94-7.66)	
rs16855789	3q26.2	A	0.900	12 (5.7)	29 (14.4)	2.79 (1.38-5.64)	5.17E-03	AA	0	2 (2.0)	–	1.21E-02
	TNIK							AG	12 (11.3)	25 (24.8)	2.58 (1.21-5.46)	
	exon 23 (synon.)							GG	94 (88.7)	74 (73.3)	0.35 (0.17-0.74)	
rs1375559	3p12.1	T	0.293	56 (26.7)	87 (42.6)	2.04 (1.35-3.09)	**9.15E-04**	TT	9 (8.6)	17 (16.7)	2.13 (0.90-5.04)	**2.03E-03**
	CADM2							CT	38 (36.2)	53 (52.0)	1.91 (1.09–3.33)	
	intron 2							CC	58 (55.2)	32 (31.4)	0.37 (0.21–0.65)	
rs13117637	4q35.1	A	0.521	85 (30.1)	118 (57.8)	2.05 (1.39-3.03)	**4.28E-04**	AA	19 (17.9)	33 (32.4)	2.19 (1.15-4.18)	**1.34E-03**
	SORBS2							AG	47 (44.3)	52 (51.0)	1.31 (0.76-2.25)	
	intron 30							GG	40 (37.7)	17 (16.7)	0.33 (0.17-0.63)	
rs1352495	4q26	C	0.429	104 (49.1)	68 (33.0)	0.51 (0.34-0.76)	**1.22E-03**	CC	24 (22.6)	10 (9.7)	0.37 (0.17-0.81)	**3.31E-03**
	intergenic							CT	56 (52.8)	48 (46.6)	0.78 (0.45-1.34)	
								TT	26 (24.5)	45 (43.7)	2.39 (1.32-4.30)	
rs7730762	5q34	A	0.058	15 (7.1)	41 (19.9)	3.26 (1.74-6.11)	**2.11E-04**	AA	0	3 (2.9)	–	**4.78E-04**
	intergenic							AG	15 (14.2)	35 (34.0)	3.12 (1.58-6.17)	
								GG	91 (85.8)	65 (63.1)	0.28 (0.14-0.55)	
rs72769818	5q12.3	A	0.090	29 (13.9)	10 (4.9)	0.32 (0.15-0.67)	**3.02E-03**	AA	0	0	–	NaN
	RNF180							AG	29 (27.9)	10 (9.8)	0.28 (0.13-0.61)	
	intron 1							GG	75 (72.1)	92 (90.2)	3.56 (1.63-7.77)	
rs3798793	6q23.2	T	0.634	96 (45.3)	111 (53.9)	1.41 (0.96-2.07)	9.68E-02	TT	21 (19.8)	26 (25.2)	1.37 (0.71-2.62)	1.25E-01
	VNN1							CT	54 (50.9)	59 (57.3)	1.29 (0.75–2.23)	
	intron 2							CC	31 (29.2)	18 (17.5)	0.51 (0.27–0.99)	
rs7085835	10p13	T	0.123	25 (11.8)	7 (3.4)	0.26 (0.11-0.62)	**2.34E-03**	TT	1 (0.9)	0	–	5.01E-03
	intergenic							CT	23 (21.7)	7 (6.8)	0.26 (0.11-0.64)	
								CC	82 (77.4)	96 (93.2)	4.01 (1.65-9.79)	
rs1537443	13q21.1	T	0.101	28 (14.1)	7 (3.5)	0.22 (0.09-0.51)	**3.17E-04**	TT	1 (1.0)	0	–	**6.00E-04**
	intergenic							CT	26 (26.3)	7 (6.9)	0.21 (0.09-0.51)	
								CC	72 (72.7)	94 (93.1)	5.04 (2.08-12.22)	
rs9518579	13q33.1	C	0.410	106 (50.0)	65 (32.2)	0.47 (0.32-0.71)	**3.42E-04**	CC	28 (26.4)	11 (10.9)	0.34 (0.16-0.73)	**1.80E-03**
	FGF14							CT	50 (47.2)	43 (42.6)	0.83 (0.48-1.44)	
	intron 4							TT	28 (26.4)	47 (46.5)	2.42 (1.35-4.34)	
rs2165241	15q24.1	C	0.713	98 (46.2)	35 (17.0)	0.24 (0.15-0.37)	**2.77E-10**	CC	16 (15.1)	1 (1.0)	0.06 (0.01-0.42)	**1.04E-10**
	LOXL1							CT	66 (62.3)	33 (32.0)	0.29 (0.16-0.51)	
	intron 1							TT	24 (22.6)	69 (67.0)	6.93 (3.76-12.8)	
rs12442768	15q13.3	C	0.102	33 (15.9)	56 (27.5)	2.01 (1.24-3.25)	6.19E-03	CC	1 (1.0)	4 (3.9)	4.20 (0.46-38.27)	9.06E-03
	FMN1							AC	31 (29.8)	48 (47.1)	2.09 (1.18-3.71)	
	UTR-3							AA	72 (69.2)	50 (49.0)	0.43 (0.24-0.76)	
rs2058527	16p13.3	T	0.243	62 (29.2)	31 (15.0)	0.43 (0.26-0.69)	**7.48E-04**	TT	11 (10.4)	1 (1.0)	0.08 (0.01-0.67)	**1.86E-03**
	RBFOX1							GT	40 (37.7)	29 (28.2)	0.65 (0.36-1.16)	
	intron 3							GG	55 (51.9)	73 (70.9)	2.26 (1.27-3.99)	

Allelic frequencies of all studied SNPs were in Hardy-Weinberg equilibrium. Bold denotes significant association after multiple testing adjustment (15 SNPs; $p < 3.33E-03$). MA; minor allele, MAF; MA frequency, PEXS; pseudoexfoliation syndrome, OR; odds ratio, CI; confidence interval.

[a] /SNP identifier based on NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp/).

[b] /NCBI ID of genes localized in proximity to the SNPs of interest (http://hapmap.ncbi.nlm.nih.gov/).

[c] /MAF based on NCBI SNP database (https://www.ncbi.nlm.nih.gov/snp/).

allows only for estimation of the mean allele frequencies of the pooled-DNA samples, what introduces limit on the relative allele frequency (RAF) differences that can be detected. Although, allelotyping was shown to reliably detect SNPs with large or moderate genetic effects (Gaj et al., 2012; Pearson et al., 2007; Schrauwen et al., 2009), it needs to be considered that associations with the lowest RAF differences may be masked due to the pooling-specific error. The pooling-specific error of the RAF is in particular high for the alleles with low MA frequency, but it is lower when RAF values for two groups of pools are compared, as in the case-control studies (Macgregor et al., 2008; Teumer et al., 2013). The next limitation of allolotyping is that adjusting for population stratification is practically impossible after DNA pooling, thus for such an approach little or no stratification should exist or pools should be appropriately designed in advance based on the knowledge of possible covariates. The more covariates will be taken into account when designing separate pools, the less data will be lost compared to individual genotyping.

One possible reason for discrepancies among reported results of association studies is misclassification of case or control individuals, which can reduce the power of a study as well as bias the results toward no association (Pearson and Manolio, 2008). Especially for smaller cohorts, precise diagnostic and exclusion criteria must be employed to minimize misclassification error and obtain the most homogeneous groups possible for both cases and controls. To meet this requirement in our GWAS, we have made a huge effort to rigorously select patients with pure PEXS, without any symptoms of glaucoma, what substantially increased the homogeneity of the case cohort. In addition, the PEXS and control groups were exactly age- and gender-matched (Table 1). Moreover, relatively old individuals (mean age of nearly 78 years) were enrolled in this study, which is considered to be an advantage in cases of late-onset disease, reducing the likelihood of the eventual development of PEXS among control individuals and PEXG among PEXS patients.

However, we were fully aware that the sample size of 200 subjects is

Table 3
The results of multivariate binary logistic regression analysis.

dbSNP ID[a]	OR (95% CI)	*p*-value	R^2 [b]	AUC[c]	AUC change
rs2165241	0.03 (0.01-0.11)	1.87×10^{-8}	0.302	0.672	-
rs13117637	6.74 (2.29-19.86)	.001	0.394	0.746	**0.074**
rs2058527	0.22 (0.08-0.59)	.002	0.448	0.782	**0.036**
rs1537443	0.13 (0.04-0.49)	.003	0.494	0.814	**0.032**
rs72769818	0.15 (0.04-0.52)	.003	0.543	0.831	**0.017**
rs1852211	0.19 (0.06-0.60)	.005	0.585	0.854	**0.023**
rs12442768	3.79 (1.46-9.85)	.006	0.620	0.873	**0.019**
rs1375559	3.55 (1.40-9.00)	.008	0.644	0.890	**0.017**
rs7730762	3.90 (1.30-11.71)	.015	0.667	0.892	0.002
rs1352495	0.28 (0.10-0.79)	.016	0.689	0.898	0.006

Ten significant SNPs are presented ($p < .05$), ranked according to their significance *p*-value, starting from the most significant association of *LOXL1* gene variant. SNPs providing area under the ROC curve (AUC) parameter change by ≥ 0.01 were marked with bold and included in the final 8-SNPs prediction model. Odds ratios (ORs) were calculated for the minor alleles coded in a dominant way (homozygotes of minor allele were combined with heterozygotes). CI; confidence interval.

[a] SNP identifier based on NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/).

[b] Nagelkerke pseudo-R^2 value calculated after sequential implementation of the ranked SNPs.

[c] AUC value calculated after sequential implementation of the ranked SNPs.

Table 4
Risk prediction based on 1-SNP (*LOXL1*) or 8-SNPs models.

Prediction parameters	Probability threshold	rs2165241-prediction model	8-SNPs prediction model
AUC	-	0.672 [SE 0.041]	0.890 [SE 0.022]
	50%		
Sensitivity		68.7% [68/99]	78.8% [78/99]
Specificity		79.8% [75/94]	80.9% [76/94]
PPV		78.2% [68/87]	81.3% [78/96]
NPV		70.8% [75/106]	78.4% [76/97]
	70%		
Sensitivity		73.9% [68/92]	88.0% [66/75]
Specificity		68.9% [42/61]	81.8% [63/77]
PPV		78.2% [68/87]	82.5% [66/80]
NPV		63.6% [42/66]	87.5% [63/72]
Inconclusive		20.7% [40/193]	21.2% [41/193]

AUC; area under the ROC curve, SE; standard error, PPV; positive predictive value, NPV; negative predictive value.

rather small, considering GWAS, hardly allowing to achieve associations at the generally accepted level of genome-wide significance ($p < 5 \times 10^{-8}$). Therefore, we have adopted the modified method of selecting SNPs for validation, based more on biological criteria than purely statistical. Assuming that each associating SNP is usually not independent of neighborhood, we focused on loci represented by blocks of SNPs associated with a disease at $p < 5 \times 10^{-3}$, remaining in a close distance of less than 30 kb between adjacent SNPs. Then an index SNP, associating at the highest level of significance, was selected for further validation as representative of each of these loci. Such an approach was successfully introduced by us in the recently published studies (Ostrowski et al., 2016; Paziewska et al., 2017), indicating that selection of index SNPs according to allele linkage disequilibrium (LD) reduces the number of false-positive genome-wide associations and allows for discovery of new associations.

Altogether, the applied approach allowed us to detect several loci that showed marked differences in allelic frequencies between patients with PEXS and controls, including the *LOXL1* locus, which proved the validity of the obtained data. None of the remaining significant SNPs has previously been reported to be associated with PEXS.

4.1. LOXL1 association

In our microarray analysis, the *LOXL1* locus was represented by a cluster of 20 SNPs associating with PEXS at $p < 5 \times 10^{-3}$, with intervals of less than 30 kb between any two adjacent SNPs. An association of two SNPs (rs2165241 and rs4886778) with PEXS was at the level of $p < 2.93 \times 10^{-7}$. The transcriptionally relevant SNP rs16958477 within the promoter region of *LOXL1* (Fan et al., 2011), although included in the selected cluster, showed a weaker association ($p = 4 \times 10^{-3}$). In addition, known PEXS susceptibility SNPs in coding regions, rs3825942, rs1048661, and rs41435250 (Guadarrama-Vallejo et al., 2013; Thorleifsson et al., 2007), as well as the rare nonsynonymous protective variant rs201011613, which showed significant association in single-variant analysis in just published studies (Aung et al., 2017), are not included in the Illumina microarray used in the current study. For further validation in individual samples, rs2165241 was chosen as the index SNP of the *LOXL1* locus. The allelic frequency of this variant demonstrated the strongest association with the risk of PEXS ($p = 2.77 \times 10^{-10}$) among all of the 12 SNPs that remained significant after correcting for multiple hypothesis testing (Table 2). Similarly to a previous replication study on *LOXL1* variants among 36 PEXS/PEXG Polish patients (Malukiewicz et al., 2011), the risk allele T of this SNP was more common than allele C, not only among PEXS patients (83% and 87.5%, respectively) but also in the control group (54% and 65%, respectively). The effect of allele T (OR = 4.2, 95% CI 2.67–6.61, $p = 2.77 \times 10^{-10}$) in our study was consistent with that identified in an early GWAS (OR = 3.18, 95% CI 2.12–4.76, $p = 1.9 \times 10^{-8}$) (Thorleifsson et al., 2007) or by sequencing the entire *LOXL1* gene and subsequent association analysis in Spanish individuals (OR = 4.74, 95% CI 2.72–8.28, $p = 6.3 \times 10^{-16}$), in which rs2165241 exhibited the strongest disease association among 49 SNPs showing significant differences in allelic frequencies between cases and controls (Álvarez et al., 2015). In addition, in two recent meta-analyses, rs2165241 showed a stronger association with PEXS/PEXG than both rs3825942 and rs1048661 SNPs within coding regions (Founti et al., 2015; Tang et al., 2014). It was suggested, however, that the only reason for this strong association is that allele T of rs2165241 effectively tagged the high-risk GG haplotype of the coding sequence variants (Thorleifsson et al., 2007).

Ethnicity-based differences were observed in the association of different *LOXL1* variants with PEXS/PEXG. A meta-analysis including 39 independent studies showed that rs2165241-C was a protective allele for the disease in Caucasians (13 studies; OR = 0.31), in accordance with our findings, but was a risk allele in Japanese (3 studies; OR = 7.49) and Koreans (2 studies; OR = 6.63) (Founti et al., 2015). In addition, for the SNPs rs1048661 (Founti et al., 2015) and rs4886776 (Aung et al., 2015), the direction of the association in the non-Japanese was opposite to that seen in the Japanese, suggesting that other population-based genetic factors may modify the effect of *LOXL1* variants. In addition, the possibility of *LOXL1* intragenic epistatic effects was proposed, like the one between newly identified synonymous (A310A) exon 1 risk variant rs41435250 and rs2165241 in Mexicans (Guadarrama-Vallejo et al., 2013); however, this has yet to be confirmed.

4.2. Possible functional associations with PEXS

Apart from rs2165241 in *LOXL1*, nine additional identified SNPs were within gene regions (Table 2). Although it is rather unlikely that the associated polymorphisms are directly causative, the functions of the proteins encoded by these genes suggest that they may be relevant to PEXS development. The strongest new association ($p = 6.95 \times 10^{-5}$) reported in the current study was observed for rs1852211, which is located within an intron in *CD80*. The B7 genes (*CD80* and *CD86*) were suggested to play a protective role against the development of optic neuropathy in patients with Graves' disease and a two-locus model was proposed to predict the risk of optic nerve damage (Liao et al., 2011). Both CD80 and CD86 are membrane receptors on antigen-presenting cells, activated by the binding of CD28 or cytotoxic

T-lymphocyte-associated (CTLA)-4 protein. Polymorphism within the genes belonging to the CD80/CD86-CD28/CTLA-4 pathway may modify the risk of multiple sclerosis and the interaction between certain variants of these genes was found to influence the age at disease onset (Wagner et al., 2015).

The strongest effect on PEXS susceptibility was identified for the SNP rs10888255, a nonsynonymous variant (R171P) in the first exon of an olfactory receptor (*OR11L1*) gene, of which the MA (allele C) frequency was significantly lower (OR = 0.21, 95% CI 0.08–0.51) in PEXS patients than in controls. Olfactory receptors (OlfRs) represent the largest class of G-protein-coupled receptors, which share a seven-transmembrane domain structure with many neurotransmitter and hormone receptors. They are typically considered to be detectors and transducers of signals from odorant molecules in the nose to the brain. However, OlfRs are also localized in many other organs outside the nasal cavity, playing different tissue-specific functions distinct from the perception of odors (Ferrer et al., 2016). Ectopic expression of OlfRs was recognized in neurons of the central nervous system and its alteration was identified in several neurodegenerative and mental disorders. Recently, a number of species of OlfRs were found to be expressed in murine corneal epithelium, suggesting that they play a role in sensing the ocular environment and maintaining homeostasis of the eye (Pronin et al., 2014). Specifically, it was speculated that Olfr558, which has a close human homolog, OR51E1, is involved in regulation of the arteriole diameter and blood flow in the choroid, affecting the amount of oxygen and nourishment provided to the outer layers of the retina.

The strict regulation of actin cytoskeleton remodeling is critical for the proper formation of neurites during brain development. This is controlled by multiple signaling pathways, acting as the cascades of signal transduction through a variety of regulatory proteins. Formin 1, the protein encoded by the *FMN1* gene, is an important regulator of actin fibers and microtubule dynamics during cell division, cell migration, and development and is involved in the formation of adherens junctions (Dettenhofer et al., 2008). Through the neurogenin 3 signaling pathway, it mediates the induction of dendritogenesis and synaptogenesis in mouse hippocampal neurons and can modulate actin-based synaptic structures (Simon-Areces et al., 2011). It also participates in the control of neuronal morphology and differentiation, as well as neuronal and axonal mobility. Formin 1 is involved in glutamatergic synaptic transmission, being connected mainly with excitatory post-synaptic potential. Its dysfunction has been implicated in early-onset obsessive-compulsive disorder (Cappi et al., 2014). The other actin cytoskeleton-related protein is ArgBP2, a small adaptor protein that binds c-Arg and c-Abl non-receptor-type tyrosine kinases, encoded by the sorbin and SH3 domain-containing 2 (*SORBS2*) gene. *SORBS2* is widely expressed in human tissues at actin stress fibers and the nucleus (Kioka et al., 2002). ArgBP2 is involved in the assembly of signaling complexes that play a role in the formation and stabilization of focal contacts. Among its important binding partners is vinculin, an actin-binding cytoskeletal protein associated with cell–ECM and cell–cell junctions (Kioka et al., 2002), and Fat1 protein, an atypical cadherin expressed at intercellular junctions, lamellipodia, and filopodia, the inactivation of which leads to defective neuronal development (Braun et al., 2016). Neural ArgBP2 splicing variant, exclusively expressed in the brain, is colocalized with F-actin in neural dendritic cones and spines and plays an important role in neuronal dendrite development and excitatory synaptic transmission (Zhang et al., 2016).

The signaling pathways that are involved in the control of neuritogenesis are themselves subject to control, mostly by the ubiquitination and proteasomal degradation of their key regulatory elements. Especially important in this context is inhibition of the formation of new neurites once the final neural network has been established in the mature brain. TRAF2 and NCK-interacting kinase (TNIK) is a serine/threonine kinase that modulates actin dynamics. Overexpression of wild-type *TNIK* results in the disruption of F-actin structure and cell spreading (Taira et al., 2004). TNIK is a specific effector of the small GTPase RAP2, known as a negative regulator of dendritogenesis (Kawabe et al., 2010). Interaction of TNIK with RAP2 is essential for the binding of NEDD4-1 ubiquitin E3 ligase to a signaling complex, which regulates neural dendrite extension and arborization. Ubiquitination by NEDD4-1 inhibits RAP2 function, leading to the reduction of TNIK activity and promotion of dendrite growth. TNIK is highly expressed in the brain and TNIK mRNA was shown to be upregulated in the dorsolateral prefrontal cortex of patients with schizophrenia (Glatt et al., 2005). In turn, RNF180 is a RING finger membrane-bound E3 ubiquitin ligase, tail-anchored to the endoplasmic reticulum. It is expressed in several adult tissues and in immature brain and lens (Ogawa et al., 2008). RNF180 interacts with the transcription factor ZIC2 and together with the E2 ubiquitin-conjugating enzyme UBE2E1 leads to ZIC2 ubiquitination and proteasomal degradation. Mouse Zic2 was found to be expressed in retinal ganglion cells with an uncrossed trajectory, at the time when these cells grew from the ventrotemporal retina toward the optic chiasm, and was necessary to regulate axon repulsion by cues at the optic chiasm midline (Herrera et al., 2003).

Several further associations identified in the current study are in some way related to nervous system development and function, essentially with synapse organization and synaptic transmission. Alterations in a gene encoding an RNA-binding protein, fox-1 homolog (*RBFOX1*), were reported in a range of neurobehavioral phenotypes including epilepsy, autism, and mental retardation (Bill et al., 2013; Zhou et al., 2014). By interaction with ataxin-2, the encoded protein may contribute to spinocerebellar ataxia type 2. RBFOX1 is considered a key regulator of the expression of large gene networks during neuronal development and maturation, acting on their alternative splicing or transcription programs. It plays a role in the homeostatic control of neuronal excitation upon exposure to secreted neurotransmitters (Gehman et al., 2011). It is hypothesized that the regulatory activity of RBFOX1 may be stimulated as an adaptive response to environmental or cellular stressors; thus, variation in this gene could modify disease risk under certain conditions (Bill et al., 2013). Besides the brain, heart, and muscle tissues, *RBFOX1* is expressed in the retina (Bitel et al., 2011) and its SNP variants have been implicated in refractive error (Stambolian et al., 2013) and myopia (Kiefer et al., 2013). Cell adhesion molecule 2 (CADM2) is involved in synapse organization and maintaining synaptic circuitry of the central nervous system (Frei et al., 2014). *CADM2* is almost exclusively expressed in the brain and retina (Postel et al., 2013). An intronic SNP, rs17518584, was found to significantly influence *CADM2* expression levels in the brain, especially in the frontal and anterior cingulated cortex (Ibrahim-Verbaas et al., 2016). The CADM2 protein is involved in glutamate neurosignaling pathways, gamma-aminobutyric acid (GABA) transport, and neuron cell–cell adhesion. It is positively coexpressed with many members of the voltage-gated K^+ channel group. GWASs showed an association of *CADM2* with general cognitive functions and educational attainment (Davies et al., 2016; Ibrahim-Verbaas et al., 2016).

Genetic variations in the fibroblast growth factor 14 (*FGF14*) gene have been associated with familial spinocerebellar ataxia 27 as well as schizophrenia and depression (Xiao et al., 2013). It is expressed throughout the nervous system, but the protein that it encodes is preferentially localized in the axon initial segment, which is critical for the initiation of action potentials (Xiao et al., 2013). FGF14 directly interacts with the voltage-gated Na^+ channels and mutation that disrupts this interaction results in reduced expression of the Na^+ channel α subunit at the axon initial segment and impaired neuronal excitability (Laezza et al., 2007). Furthermore, FGF14 is a potent regulator of the presynaptic P/Q-type voltage-gated Ca^{2+} channel ($Ca_v2.1$) and synaptic transmission in cerebellar neurons (Yan et al., 2013). The neuronal ion channels help to control the release of neurotransmitters and are essential for communication between neurons in the brain. It is considered that $Ca_v2.1$ channels are also involved in the survival of neuronal cells and their plasticity.

The biological activities of both *CACNA1A* and *CNTNAP2* genes, common variants of which were shown by previously conducted GWASs to be associated with PEXS/PEXG susceptibility (Aung et al., 2015; Krumbiegel et al., 2011), fit well with the processes and signaling pathways in which the genes identified in the current study are involved. For example, mutations in the *CACNA1A* gene, encoding the α1A subunit of $Ca_v2.1$ in neurons, have been implicated in spinocerebellar ataxia type 6, episodic ataxia type 2, hemiplegic migraine, and epilepsy (Tantsis et al., 2016). The G allele of the SNP rs4926244, associated with PEXS/PEXG at OR ≤ 1.29 in both GWAS and validation stages, was modestly correlated with lower CACNA1A mRNA levels in peripheral blood cells (Aung et al., 2015). It was hypothesized that altered expression and function of Ca^{2+} channels could influence the risk of PEX material aggregation by altering the concentration of Ca^{2+} ions. In the current study, however, none of the common *CACNA1A* variants showed evidence for an association with PEXS. The most likely explanation for this discrepancy is that the size of the patient cohort in our GWAS made it statistically underpowered to detect associations at an OR lower than 2, which was the case for the OR of the *CACNA1A* variant.

The *CNTNAP2* gene has also been implicated in neuropsychiatric disorders, including epilepsy, schizophrenia, autism, and mental retardation (Lu et al., 2016). CNTNAP2 is a large multi-domain transmembrane cell adhesion molecule at axo-glial and synaptic contacts. Specifically, it is localized at the juxtaparanodes of myelinated axons. As part of an extensive protein network, it mediates the trafficking and clustering of Na^+ and K^+ channels for efficient action potential propagation (Faivre-Sarrailh and Devaux, 2013). It is essential for maintaining normal synaptic transmission, neural migration, and synapse development (Lu et al., 2016; Peñagarikano et al., 2011). *CNTNAP2* was found to be expressed in all human ocular tissues, particularly in the retina (Krumbiegel et al., 2011). It is speculated that the interaction of CNTNAP2 with cytoskeletal proteins is crucial for membrane stabilization and that alterations in membrane stabilization or ion channel function may contribute to abnormal PEX aggregate formation. Although both rs2107856 and rs2141388, the SNPs in *CNTNAP2* most significantly associated with PEXS/PEXG (Krumbiegel et al., 2011), are not included in the Illumina array, a block of 13 SNPs located in this gene, with an interval of less than 30 kb between adjacent SNPs, and associating with PEXS at $p \leq 5 \times 10^{-3}$ was identified in the current study. However, none of these SNPs showed an association with PEXS at $p \leq 10^{-4}$ in order to be selected as an index SNP of this locus for further validation. The most significant association was observed for rs6961110 ($p = 9.3 \times 10^{-4}$). Consistent with this, in a previous replication study on 48 PEXS patients from a Polish population, no significant differences in allele frequencies were found for both rs2107856 and rs2141388 in *CNTNAP2*, compared to controls (Malukiewicz et al., 2012).

From five lately identified new susceptibility variants (Aung et al., 2017), one (rs3130283) is not represented in microarray data from Illumina Human Omni2.5-Exome BeadChips, which we have been using. The remaining four SNPs were not significantly associated with PEXS in our study. The only *p*-value < .1 was for SNP rs10072088 (*SEMA6A*; $p = .077$). In close vicinity (± 50 kb) to this SNP, there were 22 variants with $p < .1$ (the lowest $p = .013$). The strongest genetic effect observed for these five newly identified variants is OR = 1.24. Thus, not surprisingly none of them shows up significant, considering the statistical power of our study.

The evidence on the identified PEXS susceptibility genes presented above suggests that nervous system development and maturation, neuronal migration, synapse organization, and synaptic transmission, as well as actin cytoskeleton remodeling and interaction between ECM and cells, are possible processes that contribute to PEXS. In general, this agrees with “protein sink model”, which assumes that weakening of the blood-aqueous barrier and the leakage of serum proteins, inflammatory mediators, and ECM components into aqueous humor is a key element of the pathogenic process that eventually leads to aggregation and precipitation of PEX material (Vazquez and Lee, 2014). In addition, changes in ECM proteins and structural composition of the eye elastic tissues can lead to increased IOP and mechanical damage to the optic nerve.

Similarly, complex interaction between ECM, neuronal development, and visual signals from the retina was suggested to underlie the development of myopia, based on GWAS-identified associations involving the *RBFOX1* and *ZIC2* genes, among others (Kiefer et al., 2013). In turn, *TNIK*-included gene regulatory networks related to cell migration, cytoskeleton reorganization, development of forebrain structures, and axonal connectivity were found to be involved in schizophrenia (Potkin et al., 2010). On the other hand, increased production or reduced degradation of ECM, inflammation, and oxidative stress were suggested to contribute to the pathogenesis of PEXS, based on the presence of ECM proteins and remodeling enzymes, complement proteins, cell adhesion molecules, and stress response proteins in PEX material, as well as on the altered expression of the genes encoding such proteins in the affected ocular anterior segment tissues (Ovodenko et al., 2007; Schlötzer-Schrehardt and Naumann, 2006; Zenkel and Schlötzer-Schrehardt, 2014).

4.3. Prediction modeling

Multivariate regression analysis enabled us to select 10 SNPs that have significant independent effects on PEXS, from the set of all 15 tested DNA variants. Associations of three excluded SNPs (rs3798793, rs16855789, rs7085835) were also weak in the allele or genotype frequency analysis. The effects of the next two SNPs, rs10888255 in *OR11L1* and rs9518579 in *FGF14*, although strong in the allelic test, were probably correlated with other included variants. Since none of the remaining SNPs is located in the same LD block, an impact of intergenic epistatic interactions or other epigenetic events is a possible explanation of this inconsistency, but further studies are needed to verify any such hypotheses. Among the selected SNPs, rs2165241 in *LOXL1* alone was found to explain 30% of the overall risk of developing PEXS. An additional nine significantly associated SNPs explained another 39%, giving a total of 69% (Table 3).

It is beyond doubt that *LOXL1* can be considered a major genetic risk factor for PEXS, conferring 80–99% population attributable risk in various cohorts (Challa, 2009; Thorleifsson et al., 2007). However, the high prevalence of *LOXL1* risk variants among control individuals and the much lower proportion of the population developing a PEXS/PEXG phenotype indicates that a genetic test for *LOXL1* may be of limited clinical use. Consistent with this, the estimated ability of the G allele of two known SNPs in the *LOXL1* coding region, rs3825942 and rs1048661, to predict affection status showed 100% and 95.7% sensitivity and 3.1% and 13% specificity, respectively (Challa, 2009). Similarly, rs3825942 indicated 100% sensitivity and 13.3% specificity in a Polish population (Malukiewicz et al., 2011). In the same study, the T allele of an intronic SNP rs2165241 indicated 87.5% sensitivity and 35% specificity in a diagnostic test for PEXS. In contrast, the C allele of this variant in the 1-SNP risk prediction model developed in the current study showed 69% sensitivity and 80% specificity. However, the overall accuracy of prediction was rather low (AUC = 0.672). The inclusion of seven additional SNPs, apart from rs2165241, in the final 8-SNP prediction model increased the AUC value to 0.89. Assuming a 70% probability threshold, which is more reasonable for diagnostic purposes, the sensitivity of prediction increased by 14%, to 88%, and specificity increased by 13%, to 82%; however 21% of the surveyed individuals remained unclassified. Because of the limited number of samples, 10-fold cross-validation was used for estimating the model accuracy. However, the developed prediction model needs to be further validated on a separate, unrelated testing set of samples.

In accordance with the estimated statistical power of our GWAS, all identified PEXS susceptibility variants conferred risk with an OR ≥ 2 or

≤0.5. In this context, the following question arises: Why were SNP associations with rather substantial effects not discovered in the previously conducted GWASs on PEXS? The main difference among these studies concerns the case cohorts. In the majority of studies carried out to date, the case cohorts consisted of combined patients with either PEXS or PEXG. To the best of our knowledge, the current study is the first conducted on such a large group of over 100 patients with clear PEXS, without any symptoms of glaucoma. Given that most of the included subjects were elderly, it cannot be ruled out that PEXS patients in whom glaucoma has never developed or will appear at a later stage are overrepresented in this study. Therefore, at least some of the identified variants may be related to the prevention of the glaucomatous process rather than to PEXS itself. Notably, for most of the susceptibility variants, the MA exhibited a strong protective effect against PEXS, which matches the assumption that other common variants can modify the risk of PEXS development associated with *LOXL1* risk variants (Jonasson, 2009).

4.4. Conclusion

Several new common genetic variants associated with PEXS without glaucoma in a Polish population were revealed by a GWAS of relatively low statistical power, suggesting that there are still other unidentified associations with a lower effect size. For most of the discovered susceptibility SNPs, the MA was associated with a reduced risk of PEXS development. Apart from the known *LOXL1* variant rs2165241, nine other SNPs located in gene regions were identified. The molecular functions of the proteins encoded by these genes suggested that nervous system development and maturation, neuronal migration, synapse organization, and synaptic transmission, as well as actin cytoskeleton remodeling and interaction between ECM and cells, may contribute to the development of PEXS or to inhibition or delay of its conversion to glaucoma. The overall accuracy of the prediction model for PEXS, consisting of eight discovered susceptibility variants, is 0.89. Several lines of evidence suggest that this classifier may allow patients with PEXS but with a reduced risk of developing glaucoma to be distinguished. However, there is a need for future studies that replicate these analyses in independent study groups.

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

This work was supported by grant No. 506-1-15-03-16 from the Centre of Postgraduate Medical Education of Poland (http://www.cmkp.edu.pl/). EP is supported by the Foundation for Polish Science (FNP) programme START 2017.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.exer.2017.12.006

References

Aboobakar, I.F., Allingham, R.R., 2014. Genetics of exfoliation syndrome and glaucoma. Int. Ophthalmol. Clin. 54, 43–56. http://dx.doi.org/10.1097/IIO.0000000000000042.

Álvarez, L., García, M., González-Iglesias, H., Escribano, J., Rodríguez-Calvo, P.P., Fernández-Vega, L., Coca-Prados, M., 2015. LOXL1 gene variants and their association with pseudoexfoliation glaucoma (XFG) in Spanish patients. BMC Med. Genet. 16, 72. http://dx.doi.org/10.1186/s12881-015-0221-y.

Aung, T., Ozaki, M., Lee, M.C., Schlötzer-Schrehardt, U., Thorleifsson, G., Mizoguchi, T., Igo, R.P., Haripriya, A., Williams, S.E., Astakhov, Y.S., Orr, A.C., Burdon, K.P., Nakano, S., Mori, K., Abu-Amero, K., Hauser, M., Li, Z., Prakadeeswari, G., Bailey, J.N.C., Cherecheanu, A.P., Kang, J.H., Nelson, S., Hayashi, K., Manabe, S.-I., Kazama, S., Zarnowski, T., Inoue, K., Irkec, M., Coca-Prados, M., Sugiyama, K., Järvelä, I., Schlottmann, P., Lerner, S.F., Lamari, H., Nilgün, Y., Bikbov, M., Park, K.H., Cha, S.C., Yamashiro, K., Zenteno, J.C., Jonas, J.B., Kumar, R.S., Perera, S.A., Chan, A.S.Y., Kobakhidze, N., George, R., Vijaya, L., Do, T., Edward, D.P., de Juan Marcos, L., Pakravan, M., Moghimi, S., Ideta, R., Bach-Holm, D., Kappelgaard, P., Wirostko, B., Thomas, S., Gaston, D., Bedard, K., Greer, W.L., Yang, Z., Chen, X., Huang, L., Sang, J., Jia, H., Jia, L., Qiao, C., Zhang, H., Liu, X., Zhao, B., Wang, Y.-X., Xu, L., Leruez, S., Reynier, P., Chichua, G., Tabagari, S., Uebe, S., Zenkel, M., Berner, D., Mossböck, G., Weisschuh, N., Hoja, U., Welge-Luessen, U.-C., Mardin, C., Founti, P., Chatzikyriakidou, A., Pappas, T., Anastasopoulos, E., Lambropoulos, A., Ghosh, A., Shetty, R., Porporato, N., Saravanan, V., Venkatesh, R., Shivkumar, C., Kalpana, N., Sarangapani, S., Kanavi, M.R., Beni, A.N., Yazdani, S., Lashay, A., Naderifar, H., Khatibi, N., Fea, A., Lavia, C., Dallorto, L., Rolle, T., Frezzotti, P., Paoli, D., Salvi, E., Manunta, P., Mori, Y., Miyata, K., Higashide, T., Chihara, E., Ishiko, S., Yoshida, A., Yanagi, M., Kiuchi, Y., Ohashi, T., Sakurai, T., Sugimoto, T., Chuman, H., Aihara, M., Inatani, M., Miyake, M., Gotoh, N., Matsuda, F., Yoshimura, N., Ikeda, Y., Ueno, M., Sotozono, C., Jeoung, J.W., Sagong, M., Park, K.H., Ahn, J., Cruz-Aguilar, M., Ezzouhairi, S.M., Rafei, A., Chong, Y.F., Ng, X.Y., Goh, S.R., Chen, Y., Yong, V.H.K., Khan, M.I., Olawoye, O.O., Ashaye, A.O., Ugbede, I., Onakoya, A., Kizor-Akaraiwe, N., Teekhasaenee, C., Suwan, Y., Supakontanasan, W., Okeke, S., Uche, N.J., Asimadu, I., Ayub, H., Akhtar, F., Kosior-Jarecka, E., Lukasik, U., Lischinsky, I., Castro, V., Grossmann, R.P., Megevand, G.S., Roy, S., Dervan, E., Silke, E., Rao, A., Sahay, P., Fornero, P., Cuello, O., Sivori, D., Zompa, T., Mills, R.A., Souzeau, E., Mitchell, P., Wang, J.J., Hewitt, A.W., Coote, M., Crowston, J.G., Astakhov, S.Y., Akopov, E.L., Emelyanov, A., Vysochinskaya, V., Kazakbaeva, G., Fayzrakhmanov, R., Al-Obeidan, S.A., Owaidhah, O., Aljasim, L.A., Chowbay, B., Foo, J.N., Soh, R.Q., Sim, K.S., Xie, Z., Cheong, A.W.O., Mok, S.Q., Soo, H.M., Chen, X.Y., Peh, S.Q., Heng, K.K., Husain, R., Ho, S.-L., Hillmer, A.M., Cheng, C.-Y., Escudero-Domínguez, F.A., González-Sarmiento, R., Martinon-Torres, F., Salas, A., Pathanapitoon, K., Hansapinyo, L., Wanichwecharugruang, B., Kitnarong, N., Sakuntabhai, A., Nguyn, H.X., Nguyn, G.T.T., Nguyn, T.V., Zenz, W., Binder, A., Klobassa, D.S., Hibberd, M.L., Davila, S., Herms, S., Nöthen, M.M., Moebus, S., Rautenbach, R.M., Ziskind, A., Carmichael, T.R., Ramsay, M., Álvarez, L., García, M., González-Iglesias, H., Rodríguez-Calvo, P.P., Fernández-Vega Cueto, L., Oguz, Ç., Tamcelik, N., Atalay, E., Batu, B., Aktas, D., Kasım, B., Wilson, M.R., Coleman, A.L., Liu, Y., Challa, P., Herndon, L., Kuchtey, R.W., Kuchtey, J., Curtin, K., Chaya, C.J., Crandall, A., Zangwill, L.M., Wong, T.Y., Nakano, M., Kinoshita, S., den Hollander, A.I., Vesti, E., Fingert, J.H., Lee, R.K., Sit, A.J., Shingleton, B.J., Wang, N., Cusi, D., Qamar, R., Kraft, P., Pericak-Vance, M.A., Raychaudhuri, S., Heegaard, S., Kivelä, T., Reis, A., Kruse, F.E., Weinreb, R.N., Pasquale, L.R., Haines, J.L., Thorsteinsdottir, U., Jonasson, F., Allingham, R.R., Milea, D., Ritch, R., Kubota, T., Tashiro, K., Vithana, E.N., Micheal, S., Topouzis, F., Craig, J.E., Dubina, M., Sundaresan, P., Stefansson, K., Wiggs, J.L., Pasutto, F., Khor, C.C., 2017. Genetic association study of exfoliation syndrome identifies a protective rare variant at LOXL1 and five new susceptibility loci. Nat. Genet. 49, 993–1004. http://dx.doi.org/10.1038/ng.3875.

Aung, T., Ozaki, M., Mizoguchi, T., Allingham, R.R., Li, Z., Haripriya, A., Nakano, S., Uebe, S., Harder, J.M., Chan, A.S.Y., Lee, M.C., Burdon, K.P., Astakhov, Y.S., Abu-Amero, K.K., Zenteno, J.C., Nilgün, Y., Zarnowski, T., Pakravan, M., Safieh, L.A., Jia, L., Wang, Y.X., Williams, S., Paoli, D., Schlottmann, P.G., Huang, L., Sim, K.S., Foo, J.N., Nakano, M., Ikeda, Y., Kumar, R.S., Ueno, M., Manabe, S., Hayashi, K., Kazama, S., Ideta, R., Mori, Y., Miyata, K., Sugiyama, K., Higashide, T., Chihara, E., Inoue, K., Ishiko, S., Yoshida, A., Yanagi, M., Kiuchi, Y., Aihara, M., Ohashi, T., Sakurai, T., Sugimoto, T., Chuman, H., Matsuda, F., Yamashiro, K., Gotoh, N., Miyake, M., Astakhov, S.Y., Osman, E.A., Al-Obeidan, S.A., Owaidhah, O., Al-Jasim, L., Al Shahwan, S., Fogarty, R.A., Leo, P., Yetkin, Y., Oğuz, Ç., Kanavi, M.R., Beni, A.N., Yazdani, S., Akopov, E.L., Toh, K.-Y., Howell, G.R., Orr, A.C., Goh, Y., Meah, W.Y., Peh, S.Q., Kosior-Jarecka, E., Lukasik, U., Krumbiegel, M., Vithana, E.N., Wong, T.Y., Liu, Y., Koch, A.E.A., Challa, P., Rautenbach, R.M., Mackey, D.A., Hewitt, A.W., Mitchell, P., Wang, J.J., Ziskind, A., Carmichael, T., Ramakrishnan, R., Narendran, K., Venkatesh, R., Vijayan, S., Zhao, P., Chen, X., Guadarrama-Vallejo, D., Cheng, C.Y., Perera, S.A., Husain, R., Ho, S.-L., Welge-Luessen, U.-C., Mardin, C., Schloetzer-Schrehardt, U., Hillmer, A.M., Herms, S., Moebus, S., Nöthen, M.M., Weisschuh, N., Shetty, R., Ghosh, A., Teo, Y.Y., Brown, M.A., Lischinsky, I., Blue Mountains Eye Study GWAS Team, Wellcome Trust Case Control Consortium 2,Crowston, J.G., Coote, M., Zhao, B., Sang, J., Zhang, N., You, Q., Vysochinskaya, V., Founti, P., Chatzikyriakidou, A., Lambropoulos, A., Anastasopoulos, E., Coleman, A.L., Wilson, M.R., Rhee, D.J., Kang, J.H., May-Bolchakova, I., Heegaard, S., Mori, K., Alward, W.L.M., Jonas, J.B., Xu, L., Liebmann, J.M., Chowbay, B., Schaeffeler, E., Schwab, M., Lerner, F., Wang, N., Yang, Z., Frezzotti, P., Kinoshita, S., Fingert, J.H., Inatani, M., Tashiro, K., Reis, A., Edward, D.P., Pasquale, L.R., Kubota, T., Wiggs, J.L., Pasutto, F., Topouzis, F., Dubina, M., Craig, J.E., Yoshimura, N., Sundaresan, P., John, S.W.M., Ritch, R., Hauser, M.A., Khor, C.-C., 2015. A common variant mapping to CACNA1A is associated with susceptibility to exfoliation syndrome. Nat. Genet. 47, 387–392. http://dx.doi.org/10.1038/ng.3226.

Benitez-del-Castillo Sanchez, J., Morion-Grande, M., Marco-Garcia, M.C., Parron-Carreño, T., 2015. Epidemiology of pseudoexfoliation syndrome: demystifying legends and looking to the future. Arch. Soc. Esp. Oftalmol. 90, 455–457. http://dx.doi.org/10.1016/j.oftal.2015.07.002.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol 57, 289–300.

Bill, B.R., Lowe, J.K., Dybuncio, C.T., Fogel, B.L., 2013. Orchestration of neurodevelopmental programs by RBFOX1: implications for autism spectrum disorder. Int. Rev. Neurobiol. 113, 251–267. http://dx.doi.org/10.1016/B978-0-12-418700-9.00008-3.

Bitel, C.L., Nathan, R., Wong, P., Kuppasani, S., Matsushita, M., Kanazawa, H., Frederikse, P.H., 2011. Evidence that "brain-specific" FOX-1, FOX-2, and nPTB alternatively spliced isoforms are produced in the lens. Curr. Eye Res. 36, 321–327.

Braun, G.S., Kuszka, A., Dau, C., Kriz, W., Moeller, M.J., 2016. Interaction of atypical cadherin Fat1 with SoHo adaptor proteins CAP/ponsin and ArgBP2. Biochem. Biophys. Res. Commun. 472, 88–94. http://dx.doi.org/10.1016/j.bbrc.2016.02.069.

Cappi, C., Hounie, A.G., Mariani, D.B., Diniz, J.B., Silva, A.R.T., Reis, V.N.S., Busso, A.F., Silva, A.G., Fidalgo, F., Rogatto, S.R., Miguel, E.C., Krepischi, A.C., Brentani, H., 2014. An inherited small microdeletion at 15q13.3 in a patient with early-onset obsessive-compulsive disorder. PLoS One 9, e110198. http://dx.doi.org/10.1371/journal.pone.0110198.

Challa, P., 2009. Genetics of pseudoexfoliation syndrome. Curr. Opin. Ophthalmol. 20, 88–91. http://dx.doi.org/10.1097/ICU.0b013e328320d86a.

Conway, R.M., Schlötzer-Schrehardt, U., Küchle, M., Naumann, G.O.H., 2004. Pseudoexfoliation syndrome: pathological manifestations of relevance to intraocular surgery. Clin. Experiment. Ophthalmol 32, 199–210. http://dx.doi.org/10.1111/j.1442-9071.2004.00806.x.

CRAN - Package epitools [WWW Document], n.d. URL https://cran.r-project.org/web/packages/epitools/index.html (Accessed 12 August 2016).

CRAN - Package pwr [WWW Document], n.d. URL https://cran.r-project.org/web/packages/pwr/index.html (Accessed 30 May 2017).

Damji, K.F., Bains, H.S., Stefansson, E., Loftsdottir, M., Sverrisson, T., Thorgeirsson, E., Jonasson, F., Gottfredsdottir, M., Allingham, R.R., 1998. Is pseudoexfoliation syndrome inherited? A review of genetic and nongenetic factors and a new observation. Ophthalmic Genet. 19, 175–185.

Davies, G., Marioni, R.E., Liewald, D.C., Hill, W.D., Hagenaars, S.P., Harris, S.E., Ritchie, S.J., Luciano, M., Fawns-Ritchie, C., Lyall, D., Cullen, B., Cox, S.R., Hayward, C., Porteous, D.J., Evans, J., McIntosh, A.M., Gallacher, J., Craddock, N., Pell, J.P., Smith, D.J., Gale, C.R., Deary, I.J., 2016. Genome-wide association study of cognitive functions and educational attainment in UK Biobank (N = 112 151). Mol. Psychiatr. 21, 758–767. http://dx.doi.org/10.1038/mp.2016.45.

Dettenhofer, M., Zhou, F., Leder, P., 2008. Formin 1-isoform IV deficient cells exhibit defects in cell spreading and focal adhesion formation. PLoS One 3, e2497. http://dx.doi.org/10.1371/journal.pone.0002497.

Faivre-Sarrailh, C., Devaux, J.J., 2013. Neuro-glial interactions at the nodes of Ranvier: implication in health and diseases. Front. Cell. Neurosci. 7, 196. http://dx.doi.org/10.3389/fncel.2013.00196.

Fan, B.J., Pasquale, L.R., Rhee, D., Li, T., Haines, J.L., Wiggs, J.L., 2011. LOXL1 promoter haplotypes are associated with exfoliation syndrome in a U.S. Caucasian population. Invest. Ophthalmol. Vis. Sci. 52, 2372–2378. http://dx.doi.org/10.1167/iovs.10-6268.

Ferrer, I., Garcia-Esparcia, P., Carmona, M., Carro, E., Aronica, E., Kovacs, G.G., Grison, A., Gustincich, S., 2016. Olfactory receptors in non-chemosensory organs: the nervous system in health and disease. Front. Aging Neurosci. 8, 163. http://dx.doi.org/10.3389/fnagi.2016.00163.

Forsius, H., Forsman, E., Fellman, J., Eriksson, A.W., 2002. Exfoliation syndrome: frequency, gender distribution and association with climatically induced alterations of the cornea and conjunctiva. Acta Ophthalmol. Scand. 80, 478–484.

Founti, P., Haidich, A.-B., Chatzikyriakidou, A., Salonikiou, A., Anastasopoulos, E., Pappas, T., Lambropoulos, A., Viswanathan, A.C., Topouzis, F., 2015. Ethnicity-based differences in the association of LOXL1 polymorphisms with pseudoexfoliation/pseudoexfoliative glaucoma: a meta-analysis. Ann. Hum. Genet. 79, 431–450. http://dx.doi.org/10.1111/ahg.12128.

Frei, J.A., Andermatt, I., Gesemann, M., Stoeckli, E.T., 2014. The SynCAM synaptic cell adhesion molecules are involved in sensory axon pathfinding by regulating axon-axon contacts. J. Cell Sci. 127, 5288–5302. http://dx.doi.org/10.1242/jcs.157032.

Gaj, P., Maryan, N., Hennig, E.E., Ledwon, J.K., Paziewska, A., Majewska, A., Karczmarski, J., Nesteruk, M., Wolski, J., Antoniewicz, A.A., Przytulski, K., Rutkowski, A., Teumer, A., Homuth, G., Starzyńska, T., Regula, J., Ostrowski, J., 2012. Pooled sample-based GWAS: a cost-effective alternative for identifying colorectal and prostate cancer risk variants in the Polish population. PLoS One 7, e35307. http://dx.doi.org/10.1371/journal.pone.0035307.

Gehman, L.T., Stoilov, P., Maguire, J., Damianov, A., Lin, C.-H., Shiue, L., Ares, M., Mody, I., Black, D.L., 2011. The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. Nat. Genet. 43, 706–711. http://dx.doi.org/10.1038/ng.841.

Glatt, S.J., Everall, I.P., Kremen, W.S., Corbeil, J., Sásik, R., Khanlou, N., Han, M., Liew, C.-C., Tsuang, M.T., 2005. Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc. Natl. Acad. Sci. U. S. A 102, 15533–15538. http://dx.doi.org/10.1073/pnas.0507666102.

Gottanka, J., Flügel-Koch, C., Martus, P., Johnson, D.H., Lütjen-Drecoll, E., 1997. Correlation of pseudoexfoliative material and optic nerve damage in pseudoexfoliation syndrome. Invest. Ophthalmol. Vis. Sci. 38, 2435–2446.

Guadarrama-Vallejo, D., Miranda-Duarte, A., Zenteno, J.C., 2013. The T allele of lysyl oxidase-like 1 rs41435250 is a novel risk factor for pseudoexfoliation syndrome and pseudoexfoliation glaucoma independently and through intragenic epistatic interaction. Mol. Vis. 19, 1937–1944.

Hastie, T., Trevor, T., Tibshirani, R., Friedman, J., 2009. The Elements of Statistical Learning. Data Mining, Inference, and Prediction, second ed. Springer Series of Statistics.

Herrera, E., Brown, L., Aruga, J., Rachel, R.A., Dolen, G., Mikoshiba, K., Brown, S., Mason, C.A., 2003. Zic2 patterns binocular vision by specifying the uncrossed retinal projection. Cell 114, 545–557.

Ibrahim-Verbaas, C.A., Bressler, J., Debette, S., Schuur, M., Smith, A.V., Bis, J.C., Davies, G., Trompet, S., Smith, J.A., Wolf, C., Chibnik, L.B., Liu, Y., Vitart, V., Kirin, M., Petrovic, K., Polasek, O., Zgaga, L., Fawns-Ritchie, C., Hoffmann, P., Karjalainen, J., Lahti, J., Llewellyn, D.J., Schmidt, C.O., Mather, K.A., Chouraki, V., Sun, Q., Resnick, S.M., Rose, L.M., Oldmeadow, C., Stewart, M., Smith, B.H., Gudnason, V., Yang, Q., Mirza, S.S., Jukema, J.W., deJager, P.L., Harris, T.B., Liewald, D.C., Amin, N., Coker, L.H., Stegle, O., Lopez, O.L., Schmidt, R., Teumer, A., Ford, I., Karbalai, N., Becker, J.T., Jonsdottir, M.K., Au, R., Fehrmann, R.S.N., Herms, S., Nalls, M., Zhao, W., Turner, S.T., Yaffe, K., Lohman, K., van Swieten, J.C., Kardia, S.L.R., Knopman, D.S., Meeks, W.M., Heiss, G., Holliday, E.G., Schofield, P.W., Tanaka, T., Stott, D.J., Wang, J., Ridker, P., Gow, A.J., Pattie, A., Starr, J.M., Hocking, L.J., Armstrong, N.J., McLachlan, S., Shulman, J.M., Pilling, L.C., Eiriksdottir, G., Scott, R.J., Kochan, N.A., Palotie, A., Hsieh, Y.-C., Eriksson, J.G., Penman, A., Gottesman, R.F., Oostra, B.A., Yu, L., DeStefano, A.L., Beiser, A., Garcia, M., Rotter, J.I., Nöthen, M.M., Hofman, A., Slagboom, P.E., Westendorp, R.G.J., Buckley, B.M., Wolf, P.A., Uitterlinden, A.G., Psaty, B.M., Grabe, H.J., Bandinelli, S., Chasman, D.I., Grodstein, F., Räikkönen, K., Lambert, J.-C., Porteous, D.J., Generation Scotland, Price, J.F., Sachdev, P.S., Ferrucci, L., Attia, J.R., Rudan, I., Hayward, C., Wright, A.F., Wilson, J.F., Cichon, S., Franke, L., Schmidt, H., Ding, J., de Craen, A.J.M., Fornage, M., Bennett, D.A., Deary, I.J., Ikram, M.A., Launer, L.J., Fitzpatrick, A.L., Seshadri, S., van Duijn, C.M., Mosley, T.H., 2016. GWAS for executive function and processing speed suggests involvement of the CADM2 gene. Mol. Psychiatr. 21, 189–197. http://dx.doi.org/10.1038/mp.2015.37.

Jeng, S.M., Karger, R.A., Hodge, D.O., Burke, J.P., Johnson, D.H., Good, M.S., 2007. The risk of glaucoma in pseudoexfoliation syndrome. J. Glaucoma 16, 117–121. http://dx.doi.org/10.1097/01.ijg.0000243470.13343.8b.

Jonasson, F., 2009. From epidemiology to lysyl oxidase like one (LOXL1) polymorphisms discovery: phenotyping and genotyping exfoliation syndrome and exfoliation glaucoma in Iceland. Acta Ophthalmol. (Copenh.) 87, 478–487. http://dx.doi.org/10.1111/j.1755-3768.2009.01635.x.

Kawabe, H., Neeb, A., Dimova, K., Young, S.M., Takeda, M., Katsurabayashi, S., Mitkovski, M., Malakhova, O.A., Zhang, D.-E., Umikawa, M., Kariya, K., Goebbels, S., Nave, K.-A., Rosenmund, C., Jahn, O., Rhee, J., Brose, N., 2010. Regulation of Rap2A by the ubiquitin ligase Nedd4-1 controls neurite development. Neuron 65, 358–372. http://dx.doi.org/10.1016/j.neuron.2010.01.007.

Kiefer, A.K., Tung, J.Y., Do, C.B., Hinds, D.A., Mountain, J.L., Francke, U., Eriksson, N., 2013. Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia. PLoS Genet. 9, e1003299. http://dx.doi.org/10.1371/journal.pgen.1003299.

Kioka, N., Ueda, K., Amachi, T., 2002. Vinexin, CAP/ponsin, ArgBP2: a novel adaptor protein family regulating cytoskeletal organization and signal transduction. Cell Struct. Funct. 27, 1–7.

Krumbiegel, M., Pasutto, F., Mardin, C.Y., Weisschuh, N., Paoli, D., Gramer, E., Zenkel, M., Weber, B.H.F., Kruse, F.E., Schlötzer-Schrehardt, U., Reis, A., 2009. Exploring functional candidate genes for genetic association in German patients with pseudoexfoliation syndrome and pseudoexfoliation glaucoma. Invest. Ophthalmol. Vis. Sci. 50, 2796–2801. http://dx.doi.org/10.1167/iovs.08-2339.

Krumbiegel, M., Pasutto, F., Schlötzer-Schrehardt, U., Uebe, S., Zenkel, M., Mardin, C.Y., Weisschuh, N., Paoli, D., Gramer, E., Becker, C., Ekici, A.B., Weber, B.H.F., Nürnberg, P., Kruse, F.E., Reis, A., 2011. Genome-wide association study with DNA pooling identifies variants at CNTNAP2 associated with pseudoexfoliation syndrome. Eur. J. Hum. Genet. EJHG 19, 186–193. http://dx.doi.org/10.1038/ejhg.2010.144.

Laezza, F., Gerber, B.R., Lou, J.-Y., Kozel, M.A., Hartman, H., Craig, A.M., Ornitz, D.M., Nerbonne, J.M., 2007. The FGF14(F145S) mutation disrupts the interaction of FGF14 with voltage-gated Na+ channels and impairs neuronal excitability. J. Neurosci. Off. J. Soc. Neurosci 27, 12033–12044. http://dx.doi.org/10.1523/JNEUROSCI.2282-07.2007.

Lee, R.K., 2008. The molecular pathophysiology of pseudoexfoliation glaucoma. Curr. Opin. Ophthalmol. 19, 95–101. http://dx.doi.org/10.1097/ICU.0b013e3282f49cda.

Lemmelä, S., Forsman, E., Sistonen, P., Eriksson, A., Forsius, H., Järvelä, I., 2007. Genome-wide scan of exfoliation syndrome. Invest. Ophthalmol. Vis. Sci. 48, 4136–4142. http://dx.doi.org/10.1167/iovs.06-1092.

Liao, W.-L., Chen, R.-H., Lin, H.-J., Liu, Y.-H., Chen, W.-C., Tsai, Y., Wan, L., Tsai, F.-J., 2011. The association between polymorphisms of B7 molecules (CD80 and CD86) and Graves' ophthalmopathy in a Taiwanese population. Ophthalmology 118, 553–557. http://dx.doi.org/10.1016/j.ophtha.2010.07.021.

Lu, Z., Reddy, M.V.V.V.S., Liu, J., Kalichava, A., Liu, J., Zhang, L., Chen, F., Wang, Y., Holthauzen, L.M.F., White, M.A., Seshadrinathan, S., Zhong, X., Ren, G., Rudenko, G., 2016. Molecular architecture of contactin-associated protein-like 2 (CNTNAP2) and its interaction with contactin 2 (CNTN2). J. Biol. Chem. http://dx.doi.org/10.1074/jbc.M116.748236.

Macgregor, S., Zhao, Z.Z., Henders, A., Nicholas, M.G., Montgomery, G.W., Visscher, P.M., 2008. Highly cost-efficient genome-wide association studies using DNA pools and dense SNP arrays. Nucleic Acids Res. 36, e35. http://dx.doi.org/10.1093/nar/gkm1060.

Malukiewicz, G., Lesiewska-Junk, H., Linkowska, K., Grzybowski, T., Kaźmierczak, K., 2012. Analysis of CNTNAP2 polymorphisms in Polish population with pseudoexfoliation syndrome. Acta Ophthalmol. (Copenh.) 90, e660–661. http://dx.doi.org/10.1111/j.1755-3768.2012.02395.x.

Malukiewicz, G., Lesiewska-Junk, H., Linkowska, K., Mielnik, M., Grzybowski, T., Sulima, N., 2011. Analysis of LOXL1 single nucleotide polymorphisms in Polish population with pseudoexfoliation syndrome. Acta Ophthalmol. (Copenh.) 89, e64–66. http://dx.doi.org/10.1111/j.1755-3768.2010.02083.x.

Merkur, A., Damji, K.F., Mintsioulis, G., Hodge, W.G., 2001. Intraocular pressure decrease after phacoemulsification in patients with pseudoexfoliation syndrome. J. Cataract Refract. Surg. 27, 528–532.

Ogawa, M., Mizugishi, K., Ishiguro, A., Koyabu, Y., Imai, Y., Takahashi, R., Mikoshiba, K., Aruga, J., 2008. Rines/RNF180, a novel RING finger gene-encoded product, is a membrane-bound ubiquitin ligase. Genes Cells Devoted Mol. Cell. Mech 13, 397–409. http://dx.doi.org/10.1111/j.1365-2443.2008.01169.x.

Ostrowski, J., Paziewska, A., Lazowska, I., Ambrozkiewicz, F., Goryca, K., Kulecka, M.,

Rawa, T., Karczmarski, J., Dabrowska, M., Zeber-Lubecka, N., Tomecki, R., Kluska, A., Balabas, A., Piatkowska, M., Paczkowska, K., Kierkus, J., Socha, P., Lodyga, M., Rydzewska, G., Klopocka, M., Mierzwa, G., Iwanczak, B., Krzesiek, E., Bak-Drabik, K., Walkowiak, J., Klincewicz, B., Radwan, P., Grzybowska-Chlebowczyk, U., Landowski, P., Jankowska, A., Korczowski, B., Starzynska, T., Albrecht, P., Mikula, M., 2016. Genetic architecture differences between pediatric and adult-onset inflammatory bowel diseases in the Polish population. Sci. Rep. 6, 39831. http://dx.doi.org/10.1038/srep39831.

Ovodenko, B., Rostagno, A., Neubert, T.A., Shetty, V., Thomas, S., Yang, A., Liebmann, J., Ghiso, J., Ritch, R., 2007. Proteomic analysis of exfoliation deposits. Invest. Ophthalmol. Vis. Sci. 48, 1447–1457. http://dx.doi.org/10.1167/iovs.06-0411.

Paziewska, A., Habior, A., Rogowska, A., Zych, W., Goryca, K., Karczmarski, J., Dabrowska, M., Ambrozkiewicz, F., Walewska-Zielecka, B., Krawczyk, M., Cichoz-Lach, H., Milkiewicz, P., Kowalik, A., Mucha, K., Raczynska, J., Musialik, J., Boryczka, G., Wasilewicz, M., Ciecko-Michalska, I., Ferenc, M., Janiak, M., Kanikowska, A., Stankiewicz, R., Hartleb, M., Mach, T., Grzymislawski, M., Raszeja-Wyszomirska, J., Wunsch, E., Bobinski, T., Mikula, M., Ostrowski, J., 2017. A novel approach to genome-wide association analysis identifies genetic associations with primary biliary cholangitis and primary sclerosing cholangitis in Polish patients. BMC Med. Genom. 10, 2. http://dx.doi.org/10.1186/s12920-016-0239-9.

Pearson, J.V., Huentelman, M.J., Halperin, R.F., Tembe, W.D., Melquist, S., Homer, N., Brun, M., Szelinger, S., Coon, K.D., Zismann, V.L., Webster, J.A., Beach, T., Sando, S.B., Aasly, J.O., Heun, R., Jessen, F., Kolsch, H., Tsolaki, M., Daniilidou, M., Reiman, E.M., Papassotiropoulos, A., Hutton, M.L., Stephan, D.A., Craig, D.W., 2007. Identification of the genetic basis for complex disorders by use of pooling-based genomewide single-nucleotide-polymorphism association studies. Am. J. Hum. Genet. 80, 126–139. http://dx.doi.org/10.1086/510686.

Pearson, T.A., Manolio, T.A., 2008. How to interpret a genome-wide association study. J. Am. Med. Assoc. 299, 1335–1344. http://dx.doi.org/10.1001/jama.299.11.1335.

Peñagarikano, O., Abrahams, B.S., Herman, E.I., Winden, K.D., Gdalyahu, A., Dong, H., Sonnenblick, L.I., Gruver, R., Almajano, J., Bragin, A., Golshani, P., Trachtenberg, J.T., Peles, E., Geschwind, D.H., 2011. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. Cell 147, 235–246. http://dx.doi.org/10.1016/j.cell.2011.08.040.

Pośpiech, E., Draus-Barini, J., Kupiec, T., Wojas-Pelc, A., Branicki, W., 2012. Prediction of eye color from genetic data using Bayesian approach. J. Forensic Sci. 57, 880–886. http://dx.doi.org/10.1111/j.1556-4029.2012.02077.x.

Pośpiech, E., Karłowska-Pik, J., Marcińska, M., Abidi, S., Andersen, J.D., van den Berge, M., Carracedo, Á., Eduardoff, M., Freire-Aradas, A., Morling, N., Sijen, T., Skowron, M., Söchtig, J., Syndercombe-Court, D., Weiler, N., Schneider, P.M., Ballard, D., Børsting, C., Parson, W., Phillips, C., Branicki, W., EUROFORGEN-NoE Consortium, 2015. Evaluation of the predictive capacity of DNA variants associated with straight hair in Europeans. Forensic Sci. Int. Genet. 19, 280–288. http://dx.doi.org/10.1016/j.fsigen.2015.09.004.

Postel, K., Bellmann, J., Splith, V., Ader, M., 2013. Analysis of cell surface markers specific for transplantable rod photoreceptors. Mol. Vis. 19, 2058–2067.

Potkin, S.G., Macciardi, F., Guffanti, G., Fallon, J.H., Wang, Q., Turner, J.A., Lakatos, A., Miles, M.F., Lander, A., Vawter, M.P., Xie, X., 2010. Identifying gene regulatory networks in schizophrenia. Neuroimage 53, 839–847. http://dx.doi.org/10.1016/j.neuroimage.2010.06.036.

Pronin, A., Levay, K., Velmeshev, D., Faghihi, M., Shestopalov, V.I., Slepak, V.Z., 2014. Expression of olfactory signaling genes in the eye. PLoS One 9, e96435. http://dx.doi.org/10.1371/journal.pone.0096435.

Ringvold, A., 1999. Epidemiology of the pseudo-exfoliation syndrome. Acta Ophthalmol. Scand. 77, 371–375.

Ritch, R., 2001. Perspective on exfoliation syndrome. J. Glaucoma 10, S33–S35.

Ritch, R., Schlötzer-Schrehardt, U., 2001. Exfoliation (pseudoexfoliation) syndrome: toward a new understanding. Proceedings of the first International think tank. Acta Ophthalmol. Scand. 79, 213–217.

R: The R Project for Statistical Computing [WWW Document], n.d. URL http://www.r-project.org/ (Accessed 4 February 2015).

Schlötzer-Schrehardt, U., 2011. Genetics and genomics of pseudoexfoliation syndrome/glaucoma. Middle East Afr. J. Ophthalmol. 18, 30–36. http://dx.doi.org/10.4103/0974-9233.75882.

Schlötzer-Schrehardt, U., Hammer, C.M., Krysta, A.W., Hofmann-Rummelt, C., Pasutto, F., Sasaki, T., Kruse, F.E., Zenkel, M., 2012. LOXL1 deficiency in the lamina cribrosa as candidate susceptibility factor for a pseudoexfoliation-specific risk of glaucoma. Ophthalmology 119, 1832–1843. http://dx.doi.org/10.1016/j.ophtha.2012.03.015.

Schlötzer-Schrehardt, U., Naumann, G.O., 1995. Trabecular meshwork in pseudoexfoliation syndrome with and without open-angle glaucoma. A morphometric, ultrastructural study. Invest. Ophthalmol. Vis. Sci. 36, 1750–1764.

Schlötzer-Schrehardt, U., Naumann, G.O.H., 2006. Ocular and systemic pseudoexfoliation syndrome. Am. J. Ophthalmol. 141, 921–937. http://dx.doi.org/10.1016/j.ajo.2006.01.047.

Schrauwen, I., Ealy, M., Huentelman, M.J., Thys, M., Homer, N., Vanderstraeten, K., Fransen, E., Corneveaux, J.J., Craig, D.W., Claustres, M., Cremers, C.W.R.J., Dhooge, I., Van de Heyning, P., Vincent, R., Offeciers, E., Smith, R.J.H., Van Camp, G., 2009. A genome-wide analysis identifies genetic variants in the RELN gene associated with otosclerosis. Am. J. Hum. Genet. 84, 328–338. http://dx.doi.org/10.1016/j.ajhg.2009.01.023.

Sharma, S., Martin, S., Sykes, M.J., Dave, A., Hewitt, A.W., Burdon, K.P., Ronci, M., Voelcker, N.H., Craig, J.E., 2016. Biological effect of LOXL1 coding variants associated with pseudoexfoliation syndrome. Exp. Eye Res. 146, 212–223. http://dx.doi.org/10.1016/j.exer.2016.03.013.

Simon-Areces, J., Dopazo, A., Dettenhofer, M., Rodriguez-Tebar, A., Garcia-Segura, L.M., Arevalo, M.-A., 2011. Formin1 mediates the induction of dendritogenesis and synaptogenesis by neurogenin3 in mouse hippocampal neurons. PLoS One 6, e21825. http://dx.doi.org/10.1371/journal.pone.0021825.

Stambolian, D., Wojciechowski, R., Oexle, K., Pirastu, M., Li, X., Raffel, L.J., Cotch, M.F., Chew, E.Y., Klein, B., Klein, R., Wong, T.Y., Simpson, C.L., Klaver, C.C.W., van Duijn, C.M., Verhoeven, V.J.M., Baird, P.N., Vitart, V., Paterson, A.D., Mitchell, P., Saw, S.M., Fossarello, M., Kazmierkiewicz, K., Murgia, F., Portas, L., Schache, M., Richardson, A., Xie, J., Wang, J.J., Rochtchina, E., DCCT/EDIC Research Group,Viswanathan, A.C., Hayward, C., Wright, A.F., Polasek, O., Campbell, H., Rudan, I., Oostra, B.A., Uitterlinden, A.G., Hofman, A., Rivadeneira, F., Amin, N., Karssen, L.C., Vingerling, J.R., Hosseini, S.M., Döring, A., Bettecken, T., Vatavuk, Z., Gieger, C., Wichmann, H.-E., Wilson, J.F., Fleck, B., Foster, P.J., Topouzis, F., McGuffin, P., Sim, X., Inouye, M., Holliday, E.G., Attia, J., Scott, R.J., Rotter, J.I., Meitinger, T., Bailey-Wilson, J.E., 2013. Meta-analysis of genome-wide association studies in five cohorts reveals common variants in RBFOX1, a regulator of tissue-specific splicing, associated with refractive error. Hum. Mol. Genet. 22, 2754–2764. http://dx.doi.org/10.1093/hmg/ddt116.

Streeten, B.W., Li, Z.Y., Wallace, R.N., Eagle, R.C., Keshgegian, A.A., 1992. Pseudoexfoliative fibrillopathy in visceral organs of a patient with pseudoexfoliation syndrome. Arch. Ophthalmol. Chic. Ill 1960 (110), 1757–1762.

Taira, K., Umikawa, M., Takei, K., Myagmar, B.-E., Shinzato, M., Machida, N., Uezato, H., Nonaka, S., Kariya, K., 2004. The Traf2- and Nck-interacting kinase as a putative effector of Rap2 to regulate actin cytoskeleton. J. Biol. Chem. 279, 49488–49496. http://dx.doi.org/10.1074/jbc.M406370200.

Tang, J.-Z., Wang, X.-Q., Ma, H.-F., Ma, F.-F., Wang, B., Wang, P.-F., Peng, Z.-X., Zhou, X.-Y., 2014. Association between polymorphisms in lysyl oxidase-like 1 and susceptibility to pseudoexfoliation syndrome and pseudoexfoliation glaucoma. PLoS One 9, e90331. http://dx.doi.org/10.1371/journal.pone.0090331.

Tantsis, E.M., Gill, D., Griffiths, L., Gupta, S., Lawson, J., Maksemous, N., Ouvrier, R., Riant, F., Smith, R., Troedson, C., Webster, R., Menezes, M.P., 2016. Eye movement disorders are an early manifestation of CACNA1A mutations in children. Dev. Med. Child Neurol. 58, 639–644. http://dx.doi.org/10.1111/dmcn.13033.

Teumer, A., Ernst, F.D., Wiechert, A., Uhr, K., Nauck, M., Petersmann, A., Völzke, H., Völker, U., Homuth, G., 2013. Comparison of genotyping using pooled DNA samples (allelotyping) and individual genotyping using the affymetrix genome-wide human SNP array 6.0. BMC Genom. 14, 506. http://dx.doi.org/10.1186/1471-2164-14-506.

Thomassin, L., Werneck, C.C., Broekelmann, T.J., Gleyzal, C., Hornstra, I.K., Mecham, R.P., Sommer, P., 2005. The Pro-regions of lysyl oxidase and lysyl oxidase-like 1 are required for deposition onto elastic fibers. J. Biol. Chem. 280, 42848–42855. http://dx.doi.org/10.1074/jbc.M506832200.

Thorleifsson, G., Magnusson, K.P., Sulem, P., Walters, G.B., Gudbjartsson, D.F., Stefansson, H., Jonsson, T., Jonasdottir, A., Jonasdottir, A., Stefansdottir, G., Masson, G., Hardarson, G.A., Petursson, H., Arnarsson, A., Motallebipour, M., Wallerman, O., Wadelius, C., Gulcher, J.R., Thorsteinsdottir, U., Kong, A., Jonasson, F., Stefansson, K., 2007. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science 317, 1397–1400. http://dx.doi.org/10.1126/science.1146554.

Vazquez, L.E., Lee, R.K., 2014. Genomic and proteomic pathophysiology of pseudoexfoliation glaucoma. Int. Ophthalmol. Clin. 54, 1–13. http://dx.doi.org/10.1097/IIO.0000000000000047.

Wagenseil, J.E., Mecham, R.P., 2007. New insights into elastic fiber assembly. Birth Defects Res. Part C Embryo Today Rev. 81, 229–240. http://dx.doi.org/10.1002/bdrc.20111.

Wagner, M., Sobczyński, M., Karabon, L., Bilińska, M., Pokryszko-Dragan, A., Pawlak-Adamska, E., Cyrul, M., Kuśnierczyk, P., Jasek, M., 2015. Polymorphisms in CD28, CTLA-4, CD80 and CD86 genes may influence the risk of multiple sclerosis and its age of onset. J. Neuroimmunol. 288, 79–86. http://dx.doi.org/10.1016/j.jneuroim.2015.09.004.

Walsh, S., Liu, F., Ballantyne, K.N., van Oven, M., Lao, O., Kayser, M., 2011. IrisPlex: a sensitive DNA tool for accurate prediction of blue and brown eye colour in the absence of ancestry information. Forensic Sci. Int. Genet. 5, 170–180. http://dx.doi.org/10.1016/j.fsigen.2010.02.004.

Wiggs, J.L., Pasquale, L.R., 2014. Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome. J. Glaucoma 23, S62–S63. http://dx.doi.org/10.1097/IJG.0000000000000124.

Xiao, M., Bosch, M.K., Nerbonne, J.M., Ornitz, D.M., 2013. FGF14 localization and organization of the axon initial segment. Mol. Cell. Neurosci. 56, 393–403. http://dx.doi.org/10.1016/j.mcn.2013.07.008.

Yan, H., Pablo, J.L., Pitt, G.S., 2013. FGF14 regulates presynaptic Ca2+ channels and synaptic transmission. Cell Rep 4, 66–75. http://dx.doi.org/10.1016/j.celrep.2013.06.012.

Zenkel, M., Schlötzer-Schrehardt, U., 2014. Expression and regulation of LOXL1 and elastin-related genes in eyes with exfoliation syndrome. J. Glaucoma 23, S48–S50. http://dx.doi.org/10.1097/IJG.0000000000000120.

Zhang, Q., Gao, X., Li, C., Feliciano, C., Wang, D., Zhou, D., Mei, Y., Monteiro, P., Anand, M., Itohara, S., Dong, X., Fu, Z., Feng, G., 2016. Impaired dendritic development and memory in Sorbs2 knock-out mice. J. Neurosci. Off. J. Soc. Neurosci 36, 2247–2260. http://dx.doi.org/10.1523/JNEUROSCI.2528-15.2016.

Zhou, H., Mangelsdorf, M., Liu, J., Zhu, L., Wu, J.Y., 2014. RNA-binding proteins in neurological diseases. Sci. China Life Sci. 57, 432–444. http://dx.doi.org/10.1007/s11427-014-4647-9.