Introduction

Pseudoexfoliation syndrome (PEX) represents a complex, multifactorial, age-related disease of worldwide significance and often associated with elevated intraocular pressure. The average prevalence of PEX is 10-20% of the general population over the age of 60 years (1). The incidence of PEX varies among ethnic groups (2). Genetic and nongenetic factors are known to be involved in the etiopathogenesis of PEX. Some evidence

Genetic background analysis of pseudoexfoliation syndrome in Polish population — summary overview

Analiza podłoża genetycznego zespołu pseudoeksfoliacji w populacji polskiej — podsumowanie

Grażyna Malukiewicz1, Hanna Lesiewska1, Joanna Stafiej1, Katarzyna Linkowska2, Jacek Swohodziński2, Tomasz Grzybowski2

1 Department of Ophthalmology Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz, Poland
Head: Professor Grażyna Malukiewicz, MD, PhD
2 Institute of Forensic Medicine, Department of Molecular and Forensic Genetics
Head: Professor Tomasz Grzybowski, MD, PhD

Abstract:

Aim: To evaluate Contactin Associated Protein-Like 2 (CNTNAP2), Contactin-Associated Protein-Like 4 (CNTNAP4), Lysyl Oxidase-Like Protein 1 (LOXL1) and superoxide dismutase 1 (SOD1) gene polymorphisms in patients with pseudoexfoliation syndrome (PEX).

Material and methods: The study group consisted of 73 cataract patients with PEX and 111 controls with cataract but without PEX. Blood samples were obtained from each participant via peripheral venepuncture and genomic DNA was isolated according to the standard procedures. Genotypes of the CNTNAP4 esv12669 was determined using a commercially available assay. Previously reported chosen gene polymorphisms assessed by us in PEX patients were overviewed.

Results: There was no difference in both allele and haplotype frequencies of single-nucleotide polymorphisms (SNPs) in CNTNAP2 (rs2107856 and rs214138) and in SOD1 (rs10432782 and rs2070424) between PEX patients and controls. There was no difference in in frequencies of copy-number variations (CNVs) alleles esv12669 in the CNTNAP4 and esv11910 in the CNTNAP2 between PEX patients and controls. There were significant associations between PEX and SNPs in LOXL1 for the G allele of rs3825942 (p = 0.0047) and for the T allele of rs216541 (p = 0.021). The haplotype (GGT) consisting of all three risk alleles was significantly overrepresented (87.5%) in patients with PEX.

Conclusions: Our studies confirm a genetic basis for PEX with the significant association between the assessed LOXL1 SNPs and PEX in Polish population.

Key words: Pseudoexfoliation syndrome, PEX, gene polymorphisms, Polish population, CNTNAP, LOXL1, SOD1.
exists for the contribution of the genes with relatively small effects, e.g. clusterin (CLU), apolipoprotein E (APOE), glutathione S-transferases (GSTs), and tumor necrosis factor-alpha (TNFA), in certain study populations (3). Three single nucleotide polymorphisms (SNPs) in the lysyl oxidase-like 1 gene (LOXL1) have been shown to be associated with PEX (1, 4, 5, 6). Krumbiegel et al. showed that APOE genotypes are not associated with PEX in either Germans or Italians (7). Some studies suggest an association between PEX and two SNPs, rs2107856 and rs2141388, of the CNTNAP2 contactin-associated protein-like 2 gene. This correlation was observed in German patients; however, it was not evident in an Italian cohort (8,9). Interestingly, it was shown that some SNPs of CNTNAP2 and CNTNAP4 genes are associated with aging and age-related disorders such as Alzheimer’s and Parkinson’s diseases (10,11).

There is increasing evidence that oxidative stress is involved in the pathobiology of PEX. Polymorphisms in genes encoding antioxidant enzymes may result in reduced enzyme activity and increased levels of reactive oxygen species (12, 13).

In this study we evaluated the CNTNAP4, contactin-associated protein-like 4 gene, and current findings concerning the association between the chosen SNPs of LOXL1, CNTNAP2, and SOD1 gene polymorphisms in PEX.

**Materials and methods**

We studied 73 patients and 111 age-matched control subjects who presented to the Department of Ophthalmology Collegium Medicum UMK in Bydgoszcz for cataract surgery. Patients were enrolled in the study, if they had no other ocular or systemic disease (e.g. glaucoma, age related macular degeneration – AMD, diabetes, dyslipidemia, hypertension, mental health disorders) except for cataract and PEX. The diagnosis of PEX was confirmed in each case by a thorough slit lamp examination. Pseudoexfoliation changes were identified as the presence of typical PEX material on the anterior surface of the lens, iris, or corneal endothelium in either eye. Controls were individuals without any evidence of pseudoexfoliation deposits on intraocular tissues and no evidence of any systemic disease. The participants gave their informed written consent for enrolment. The study protocol was approved by the Ethical Committee of Collegium Medicum in Bydgoszcz.

Genomic DNA was extracted from blood samples collected from patients and controls according to the standard procedures. Esv11910 in CNTNAP2 gene and Esv12669 in CNTNAP4 gene were investigated. Genomic DNA was isolated using GeneMatrix Bio-Trace DNA Purification Kit according to the manufacturer’s protocols (Eurx). DNA quantity was assessed by real time quantitative PCR using a Viia™ 7 Real-Time PCR System (Life Technologies) following the manufacturer’s protocols (Eurx). DNA quantity was assessed by real time quantitative PCR using a Viia™ 7 Real-Time PCR System (Life Technologies) following the manufacturer’s protocols (Eurx). DNA quantity was assessed by real time quantitative PCR using a Viia™ 7 Real-Time PCR System (Life Technologies) following the manufacturer’s protocols (Eurx). DNA quantity was assessed by real time quantitative PCR using a Viia™ 7 Real-Time PCR System (Life Technologies) following the manufacturer’s protocols (Eurx).

**Table I.**

| Primer | Sequence |
|--------|----------|
| Primer Forward cnv11910_F | 5’CCCGATCAATGCAAATTCTATTT3’ |
| Primer Reverse cnv11910_R | 5’GGGCCAGCACCTGAAGCT3’ |
| Primer Forward cnv12669_F | 5’TGCAACACAAAGGGAGGATCCT3’ |
| Primer Reverse cnv12669_R | 5’GCAGATAAGGGAGAGTGAGTGACA3’ |
| Primer Forward cnv11910_F | 5’CCCGATCAATGCAAATTCTATTT3’ |
| Primer Reverse cnv11910_R | 5’GGGCCAGCACCTGAAGCT3’ |

Tab. I. Primers for the CNV analysis of esv12669 in the CNTNAP4 gene and esv11910 in CNTNAP2 gene are provided in Table I.

The Fisher exact test was performed to compare possible associations between CNV allele frequency and disease status in patients and controls. Odds ratios were also calculated. The significance level for all statistical tests was 0.05. Statistical analysis was performed using Statistica software (version 8).

Genotypes of the LOXL1 SNPs: rs1048661 (R141L), rs3825942 (G153D), rs2165241, CNTNAP2 SNPs: rs2107856, rs214138 and SOD1 SNPs: rs10432782, rs2070424 were determined using a commercially available assays, as described before (4, 9, 13).

**Results**

There were no differences in frequency of CNTNAP4 esv12669 del/del variant and CNTNAP2 esv11910 del/del variant between PEX group and controls (Tab. II).

**Table II.**

| Subjects | Controls | OR | 95% CI | P value |
|----------|----------|----|--------|---------|
| CNTNAP4 esv12669 | 27 (34) | 52 (47) | 0.6547 | 0.3575-1.1988 | 0.1748 |
| CNTNAP2 esv11910 | 53 (73) | 77 (70) | 1.1357 | 0.5891-2.1895 | 0.7417 |

Tab. II. Frequencies of CNTNAP4 esv12669 del/del and CNTNAP2 esv11910 del/del variants in PEX patients and controls in the Polish population.

**Discussion**

**Association between LOXL1 and PEX**

LOXL1 belongs to extracellular copper-requiring enzymes which promote collagen and elastin cross-linking. Genome-wide association studies in the Icelandic and Swedish populations...
identified multiple SNPs from the LOXL1 gene which were strongly associated with PEX (6). The same observations were confirmed for other populations: American, Irish, Scottish, English, Finish, Maltese, Indian and Japanese (1,5). In our previous study, we confirmed a significant association of allele G of rs3825942 (p = 0.0047) and allele T of rs216541 (p = 0.021) with PEX. The allele frequencies for rs1048661 G, rs3825942 G and rs2165241 T were slightly higher in our subjects (0.90; 0.87; 0.65) than controls (0.8; 0.87; 0.65). The haplotype (GGT) consisting of all three risk alleles was significantly overrepresented (87.5%).

| SNP ID   | Gene    | Allele | Subjects | Controls | P value |
|----------|---------|--------|----------|----------|---------|
| rs10432782 | SOD1    | G      | 0.19     | 0.18     | 0.875   |
| rs2070424  | SOD1    | G      | 0.10     | 0.13     | 0.640   |
| rs1048661  | LOXL1   | T      | 0.097    | 0.20     | 0.090   |
| rs38255942 | LOXL1   | A      | 0.00     | 0.13     | 0.005*  |
| rs2165241  | LOXL1   | C      | 0.12     | 0.35     | 0.002*  |
| rs2107856  | CNTNAP2 | T      | 0.28     | 0.35     | 0.365   |
| rs2141388  | CNTNAP2 | T      | 0.28     | 0.35     | 0.365   |

* statistically significant (p < 0.05)

Tab. III. Allele frequencies of selected genes in patients with PEX and controls in the Polish population.

No association between CNTNAP2 and PEX

CNTNAP2 is a large gene on chromosome 7. This gene encompasses almost 1.5% of chromosome 7 and is one of the largest genes in the human genome. It is little known about specific function and regulation of CNTNAP2. It has been suggested as a candidate gene for various neuropsychiatric disorders (10,14). It encodes for contactin-associated protein-like 2, a neuronal membrane protein and member of the neurexin superfamily (15). Krumbiegel et al. revealed two SNPs, rs2107856 and rs2141388, located in intron 11 of the CNTNAP2 gene which were strongly associated with PEX in the German but not the Italian cohort (8). Despite this report, we were unable to show association between the CNTNAP2 SNPs (rs2107856, rs2141388) gene and PEX syndrome in Polish patients, as presented in our previous paper (9). These results are in harmony with results for Italian and Japanese cohorts (16). However, the risk that these two SNPs confer to the disease, with an OR of about 1.4, corresponds to the data of Krumbiegel et al. for the German population and is typical of many susceptibility variants identified in complex diseases (8).

The prevalence of clinical exfoliation syndrome increases with age, particularly in the population above the age of 60 (5). Aging is a biological process strongly determined by genetics. A few single nucleotide polymorphisms (SNPs) have been reported to be consistently associated with aging. The study of lakoubov et al. showed that CNTNAP2 in general, and its esv11910 del/del in particular, are associated with healthy aging in humans relative to the current mean life expectancy. Our study did not identify any association between the CNTNAP2 esv11910 CNV and PEX syndrome in Polish patients.

No association between CNTNAP4 and PEX

A new association with systemic diseases and limited survival past 80 years was recently reported for a copy number variation (CNV) in the CNTNAP4 gene from the neurexin superfamily (10). Iakoubov et al. have demonstrated associations between the CNTNAP4 gene and its esv12669 del/del polymorphic variant and longevity, healthy aging, as well as age-related pathologies such as cognitive impairment and, tentatively, Alzheimer’s and Parkinson’s diseases (11). We analyzed the association between CNTNAP4 esv12669 polymorphism with PEX and found no correlation with this disease.

No association between SOD1 and PEX

Many studies have shown possible involvement of oxidative stress in the pathogenesis of PEX (12, 17). Superoxide dismutase (SOD) is one of the crucial enzymes providing the first line of antioxidant defense which prevents free radical formation. The encoded isoform is a soluble cytoplasmic protein, acting as a homodimer to convert naturally occurring but harmful superoxide radicals to molecular oxygen and hydrogen peroxide so changes in the activities of this enzyme, can lead to reduced protection against oxidative stress (18). Uçakhan et al. revealed higher activity of SOD in the anterior segment ocular tissue of PEX patients (17). In our previous study, we demonstrated an increased erythrocyte SOD1 activity in PEX patients compared with those without PEX. We analyzed the association between SOD1 rs10432782 and rs2070424 polymorphisms with the risk of PEX, demonstrating that neither was associated with an increased risk of PEX (13).

Conclusion

To conclude, our studies confirm a genetic basis for PEX, as the significant association between the assessed LOXL1 SNPs and PEX was found in the Polish population. However, they showed no association between CNTNAP2, CNTNAP4, SOD1 SNPs or CNVs and PEX.

The statistical power of presented studies was weak due to relatively small sample sizes, which is why further studies searching for genetic factors contributing to PEX are required. The identification of PEX-associated SNPs would be desirable, as it will enable early detection of PEX-glaucoma, even before the onset of elevated IOP.
References:

1. Ramprasad VL, George R, Soumittra N, Sharmila F, Vijaya L, Kumaramanickavel G: Association of non-synonymous single nucleotide polymorphisms in the LOXL1 gene with pseudoexfoliation syndrome in India. Mol Vis. 2008;14:318–322.

2. Challa P, Schmidt S, Liu Y, Qin X, Vann RR, Gonzalez P, et al.: Analysis of LOXL1 polymorphisms in a United States population with pseudoexfoliation glaucoma. Mol Vis. 2008;14:146–149.

3. Schlötzer-Schrehardt U: Genetics and genomics of pseudoexfoliation syndrome/glaucoma. Middle East Afr J Ophthalmol. 2011;18:30–36.

4. Malukiewicz G, Lesiewska-Junk H, Linkowska K, Mielnik M, Grzybowski T, Sulima N: Analysis of LOXL1 single nucleotide polymorphisms in Polish population with pseudoexfoliation syndrome. Acta Ophthalmol. 2011;89:64–66.

5. Tanito M, Minami M, Akahori M, Kaidzu S, Takai Y, Ohira A, et al.: LOXL1 variants in elderly Japanese patients with exfoliation syndrome/glaucoma, primary open-angle glaucoma, normal tension glaucoma, and cataract. Mol Vis 2008;14:1898–1905.

6. Thorleifsson G, Magnusson KP, Sulem P, Walters GB, Gudbjartsson DF, Stefansson H, et al.: Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science. 2007;317:1397–1400.

7. Krumbiegel M, Pasutto F, Mardin CY, Weisschuh N, Paoli D, Gramer E, et al.: Apolipoprotein E genotypes in pseudoexfoliation syndrome and pseudoexfoliation glaucoma. J Glaucoma. 2010;19:561–565.

8. Krumbiegel M, Pasutto F, Schlötzer-Schrehardt U, Uebe S, Zenkel M, Mardin CY, et al.: Genome-wide association study with DNA pooling identifies variants at CNTNAP2 associated with pseudoexfoliation syndrome. Eur J Hum Gen. 2011;19:186–193.

9. Malukiewicz G, Lesiewska-Junk H, Linkowska K, Grzybowski T, Kazmierczak K: Analysis of CNTNAP2 polymorphisms in Polish population with pseudoexfoliation syndrome. Acta Ophthalmol. 2012;90:660–661.

10. Iakoubov L, Mossakowska M, Duan Z, Sesti F, Puzianowska-Kuznicka M: A Common Copy Number Variation (CNV) Polymorphism in the CNTNAP4 Gene: Association with Aging in Females PLOS One. 2013;8:79790.

11. Iakoubov L, Mossakowska M, Szwed M, Puzianowska-Kuznicka M: A common copy number variation polymorphism in the CNTNAP2 gene: sexual dimorphism in association with healthy aging and disease. Gerontology. 2015;61:24–31.

12. Yağcı R, Gürel A, Ersöz I, Keseuc UC, Hepşen IF, Duman S, et al.: Oxidative stress and protein oxidation in pseudoexfoliation syndrome. Curr Eye Res. 2006;31:1029–1032.

13. Lesiewska H, Malukiewicz G, Linkowska K, Grzybowski T: Analysis of SOD1 polymorphisms in Polish population with pseudoexfoliation syndrome. Acta Ophthalmol. 2015;93:322–323.

14. Friedman JL, Vreinhoek T, Markx S, Janssen IM, van der Vliet WA, Faas BH, et al.: CNTNAP2 gene dosage variation is associated with schizophrenia and epilepsy. Mol Psychiatry. 2008;13:261–263.

15. Poliak S, Gollan L, Martinez R, Custer A, Einheber S, Salzer JL, et al.: Caspr2, a new member of the neurexin superfamily, is localized at the juxtaparanodes of myelinated axons and associates with K+ channels. Neuron. 1999;24:1037–1047.

16. Shimizu A, Takano Y, Shi D, Yokokura S, Yokoyama Y, Zheng X, et al.: Evaluation of CNTNAP2 gene polymorphisms for exfoliation syndrome in Japanese. Mol Vis. 2012;18:1395–1401.

17. Uçakhan OO, Karel F, Kanpolat A, Devrim E, Durak I: Superoxide dismutase activity in the lens capsule of patients with pseudoexfoliation syndrome and cataract. J Cataract Refract Surg. 2006;32:618–622.

18. Zhang Y, Zhang L, Sun D, Li Z, Wang L, Liu P: Genetic polymorphisms of superoxide dismutases, catalase, and glutathione peroxidase in age-related cataract. Mol Vis. 2011;17:2325–2332.

The paper was originally received 24.03.2019 (KO-00196-2019)/Praca wpłynęła do Redakcji 24.03.2019 (KO-00196-2019)
Accepted for publication 12.04.2019/Zakwalifikowano do druku 12.04.2019

Reprint requests to (Adres do korespondencji):
Hanna Lesiewska
Department of Ophthalmology, Nicolaus Copernicus University, Collegium Medicum in Bydgoszcz, Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland
e-mail: hanjot@op.pl
Tel/fax: 48 52 585 4033