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PSEUDOEXFOLIATIVE GLAUCOMA AND MOLECULAR GENETIC CHARACTERISTICS OF VITAMIN D METABOLISM

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✧ ***Purpose.*** To study the possible association of 25-hydroxyvitamin D level, and vitamin D receptor (VDR) gene polymorphisms (*BsmI*, *ApaI*, *TaqI*, *FokI*) with pseudoexfoliative glaucoma (PEG) clinical manifestations. ***Methods.*** We examined 160 subjects (72 males (45%), and 88 females (55%)) aged from 55 to 75 years, residents of St. Petersburg and Leningrad region. 122 patients with PEG were enrolled in the main study group, the control group comprised 38 subjects without PEG, primary open angle glaucoma (POUG) and pseudoexfoliation syndrome (PES). 25(OH)D serum levels were assessed by chemiluminescent immunoassay (CLIA) method. Detection of *VDR* gene allele polymorphisms (*ApaI*, *BsmI*, *FokI*, and *TaqI*) was carried out using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technique. ***Results.*** Patients with PEG had lower 25(OH)D serum levels compared to patients in the control group (39.3 ± 1.2 and 52.7 ± 2.1 nMol/l, respectively, $p < 0.01$). The prevalence of vitamin D deficiency was found to be higher among PEG patients than among healthy subjects (86.4% and 59.5%, respectively, $p < 0.01$). The prevalence of *b* allele ($p < 0.001$) and *bb* genotype ($p < 0.001$) (*BsmI* polymorphism), as well as of *f* allele and *ff* genotype ($p < 0.05$) (*FokI* polymorphism) in PEG patients were higher compared to healthy subjects. We found that the *F* allele carriers (*FokI* polymorphism) had greater corneal thickness than the *ff* genotype carriers (547.3 ± 4.1 μm and 502.1 ± 25.8 μm, respectively, $p < 0.01$). It was revealed, that *bb* genotype, *Bb* genotype (*BsmI* polymorphism), and *ff* genotype (*FokI* polymorphism) were associated with the increased risk of PEG (OR = 8.2, CI 95%: 3.4-19.9; OR = 3.9, CI 95%: 1.7-9.0; OR = 2.3, CI 95%: 1.2-4.5, respectively). ***Conclusions.*** Results of this study for the first time ever showed the association between *BsmI* and *FokI* VDR gene polymorphisms and pseudoexfoliative glaucoma.

✧ ***Keywords:*** pseudoexfoliative glaucoma; 25(OH)D; vitamin D receptor gene polymorphisms.

ПСЕВДОЭКСФОЛИАТИВНАЯ ГЛАУКОМА И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ОСОБЕННОСТИ ОБМЕНА ВИТАМИНА D

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✧ ***Цель работы*** — изучить возможную ассоциацию уровня 25-гидроксивитамина D и полиморфизмов *BsmI*, *ApaI*, *TaqI* и *FokI* гена рецептора витамина D (*VDR*) с клиническими проявлениями псевдоэксфолиативной глаукомы (ПЭГ). ***Методы.*** Обследовано 160 жителей Санкт-Петербурга и Ленинградской области в возрасте от 55 до 75 лет: 72 мужчины (45 %) и 88 женщин (55 %). Основную группу составили 122 пациента с диагнозом ПЭГ, группу контроля — 38 человек без ПЭГ, первичной открытоугольной глаукомы и псевдоэксфолиативного синдрома. Уровень 25(OH) D в сыворотке крови определяли методом иммунохемилюминесцентного анализа. Детекцию аллельных полиморфизмов *ApaI*, *BsmI*, *TaqI* и *FokI* гена рецептора витамина D проводили методом полимеразной цепной реакции — полиморфизма длин рестрикционных фрагментов (ПЦР-ПДРФ). ***Результаты.*** У пациентов с ПЭГ обнаружена более низкая концентрация 25(OH)D в сыворотке крови по сравнению с группой контроля (39,3 ± 1,2 и 52,7 ± 2,1 нМоль/л соответственно, $p < 0,01$). Количество больных с дефицитом витамина D в группе ПЭГ было значительно выше, чем в контрольной группе (86,4 и 59,5 % соответственно, $p < 0,01$). Выявлено, что у пациентов с ПЭГ чаще встречался *b*-аллель ($p < 0,001$) и *bb*-генотип ($p < 0,001$) полиморфизма *BsmI* и *f*-аллель и *ff*-генотип ($p < 0,05$) полиморфизма *FokI* гена *VDR* по сравнению с общей популяцией. Установлено, что у носителей F-аллеля полиморфизма *FokI* толщина роговой оболочки была больше, чем у носителей *ff*-генотипа (547,3 ± 4,1 и 502,1 ± 25,8 мкм соответственно, $p < 0,01$). Было выявлено, что генотипы *bb* и *Bb* полиморфизма *BsmI* и генотип *ff* полиморфизма *FokI* ассоциированы с увеличением риска ПЭГ (OR = 8,2, CI 95 %: 3,4–19,9; OR = 3,9, CI 95 %: 1,7–9,0 и OR = 2,3, CI 95 %: 1,2–4,5 соответственно). ***Выводы.*** В результате проведённого исследования впервые показана ассоциация между полиморфизмами *BsmI* и *FokI* гена рецептора витамина D и псевдоэксфолиативной глаукомой.

✧ ***Ключевые слова:*** псевдоэксфолиативная глаукома; 25(OH)D; полиморфизм гена рецептора витамина D.

INTRODUCTION

Pseudoexfoliation glaucoma (PEG) is the most aggressive form of primary open-angle glaucoma (POAG), which is often resistant to pharmacotherapy and surgical treatment. The highest prevalence of PEG is seen in Scandinavian countries and Northwestern and Central regions of the Russian Federation [7]. Pathogenesis of PEG and pseudoexfoliation syndrome (PEX) is driven by disorders of the extracellular matrix, oxidative stress, immunological disorders, apoptosis, genetic determinants, and environmental factors [22, 24, 27].

Vitamin D is a fat-soluble vitamin that is similar in its chemical structure and mechanism of action to steroids. Hydroxylated derivatives of vitamin D exhibit hormonal activity [6]. The main role of vitamin D is regulating calcium/phosphorus homeostasis; it has many pleiotropic effects on the inflammatory and immune processes, cell proliferation, differentiation, and apoptosis in various organs and tissues, including the eye [14, 18].

Vitamin D synthesis has been extensively investigated. Its inactive precursors are cholecalciferol, produced from 7-dehydrocholesterol in the skin via exposure to ultraviolet rays (270–310 nm), and ergocalciferol, obtained from food. The first stage of precursor activation occurs in the liver. Hepatic 25-hydroxylase (CYP27A1 and CYP2R1) converts the precursors to calcidiol, or 25-hydroxyvitamin D [25(OH)D], which is the main form circulating in the blood [6]. Then, 1-alpha-hydroxylase (CYP27B1) converts calcidiol into its active form 1,25-dihydroxyvitamin D3, also known as calcitriol [$1,25(OH)_2D$), or D-hormone]. This second stage of hydroxylation occurs in kidneys [8]. However, hydroxylation enzymes were also found in other organs and tissues; therefore, active D-hormone can be produced elsewhere. Several experimental studies and clinical trials have shown $1,25(OH)_2D$ production in the corneal endothelium (CE), non-pigmented ciliary epithelium (NPCE), retinal pigment epithelium (RPE), and scleral fibroblasts (SF) [10]. The ability of ocular barrier epithelial cells to produce the active form of vitamin D is comparable to that of the metabolically active tissues, including the respiratory, bladder, and breast epithelia [10, 19, 28].

Most of the biological effects of $1,25(OH)_2D$ are mediated by a specific nuclear receptor NR1I1 [vitamin D receptor (VDR)] [15]. VDR is a DNA-binding transcription factor that regulates multiple physiological processes, including cell differentiation, apoptosis, immune reactions, and lipid and carbohydrate metabolism, thereby acting as a protective agent for cardiovascular, autoimmune, oncological, and other diseases [1, 4, 14, 23].

So far, few studies have reported the presence of VDRs in the ocular tissues. VDR mRNA was found in the corneal and limbal epithelium [19, 28]. Alsalem et al. also reported that cells from the SF, CE, NPCE, and RPE are capable of expressing mRNA and the VDR protein [10]. The highest expression was observed in CE and NPCE, which plays an important role in intraocular fluid production [10].

VDR polymorphism is believed to be involved in the pathogenesis of ocular diseases [1, 2, 17, 23]. The following single nucleotide polymorphisms (SNPs) of *VDR* are well researched: *Cdx2* (*rs*11568820) in exon 1, *FokI* (*rs*10735810) in exon 2, *BsmI* (*rs*1544410) and *ApaI* (*rs*7975232) in intron 8, and *TaqI* (*rs*731236) in exon 9 [12, 26]. There is an association between these SNPs and Parkinson's disease, diabetes mellitus, cancer, and Alzheimer's disease [17, 23, 26]. Only few studies have explored an association between *VDR* polymorphisms and ocular disorders [2, 20]. Understanding the mechanisms underlying the development of open-angle glaucoma (including PEG and POAG) in the context of vitamin D action will further expand the knowledge in this area.

This study aimed to explore the association between the level of 25(OH)D, *VDR* polymorphisms (*BsmI*, *ApaI*, *TaqI*, and *FokI*), and clinical manifestations of PEG.

MATERIALS AND METHODS

The study included 160 residents of Saint Petersburg and the Leningrad region of Caucasian origin (aged between 55 and 75 years). They comprised 72 males (45%) and 88 females (55%) who signed an informed consent to participate in the study. All participants were divided into two groups. The study group included 122 patients (42% males and 58% females) with stage I–III PEG, whereas the control group comprised 38 patients (55% males and 45% females) with POAG and PEX, but without PEG.

Patients who had one or more of the following conditions were excluded: diabetes mellitus; cancer; autoimmune disorders; severe comorbidity therapy with corticosteroids, immunosuppressive agents, or vitamin D; history of uveitis; acute circulatory disorders in the central retinal artery/vein; eye trauma; corneal disorders; or a wet form of age-related macular degeneration.

Ophthalmologic examination was performed in the Polyclinic and the Department of Ophthalmology at the I.P. Pavlov First Saint Petersburg State Medical University. All patients underwent autorefractometry, visual acuity testing, Maklakov's applanation tonometry, biomicroophthalmoscopy, and automated perimetry. The optic disc (OD) parameters were evaluated using the Heidelberg Retinal Tomography II. The length of the anteroposterior axis (APA) of the eyeball and central corneal thickness (CCT) were measured using an ultrasonic AL-3000 Bio & Pachymeter (Tomey Corporation, Japan).

Molecular and biochemical tests were performed in the Laboratory of Human Biochemical Homeostasis at the Research Institute of Nephrology, I.P. Pavlov First Saint Petersburg State Medical University.

Serum 25(OH)D levels were measured by immunochemiluminescence using the Architect i2000 SR System (Abbott Laboratories, USA). The recommendations of the Russian Association of Endocrinologists (2015) were used to assess vitamin D deficiency: serum 25(OH)D levels of ≥75 nmol/L (normal); 50–75 nmol/L (insufficient); <50 nmol/L (deficient) [6].

VDR polymorphisms (including *BsmI*, *ApaI*, *TaqI*, and *FokI*) were measured in 120 patients with PEG (50 males and 70 females) using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with restriction endonucleases (SibEnzyme, Novosibirsk). PCR was performed using the iCycler System (BioTeck, United Kingdom). The specificity of the primers was verified using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primerblast) (Table 1). *FokI* polymorphism was visualized using a 3% agarose gel, whereas *ApaI*, *BsmI*, and *TaqI* polymorphisms were visualized using a 12% polyacrylamide gel. We used the 100 bp + 50 bp DNA ladder (SibEnzyme, Novosibirsk) to assess the size of PCR products.

The lengths of the amplicon and restriction endonuclease recognition sites were determined using National Center for Biotechnology Information and NebCutter resources. Standard nomenclature for *BsmI*, *ApaI*, *TaqI*, and *FokI* polymorphisms are used, and the alleles without the restriction sites are indicated by capital letters (*B*, *A*, *T*, *F*). The alleles with restriction sites are indicated by lowercase letters (*b*, *a*, *t*, *f*) [12].

The results of molecular testing of patients with PEG were compared with those of 212 healthy controls (145 males and 67 females) examined in the Almazov National Research Centre [4]. The comparison group was considered to be a general population.

Table 1

Primers for *VDR* polymorphisms (*FokI*, *ApaI*, *BsmI,* and *TaqI*)

Таблица 1

Структура олигопраймеров для определения аллельных полиморфизмов *FokI*, *ApaI*, *BsmI* и *TaqI* в гене *VDR*

Gene	Location	Polymorphism	Structure of oligoprimers
VDR	12q13-14	*FokI* (T>C)	F: 5-AGCTGGCCCTGGCACTGACTCTGGCTCT3 R: 5-ATGGAAACACCTTGCTTCTTCTCCCTC-3
		ApaI (A>C)	F: 5-GTATCACCGGTCAGCAGTCATAGA-3 R: 5-TGTACGTCTGCAGTGTGTTGGA-3
		BsmI (A>G)	F: 5-GAGCCCAGTTCACGCAAGAG-3 R: 5-GGGGGGATTCTGAGGAACTAGATA-3
		TaqI (C>T)	F: 5-GTATCACCGGTCAGCAGTCATAGA-3 R: 5-TGTACGTCTGCAGTGTGTTGGA-3

STATISTICAL ANALYSIS

Statistical analysis was performed using STATISTICA 9.0 for Windows. The Pearson χ^2 test, including the Yates correction for small groups, and the Fisher exact test were used to compare categorical variables. Continuous variables were compared using the Mann–Whitney U-test, Wald test, median χ^2, and analysis of variance. To assess the relative risk of developing the disease, odds ratios (ORs) and confidence intervals (CIs, the intervals that contain the true OR with a probability of 95%) were assessed. An OR = 1 indicates no risk, an OR < 1 indicates a negative association (protective factor), and an OR > 1 indicates a positive association between the allele/genotype and the disease (risk factor). CIs for categorical variables were calculated using the Fisher exact test. Descriptive statistics are shown as means with standard deviations ($M \pm m$).

RESULTS

Analysis of biometric parameters, including the eye length (mm), CCT (μm), and OD size (mm^2), demonstrated no significant differences between the experimental and control groups (Table 2).

Biochemical testing demonstrated that patients with PEG have lower serum 25(OH)D levels than controls (39.3 ± 1.2 nmol/L vs. 52.7 ± 2.1 nmol/L, respectively, $p < 0.01$). The number of patients with vitamin D deficiency was higher in the PEG group than in the control one (86.4% vs. 59.5%, respectively, $p < 0.01$). Moreover, 12.6% of the patients with PEG had severe vitamin D deficiency (<25 nmol/L).

The distribution of genotypes and prevalence of *BsmI*, *ApaI*, *TaqI*, and *FokI VDR* polymorphisms and their alleles in patients with PEG and in the general population are shown in Table 3.

The observed frequencies of all *VDR* genotypes were consistent with Hardy–Weinberg equilibrium frequencies. There was no significant difference in the distribution of genotypes and prevalence of *ApaI* and *TaqI VDR* polymorphisms and their alleles between the groups. However, patients with PEG were more likely to have the *BsmI b* allele ($p < 0.001$) and *bb* genotype as well as the *FokI f* allele and *ff* genotype ($p < 0.05$) than those in the general population. The distribution of the *VDR* genotypes between the males and females was similar in the PEG group and in general population ($p > 0.05$).

There were no differences in the serum 25(OH)D levels between patients with PEG carrying various *VDR* genotypes (Table 4).

Eye length, CCT, and OD size in patients with various genotypes of *BsmI*, *ApaI*, *TaqI*, and *FokI VDR* polymorphisms were measured (Table 5).

As shown in Table 5, there were no differences in the parameters studied between patients with PEG with various *VDR* genotypes. However, individuals with a *FokI F* allele had higher CCT than carriers of the *ff* genotype (547.3 ± 4.1 μm vs. 502.1 ± 25.8 μm, respectively, $p < 0.01$).

The risk of PEG depending on the genotypes of the *VDR* polymorphisms was assessed. Being carriers of the *BsmI bb* genotype increased the risk of PEG by 8.2 times [95% CI: 3.4–19.9], whereas the *BsmI Bb* genotype increased the risk of PEG by 3.9 times [95% CI: 1.7–9.0]. Therefore, PEG is associated with the

Table 2

Eye length, central corneal thickness, and optic disc size in patients with pseudoexfoliation glaucoma and controls

Таблица 2

Длина передне-задней оси глазного яблока, толщина роговицы в центре и площадь диска зрительного нерва у больных псевдоэксфолиативной глаукомой и в группе контроля

Parameter	Patients with pseudoexfoliation glaucoma	Controls	*p*
Anteroposterior axis, mm	22.5 ± 0.4	22.3 ± 0.6	> 0.05
Central corneal thickness, μm	537.8 ± 7.9	560.3 ± 5.9	0.09
Optic disc size, mm^2	1.7 ± 0.1	1.6 ± 0.1	> 0.05

Table 3

Distribution of genotypes and prevalence of *BsmI*, *ApaI*, *TaqI*, and *FokI VDR* polymorphisms and their alleles in patients with pseudoexfoliation glaucoma and in general population

Таблица 3

Распределение генотипов и встречаемость аллелей полиморфизмов *BsmI*, *ApaI*, *TaqI* и *FokI* гена рецептора витамина D у пациентов с псевдоэксфолиативной глаукомой и в общей популяции

Polymorphisms	Distribution of genotypes (%) and prevalence of alleles					*p*
BsmI	***BB***	***bb***	***Bb***	***B*-allele**	***b*-allele**	
Patients with pseudoexfoliation glaucoma	5.8	43.3	50.8	0.31	0.69	< 0.001
Controls	24.1	21.7	54.2	0.51	0.49	
ApaI	***AA***	***aa***	***Aa***	***a*-allele**	***a*-allele**	
Patients with pseudoexfoliation glaucoma	17.5	27.5	55.0	0.45	0.55	> 0.05
Controls	26.0	22.6	51.4	0.52	0.48	
TaqI	***TT***	***tt***	***Tt***	***T***	***t***	
Patients with pseudoexfoliation glaucoma	39.2	15.0	45.8	0.62	0.38	> 0.05
Controls	45.3	9.4	45.3	0.68	0.32	
FokI	***FF***	***ff***	***Ff***	***F***	***f***	
Patients with pseudoexfoliation glaucoma	25.0	25.0	50.0	0.5	0.5	< 0.05
Controls	34.0	14.6	51.4	0.6	0.4	

b allele of the *BsmI VDR* polymorphism. Moreover, carriers of the *FokI ff* genotype are 2.3 more likely to develop PEG [95% CI: 1.2–4.5].

DISCUSSION

A thin cornea is one of the risk factors for glaucoma [13]. Many studies have reported that patients with PEX/PEG often have reduced CCT, which can likely be attributed to a decrease in the density of stromal cells and loss of endothelial cells [9, 25]. In our study, patients with PEG tended to have lower CCT than those in the control group (537.8 ± 7.3 μm vs 560.3 ± 5.9 μm, respectively), although this difference was not significant ($p = 0.09$).

The true prevalence of vitamin D deficiency among patients with various forms of glaucoma is largely unknown because few studies are currently available. Our findings suggested that patients with

Table 4

Serum 25(OH)D levels in patients with pseudoexfoliation glaucoma carrying different genotypes with *BsmI*, *ApaI*, *TaqI*, and *FokI* VDR polymorphisms

Таблица 4

Концентрация 25(OH)D в сыворотке крови у больных псевдоэксфолиативной глаукомой, носителей различных генотипов полиморфизмов *BsmI*, *ApaI*, *TaqI* и *FokI* гена рецептора витамина D

Polymorphisms	25(OH)D, nmol/L			*p*
BsmI	***BB***	***Bb***	***bb***	
	30.4 ± 6.2	39.5 ± 1.6	40.05 ± 2.0	> 0.05
ApaI	***AA***	***Aa***	***aa***	
	40.4 ± 4.2	39.1 ± 1.4	38.6 ± 2.7	> 0.05
TaqI	***TT***	***Tt***	***tt***	
	39.9 ± 2.2	39.1 ± 1.6	37.7 ± 3.7	> 0.05
FokI	***FF***	***Ff***	***ff***	
	41.9 ± 2.1	38.5 ± 1.8	38.0 ± 2.9	> 0.05

Table 5

Eye length, central corneal thickness, and optic disc size in patients with different genotypes of *BsmI*, *ApaI*, *TaqI*, and *FokI* VDR polymorphisms

Таблица 5

Длина передне-задней оси глазного яблока, центральная толщина роговицы и площадь диска зрительного нерва у больных псевдоэксфолиативной глаукомой, носителей различных генотипов полиморфизмов *BsmI*, *ApaI*, *TaqI* и *FokI* гена рецептора витамина D

Parameter	Genotypes			*p*
BsmI polymorphism of the vitamin D receptor gene				
	BB	*Bb*	*bb*	
APA, mm	23.4 ± 0.3	22.1 ± 0.7	22.8 ± 0.5	> 0.05
CCT, μm	544.8 ± 8.6	538.6 ± 8.6	533.7 ± 11.5	> 0.05
OD size, mm^2	1.6 ± 0.3	1.6 ± 0.1	1.7 ± 0.1	> 0.05
ApaI polymorphism of the vitamin D receptor gene				
	AA	*Aa*	*aa*	
APA, mm	21.9 ± 1.2	22.3 ± 0.6	23.2 ± 0.2	> 0.05
CCT, μm	546.3 ± 9.9	530.9 ± 12.6	543.1 ± 6.5	> 0.05
OD size, mm^2	1.48 ± 0.2	1.66 ± 0.1	1.69 ± 0.1	> 0.05
TaqI polymorphism of the vitamin D receptor gene				
	TT	*Tt*	*tt*	
APA, mm	22.7 ± 0.5	22.1 ± 0.8	23.2 ± 0.2	> 0.05
CCT, μm	532.9 ± 0.2	533.8 ± 11.7	556.8 ± 8.4	> 0.05
OD size, mm^2	1.7 ± 0.1	1.65 ± 0.1	1.48 ± 0.2	> 0.05
FokI polymorphism of the vitamin D receptor gene				
	FF	*Ff*	*ff*	
APA, mm	23.2 ± 0.2	22.1 ± 0.7	22.6 ± 0.8	> 0.05
CCT, μm	543.3 ± 5.4	536.8 ± 10.7	530.7 ± 19.9	> 0.05
OD size, mm^2	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	> 0.05

Note: APA, anteroposterior axis (eye length); CCT, central corneal thickness; OD, optic disc

PEG/POAG/PEX have a higher prevalence of vitamin D deficiency than those without glaucoma or PEX ($p < 0.01$) [3]. Our results were consistent with those observed in previous publications. A French study assessed 25(OH)D serum levels in 314 individuals with POAG and reported a high prevalence of vitamin D deficiency regardless of the time of year. The authors also showed that an increase in 25(OH)D levels by 10 nmol/L reduced the risk of POAG, whereas vitamin D deficiency increased the risk of POAG by 2.1 times [95% CI: 1.06–4.12] [16]. The results of a Korean study comprising 6094 patients, aged >45 years, also suggested that low vitamin D levels significantly increased the risk of glaucoma and were associated with clinical manifestations of optic nerve dysfunction in patients with POAG [29].

In our study, patients with both PEG and POAG were likely to have vitamin D deficiency, and the most severe forms of it (86.4% in patients with PEG and 59.5% in controls, $p < 0.01$). Vitamin D deficiency may affect the renewal of the extracellular matrix, the progression of apoptosis in retinal ganglion cells, and the state of the endothelium and vascular basement membranes in structures responsible for eye hydrodynamics and production of pseudoexfoliation material, leading to impairments typical of PEX/PEG [10, 18, 21, 30].

Considering the association between the genetic characteristics of VDR and various diseases [12, 17, 23], we analyzed four *VDR* polymorphisms in patients with PEG. The distribution of *VDR* genotypes between males and females was similar in the PEG group and in general population, which was consistent with the result obtained from other Russian studies [4]. Patients with PEG are more likely to carry the *BsmI b* allele and *bb* genotype and the *FokI f* allele and *ff* genotype. Previous molecular studies on *VDR* polymorphisms in patients with ocular disorders primarily focused on diabetic retinopathy, age-related macular degeneration, and myopia [2, 23] with few studies evaluating *VDR* genotypes in patients with glaucoma. A Chinese study assessed *BsmI*, *TaqI*, *FokI*, and *Cdx2 VDR* polymorphisms in Chinese Han patients with POAG ($n = 71$), and the results reported the predominance of the *BsmI Bb* genotype and *B* allele ($p = 0.001$ and $p = 0.002$, respectively) and the *TaqI Tt genotype* and *t* allele ($p = 0.013$ and $p = 0.018$, respectively) compared with healthy controls ($n = 73$) [20]. We also showed an association between PEG and *BsmI* polymorphisms; however, Caucasian patients with glaucoma predominantly have the *bb* genotype. There were significant differences in the distribution of *FokI* genotypes between the groups. This can likely be attributed to ethnic specificity, which was confirmed by literature data including those obtained from the Russian Federation [5].

None of the genotypes studied were associated with serum 25(OH)D levels. The prevalence of vitamin D deficiency was high in both the study and the control group.

There were no differences in the eye length and OD size between the patients with PEG carrying various genotypes of *BsmI*, *ApaI*, *TaqI*, and *FokI VDR* polymorphisms. Patients with the *FokI ff* genotype had thinner cornea than those carrying the *F* allele (CCT, 502.05 ± 141.72 μm vs. 547 ± 37.97 μm, respectively, $p < 0.01$). This could be due to the specific characteristics of VDR protein in *FokI* polymorphism carriers. *FokI* polymorphism is located in the coding region of *VDR* and determines the structure of VDR protein. This polymorphism is considered as an independent marker for *VDR* [12, 26]. The shorter variant of VDR protein (424 amino acids, encoded by the *F* allele) has a higher (1.7 times) transcription activity than the longer one (427 amino acids, encoded by *f* allele), which may potentially affect the synthesis and function at the transcriptional and posttranslational levels of many molecules, such as various types of collagen, matrix metalloproteinases, and transforming growth factors, involved both in PEG and POAG pathogenesis [11].

Analyzing the correlation between PEG and *VDR* polymorphisms, the *BsmI* bb and Bb genotypes were shown to increase PEG risk by 8.24 and 3.9 times, respectively. Carriers of *FokI ff* genotype are 2.3 times more likely to develop PEG. Therefore, these genotypes should be considered as risk factors for glaucoma. The *BsmI* polymorphism, located in the 3′ untranslated region, affects the functioning of VDR protein. Therefore, it can be associated with the disease due to the linkage to unknown important allele areas located in distant regions in the nearest gene. Similar to the *FokI* polymorphism (*ff* genotype), SNPs located in the untranslated regions of *VDR* can potentially affect *VDR* mRNA stability, thereby triggering a number of ocular diseases [12, 26].

CONCLUSION

Our findings suggest that the majority of patients with PEG of Caucasian origin residing in Saint Petersburg and Leningrad region has mild to severe vitamin D deficiency that is not associated with any *VDR* polymorphisms, including *BsmI*, *ApaI*, *TaqI*, and

FokI. This study also demonstrated an association between the *FokI ff* genotype and lower CCT, as well as a correlation between the *BsmI bb/Bb* and *FokI ff* genotypes, and the increased risk of PEG.

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