Selected RANKL/RANK/OPG system genetic variants in diabetic foot patients

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Abstract

Purpose Diabetic foot is a complication of long-lasting diabetes mellitus affecting up to 15% of patients, both in type 1 and type 2 diabetes. Osteoprotegerin is involved in osteogenesis and calcification. The aim of the study was to assess the role of selected osteoprotegerin gene variants in diabetes patients with diabetic foot.

Methods The study involved 300 patients with diabetes and diabetic foot and 968 healthy controls. The study group was formed by 243 patients with diabetic foot of neuropathic origin, 102 with diabetic foot of neuroischemic origin and 77 with Charcot neuroarthropathy.

Results Compared to controls, rs1872426 and rs1485286 showed correlation with diabetic foot in diabetes subjects. Significant associations between rs2073618, rs1872426, rs7464496 and rs1485286 in men were reported. The aforementioned correlations were also present in type 2 diabetes patient subgroup. Variant rs1485286 was associated to diabetic foot of neuropathic origin. Sex-specificity for females was present for rs6993813 in patients with diabetic foot of neuropathic origin and type 1 diabetes. Variants rs1872426, rs2073617 and rs1485286 were correlated with CN. We found that age, body weight, body mass index, waist circumference, hip circumference and waist-hip ratio were among the basic risk factors of diabetic foot.

Conclusions The following variants *TNFRSF11B* (rs2073618, rs2073617, rs1872426, rs1032128, rs7464496, rs11573829 and rs1485286), *COLEC10* (rs6993813, rs3134069) and *TNFSF11* (rs9533156) present differences in allele frequencies in diabetic foot patients and show correlation with gender, diabetes type and diabetic foot etiology.

Keywords Diabetes · Genetic · Osteoprotegerin · Polymorphism

Introduction

Diabetic foot (DF) is a complication of long-lasting diabetes affecting up to 15% of patients, both in type 1 and type 2 diabetes mellitus (DM) [1, 2]. DF incidence varies worldwide with the lowest percentage in Australia (1.5%) and the highest in Belgium (16.6%) [3]. There is a difference in DF incidence depending on DM type with 6.4% in type 2 DM and 5.5% in type 1 DM. Moreover, the incidence of DF appears higher in

males at 4.5%, while in females it is 3.5%, putting men at a slightly higher risk [3]. Charcot neuroarthropathy (CN), called also Charcot's joint, is a rare complication of long-lasting DM occurring concurrently with vascular damage. In CN, joint degeneration of the ankle and foot are observed. The consequence of CN is foot deformity, which is diagnosed in 0.8–8.0% of DM patients [4, 5]. In the majority of cases, CN progression leads to chronic foot ulcerations and amputation of the lower limb. While many molecular pathways leading to CN have been described, it remains unclear how exactly such mechanisms influence DF etiologies. As bone destruction is an important clinical feature of CN, cellular mediators involved in bone metabolism must be involved in DF pathology. Osteoprotegerin (OPG) is a secreted protein that is part of the RANKL/RANK/OPG system involved in the regulation of bone resorption [6]. OPG is encoded by the tumor necrosis factor receptor superfamily member 11B gene (*TNFRSF11B*), however some genetic variants are classically described as

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collectin subfamily members 10 (*COLEC10*). OPG protects receptor activator for nuclear factor κ B (RANK) against the RANK ligand (RANKL) [7]. Results of in vitro and in vivo studies indicate that in the absence of OPG, RANKL cytokine binds RANK causing bone tissue resorption [8]. Moreover, the antagonizing role of OPG for RANKL activity against resorption has been proven [9–11]. The analyzed genetic variants of the RANK gene belong to the TNF superfamily member 11 gene (*TNFSF11*).

A recent study revealed a correlation of RANKL/RANK/OPG allelic variants in CN (rs2073618 and rs3134069) [12]. There was also a correlation of RANKL/RANK/OPG variants with DF in the type 2 DM population studied [13]. The study showed no correlation of *TNFRSF11B* and *COLEC10* variant allele distribution in DM and healthy subjects, and a similar correlation between DF vs DM patient and DF vs healthy controls, which was the impetus to focus further investigations on cases with DF and healthy controls without an intermediate group of patients with DM without DF. The aim of our study was focused on the role of selected RANKL/RANK/OPG variants in DF pathology in type 1 DM and type 2 DM patients.

Methods

Three hundred adult patients with DM hospitalized in the Department of Diabetology and Internal Diseases, Medical University of Warsaw, Poland, were recruited into the study. The control group genotype originated from WOBASZ I (*pl. Wieloośrodkowe Ogólnopolskie Badanie Stanu Zdrowia Ludności*), a study held by the Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion, Institute of Cardiology, Warsaw, Poland [14]. The inclusion criteria for controls were negative history of congestive heart failure, myocardial infarction, coronary heart disease and thrombotic events, as well as a lack of DM. Groups were matched by gender.

Diagnosis of DF was established according to the International Consensus on the Diabetic Foot and Practical Guidelines on the Management and Prevention of the Diabetic Foot 2007. The Toronto Clinical Neuropathy Scoring System was used for peripheral sensorimotor neuropathy diagnosis. Detection of pulse on posterior tibial and dorsal artery of the foot was used to assess the presence of an ischemic component. Doppler ultrasound was used for ischemia confirmation.

The following single nucleotide polymorphisms (SNPs) were studied: rs2073618, rs1872426, rs1032129, rs11573829, rs7464496, rs6469788, rs6415470, rs3134063, rs1485286, rs1032128, rs2073617, rs10101385 and rs6993813 from *TNFRSF11B*, rs6993813 and rs3134069 from *COLEC10* and rs9533156 from *TNFSF11*.

DNA was isolated from whole blood samples collected in EDTA probes using the salting-out method. SNP genotyping was performed using *TaqMan SNP Genotyping Assay* (Applied Biosystems, USA). Analysis of allelic variants was performed by real-time polymerase chain reaction (RT-PCR) with the use of QuantStudio™ 12 K Flex (Life Technologies, Carlsbad, USA) according to the manufacturer's protocol.

Statistical analysis was performed using STATISTICA 13.1PL (StatSoft, Inc. 2017) software. Normal distribution and variances of the data were examined using the Kolmogorov–Smirnov, Shapiro–Wilk and Lilliefors tests. For quantitative variables that met the normal distribution criteria, a parametric Student's *t* test was used. For variables that did not meet the criteria for normal distribution, the Mann–Whitney *U* or Kruskal–Wallis tests were used. Statistical significance of was set at $p = 0.05$. Univariate logistic regression with the quasi-Newton method was used to assess statistically significant quantitative variables. The missing data were removed in pairs. The Hardy–Weinberg equilibrium (HWE) of allele frequencies in the studied groups was assessed using χ^2 or Fisher's exact test. The distribution of alleles in allelic variants was analyzed with PLINK 2.0 alpha software (Christopher Chang, Human Longevity, Inc. Data Science team, Stanford University's Department of Biomedical Data Science). The effective models were defined as dominant (RA + RR vs AA), additive (RR vs RA vs AA) and recessive (RR vs AA+RA), where R stands for 'risk allele'. The study was approved by the Ethic Committee of Medical University of Warsaw.

Results

In this study, we included 300 patient with diabetes (252 patients with type 2 DM, 43 with type 1 DM and 5 with other types of DM). Neuropathy was present in 261 patients and peripheral arterial disease in 122 (87% and 40.6%, respectively). Out of these, 166 patients were diagnosed with DF of neuropathic origin and 102 with DF of neuroischemic origin. CN was diagnosed in 77 patients (25.7% of the study group). Two hundred fifty patients were treated with insulin therapy. The most common complications of DM were diabetic retinopathy (195 patients, 65%) and diabetic nephropathy (141 patients, 47%). A history of coronary heart disease was present in 132 patients (44%), thrombotic events in 45 patients (15%), myocardial infarction in 104 patients (34.7%), hypertension in 254 patients (84.7%), dyslipidemia in 201 patients (67%), and lower limb amputation (LLA) in 107 patients (35.7%). Statistically significant differences between DF and controls were in age, weight, height, BMI, hip circumference and waist-hip ratio (WHR). Characteristics and differences between anthropometric features of DF and controls are presented in Table 1.

Table 1 Characteristic of basic anthropometric features between patients with diabetic foot and controls

	Cases (<i>n</i> = 300)			Controls (<i>n</i> = 968)			*, **	Regression	
	Mean	SD	Median	Mean	SD	Median		OR	95% CI
Age, y*	61.3	11.5	62.0	53.6	13.1	55.4	<10 ^{−5}	1.06	1.04–1.07
Weight, kg*	91.6	19.0	91.0	74.7	14.5	73.3	<10 ^{−5}	1.06	1.05–1.08
Height, cm*	171.9	9.2	173.0	167.5	8.6	168.0	<10 ^{−5}	1.06	1.05–1.08
BMI, kg/m ² *	30.94	5.65	30.64	26.58	4.57	26.04	<10 ^{−5}	1.19	1.16–1.23
Waist circ., cm**	104.3	15.7	104.0	92.2	13.1	93.0	<10 ^{−5}	1.12	1.10–1.15
Hip circ., cm*	107.5	13.4	105.0	104.2	9.2	103.0	<10 ^{−4}	1.03	1.02–1.05
WHR*	0.97	0.08	0.98	0.88	0.09	0.89	<10 ^{−5}	–	–

BMI body mass index, WHR waist-hip ratio, OR odds ratio

*Mann-Whitney *U* test

**Student *t* test

Hypertension, smoking and dyslipidemia were significantly more frequent in the DF group. Differences in quantitative variables are presented in Supp. Table 1.

Our analysis showed differences in allele frequencies of *TNFRSF11B* (rs2073618, rs2073617, rs1872426, rs1032128, rs7464496, rs11573829 and rs1485286), *COLEC10* (rs6993813, rs3134069) and *TNFSF11* (rs9533156) variants. Risk allele frequencies of investigated variants in studied groups is showed in Table 2.

Besides the fact that in the HapMap for the Central European population, the T allele frequency of the rs3134063 variant was 0.49, our analysis showed there were only CC homozygotes in the studied groups. In variant rs1485286, alleles were in disequilibrium.

Allele C of rs2073618 was more frequently present in DF males, DF males with type 2 DM and DF males without DM

family history when compared to controls. Moreover, the presence of allele C was more frequent in type 2 DM DF patients compared to type 1 DM DF patients. Allele C was also more frequent in DF males without DM family history and in DF males with diabetic nephropathy and with diabetic retinopathy.

Allele C of rs2073617 was more frequent in males with DF of neuropathic origin and type 2 DM, whereas it was less frequent in CN patients with type 2 DM compared to DF of neuroischemic origin and in DF females who underwent LLA compared to control females (Table 3).

Allele A of rs1872426 was more frequent in DF patients compared to controls, and this correlation was also present in selected groups of males and patients with type 2 DM, irrespective of DF etiology. Moreover, allele A of rs1872426 was more frequent in DF males with diabetic nephropathy and in DF males with diabetic retinopathy.

Table 2 Risk allele frequencies of investigated variants in studied groups

	Risk allele	RAF cases	RAF controls	χ^2	<i>P</i>	Correlation with DF
rs2073618 (C/G)	C	0.49	0.46	1.07	0.30	present
rs2073617 (C/T)	C	0.46	0.44	0.38	0.54	present
rs10101385 (A/G)	G	0.48	0.50	0.50	0.48	–
rs1872426 (A/C)	A	0.49	0.47	0.88	0.35	present
rs1032128 (A/G)	A	0.29	0.29	0.03	0.97	present
rs1032129 (A/C)	G	0.46	0.46	0.002	0.95	–
rs11573829 (A/G)	G	0.46	0.46	0.002	0.97	present
rs7464496 (C/T)	C	0.48	0.46	0.75	0.39	present
rs6469788 (A/C)	A	0.46	0.45	0.004	0.95	–
rs6415470 (A/G)	G	0.46	0.46	0.002	0.97	–
rs3134063 (C/T)	T	0.49	0.47	1.20	0.27	–
rs1485286 (C/T)	C	0.27	0.32	5.35	0.02	present
rs6993813 (C/T)	C	0.52	0.49	2.13	0.15	present
rs3134069 (A/C)	C	0.06	0.07	1.15	0.28	present
rs9533156 (C/T)	C	0.50	0.48	0.76	0.39	present

DF diabetic foot, RAF risk allele frequency

Table 3 Differences in *TNFRSF11B* (rs2073618 and rs2073617) alleles frequencies between selected groups

	Risk allele frequency (RAF)		OR (95% CI) <i>P</i>		
	Cases	Controls	Dominant	Additive	Recessive
rs2073618 (C/G) Risk allele: C					
DF males vs control males	0.50	0.45	1.57 (1.07–2.30) 0.02	–	–
DF type 2 DM males vs control males	0.51	0.45	1.73 (1.15–2.61) 0.01	–	–
DF type 2 DM vs DF type 1 DM	0.50	0.42	–	–	2.47 (0.93–6.55) 0.07*
DF males without DM in family vs control males	0.51	0.47	2.08 (1.08–3.99) 0.03	–	–
DF males + nephropathy vs control males	0.51	0.47	1.79 (1.05–3.07) 0.03	–	–
DF males + retinopathy vs control males	0.50	0.47	1.59 (1.01–2.50) 0.04	–	–
rs2073617 (C/T) Risk allele: C					
Neuropathic DF type 2 DM males vs control males	0.48	0.44	1.76 (1.02–3.02) 0.04	–	–
CN type 2 DM vs neuroischemic DF	0.39	0.47	–	–	0.35 (0.13–1.01) 0.05*
DF females + LLA vs control females	0.38	0.47	–	0.56 (0.31–1.01) 0.05*	0.15 (0.02–1.09) 0.06*

CN Charcot's neuroarthropathy, LLA lower limb amputation, DM diabetes mellitus

*- tendency

for others, see Table 1 and Table 2

Allele C of rs1485286 was less frequent in DF patients, both in males and in patients with type 2 DM. This allele was also less frequent in patients with DF of neuropathic origin and in subgroups of males and patients with type 2 DM. Allele C of rs1485286 was less frequent in DF patients and in males who underwent LLA. There was also a lower frequency of this allele in DF patients with diabetic nephropathy and retinopathy, however, the correlation with nephropathy was sex-specific for females, whereas the correlation with retinopathy was sex-specific for males (Table 4).

Allele C of rs7464496 was more frequent in DF males and DF males with type 2 DM. There was also a higher frequency of this allele in type 2 DM DF patients compared to type 1 DM DF individuals. This allele was also more frequent in males with DF and diabetic nephropathy.

Allele C of rs6993813 was more frequent in females with DF of neuropathic origin and in type 1 DM females with CN.

However, allele C of this SNP was less frequent in males with DF and diabetic nephropathy.

Allele C of rs3134069 was less frequent in DF patients with nephropathy, with sex-specificity for males and in DF patients with retinopathy.

Allele C of rs9533156 was less frequent in females with CN, type 2 DM females with CN and in females with DF of neuroischemic origin. Allele C was shown to be more frequent in patients with DF of neuropathic origin compared to patients with DF of neuroischemic etiology and a lower frequency was found in DF females with diabetic nephropathy compared to control females. Allele C of rs9533156 was more frequent in DF patients who underwent LLA, and this correlation was also sex-specific for males (Table 5.)

Haplotype analysis showed that the GACTAA haplotype of rs1032128 | rs11573829 was more frequent in DF with type 2 DM patients compared to DF and type 1 DM individuals (OR = 0.37; *p* = 0.02). A similar correlation was present

Table 4 Differences in *TNFRSF11B* (rs1872426 and rs1485286) alleles frequencies between selected groups

	Risk allele frequency (RAF)		OR (95% CI) <i>P</i>		
	Cases	Controls	Dominant	Additive	Recessive
rs1872426 (A/C) Risk allele: A					
DF vs controls	0.49	0.48	1.4 (1.00–1.96) 0.05	–	–
DF males vs control males	0.51	0.46	1.89 (1.20–2.96) 0.006	–	–
DF type 2 DM vs controls	0.50	0.46	1.49 (1.04–2.13) 0.03	–	–
DF type 2 DM males vs control males	0.52	0.46	2.04 (1.27–3.30) 0.003	–	–
Neuropathic DF males vs control males	0.50	0.46	1.91 (1.07–3.39) 0.03	–	–
Neuroischemic DF males vs control males	0.52	0.47	3.05 (1.17–7.95) 0.02	–	–
DF males + nephropathy vs control males	0.51	0.47	2.06 (1.08–3.96) 0.03	–	–
DF males + retinopathy vs control males	0.51	0.47	2.20 (1.25–3.59) 0.007	–	–
rs1485286 (C/T) Risk allele: C					
DF vs controls	0.34	0.37	–	0.80 (0.65–0.97) 0.02	0.46 (0.27–0.76) 0.003
DF males vs control males	0.28	0.31	–	–	0.41 (0.20–0.81) 0.008
DF type 2 DM vs controls	0.28	0.32	–	0.81 (0.66–1.00) 0.05*	0.52 (0.30–0.88) 0.01
DF type 2 DM males vs control males	0.28	0.31	–	–	0.46 (0.23–0.32) 0.03
Neuropathic DF vs controls	0.27	0.32	–	–	0.36 (0.17–0.75) 0.007
Neuropathic DF males vs control males	0.27	0.31	–	–	0.34 (0.14–0.87) 0.02
Neuropathic DF type 2 DM vs controls	0.28	0.32	–	–	0.43 (0.21–0.81) 0.03
DF + LLA vs controls	0.31	0.37	–	0.68 (0.49–0.94) 0.02	0.27 (0.10–0.76) 0.01
DF males + LLA vs control males	0.31	0.37	–	0.69 (0.47–1.01) 0.05*	0.29 (0.09–0.95) 0.04
DF + nephropathy vs controls	0.31	0.37	–	0.69 (0.52–0.92) 0.01	0.32 (0.14–0.74) 0.008

Table 4 (continued)

	Risk allele frequency (RAF)		OR (95% CI) <i>P</i>		
	Cases	Controls	Dominant	Additive	Recessive
DF females + nephropathy vs control females	0.29	0.38	0.52 (0.28–0.97) 0.04	–	–
DF + retinopathy vs controls	0.33	0.37	–	0.76 (0.60–0.96) 0.02	0.43 (0.23–0.81) 0.01
DF males + retinopathy vs control males	0.33	0.37	–	0.30 (0.12–0.76) 0.01	0.76 (0.56–1.03) 0.07*

See Tables 1, 2 and 3

between DF patients with type 2 DM and healthy controls (OR = 0.64; $p = 0.05$) and between neuropathic DF with type 2 DM and healthy controls (OR = 0.5; $p = 0.03$). The described haplotype was more frequent in women with Charcot neuroarthropathy with type 1 DM compared to healthy controls (OR = 3.19; $p = 0.06$) and in Charcot neuroarthropathy patients with type 2 DM and DF of neuropathic origin (OR = 7.5; $p = 0.08$). Moreover, the described haplotype was less frequent in men with Charcot neuroarthropathy compared to controls (OR = 0.26; $p = 0.06$) and in neuropathic DF with type 2 DM and controls (OR = 0.52; $p = 0.08$).

Haplotype GACCAA of rs1032128|rs11573829 was less frequent in Charcot neuroarthropathy in type 2 DM patients compared to DF of neuroischemic origin (OR = 0.49; $p = 0.04$). It was also less frequent in DF of neuroischemic origin in type 2 DM patients compared to controls (OR = 1.46; $p = 0.06$) and in women with Charcot neuroarthropathy compared to DF of neuroischemic origin (OR = 0.27; $p = 0.06$).

Haplotype CA of rs2073618|rs134069 was shown to be more frequent in DF with type 2 DM patients compared to controls (OR = 1.2; $p = 0.06$) and in women with DF and type 2 DM compared to controls (OR = 1.26; $p = 0.06$).

Moreover, analysis showed the GA haplotype of rs2073618|rs134069 was less frequent in men with type 2 DM compared to controls (OR = 0.80; $p = 0.06$).

There were no differences in allele distribution of *TNFRSF11B* (rs10101385, rs1032129, rs6469788, rs6415470 and rs134063) variants in any of the comparisons.

Discussion

We showed differences in allele frequencies of *TNFRSF11B* (rs2073618, rs2073617, rs1872426, rs1032128, rs7464496, rs11573829 and rs1485286), *COLEC10* (rs6993813,

rs134069) and *TNFRSF11* (rs9533156) variants in the studied groups.

The correlation between *TNFRSF11B* and *COLEC10* variants rs2073618 and rs134069 with CN was reported by Pitocco et al. in 2009 in a study that included 59 CN patients, 41 with DF of neuropathic origin and 103 healthy controls [12]. Several other reports showed the association of *TNFRSF11B* and *COLEC10* variants with DF [15–17]. More recently, Korzon-Burakowska et al. showed significant differences in the distribution of rs2073617 and rs134069 alleles in CN and rs2073618, rs2073617 and rs134069 in DF of neuropathic origin compared to healthy controls [18]. The presented study showed that allele C of rs2073618 was more frequent in DF patients compared to controls and, moreover, this effect was sex-specific for males. With regard to the *TNFRSF11B* variant sex-specificity effect, we showed that allele A of rs2073617 was less frequent in females with DF. Furthermore, we have shown that the frequency of allele C of rs2073618 was limited mainly to DF males with type 2 DM and allele C of rs2073617 was present mainly in males with DF of neuropathic origin. Variant rs2073618 is localized in exon 1 and may possibly affect OPG function. Transversion of C/G nucleotides affects transcription of aspartic acid into lysine on amino 3' end (N3K) in the signal region, resulting in an increased activity of the produced protein [19]. Variant rs2073617 is localized in the *TNFRSF11B* intron in the untranslated region 5' (5'UTR) and results in the transversion of C/G nucleotides with no effect on the amino acid chain.

The differences we observed in rs134069 variant allele distribution is in line with previous reports [12, 18]. Mrozikiewicz-Rakowska et al. found that allele C of rs134069 is less frequent in DF patients with type 2 DM and chronic kidney disease (CKD) [20]. However, this study could not determine a correlation between the rs2073618 variant and CKD in DF and the type 2 DM population. Our previous report showed that allele A of rs134069 and allele C of rs2073618 were less frequent in type 2 DM DF patients

Table 5 Differences in *TNFRSF11B* (rs7464496), *COLEC10* (rs6993813 and rs3134069) and *TNFSF11* (rs9533156) alleles frequencies between selected groups

	Risk allele frequency (RAF)		OR (95% CI) <i>P</i>		
	Cases	Controls	Dominant	Additive	Recessive
rs7464496 (C/T) Risk allele: C					
DF males vs control males	0.49	0.46	1.46 (1.01–2.13) 0.05*	–	–
DF type 2 DM males vs control males	0.51	0.46	1.60 (1.07–2.39) 0.02	–	–
DF type 2 DM vs DF type 1 DM	0.50	0.42	–	–	2.44 (0.92–6.50) 0.07*
DF males + nephropathy vs control males	0.51	0.47	1.77 (1.04–3.07) 0.03	–	–
rs6993813 (C/T) Risk allele: C					
Neuropathic DF females vs control females	0.50	0.49	–	2.89 (0.99–8.45) 0.05*	–
CN type 1 DM females vs control females	0.70	0.49	–	2.58 (0.95–7.01) 0.06*	–
DF males + nephropathy vs control males	0.43	0.51	0.60 (0.37–0.98) 0.04	0.65 (0.47–0.90) 0.01	–
rs3134069 (A/C) Risk allele: C					
DF + nephropathy vs controls	0.07	0.12	0.48 (0.25–0.94) 0.03	0.78 (0.25–0.92) 0.03	–
DF males + nephropathy vs control males	0.05	0.12	0.36 (0.14–0.92) 0.03	0.37 (0.15–0.91) 0.03	–
DF + retinopathy vs controls	0.08	0.12	0.56 (0.32–0.96) 0.03	–	–
rs9533156 (C/T) Risk allele: C					
CN females vs control females	0.40	0.50	0.51 (0.24–1.09) 0.08*	–	–
CN type 2 DM females vs control females	0.36	0.50	0.42 (0.16–1.10) 0.08*	–	–
Neuroischemic DF females vs control females	0.41	0.50	0.54 (0.27–1.08) 0.08*	–	–
Neuropathic DF females vs neuroischemic DF females	0.51	0.41	2.33 (0.92–5.89) 0.07*	–	–
DF + LLA vs controls	0.54	0.49	2.00 (1.17–3.42) 0.01	1.37 (1.03–1.81) 0.03	–
DF males + LLA vs control males	0.53	0.48	2.03 (1.09–3.79) 0.03	–	–
DF females + nephropathy vs control females	0.42	0.50	0.53 (0.29–0.99) 0.05*	0.63 (0.41–0.97) 0.03	–

See Tables 1, 2 and 3

with diabetic retinopathy [21]. Variant rs3134069 is localized in the *COLEC10* intron and results in A/C nucleotide transition with no effect on amino acids.

Furthermore, variant rs3134063 was not previously assessed in DF subjects and our findings suggest no correlation in this cohort. Genre et al. showed that the CGA haplotype of rs3134063, rs2073618 and rs3134069 may play a protective role against neuro-vascular incidents in anti-CCP negative rheumatoid arthritis patients [22]. In diabetic patients, a linkage between rs3134063 and vascular complications in chronic inflammatory disorders may be suspected but has not yet been proven. According to Veyrieras et al., the rs1872426 variant (as well as the rs3134063 and rs2073617 variants) belong to cis-acting expression quantitative trait loci (cis-eQTL), which may explain the influence of this variant in quantitative gene expression [23]. Hulse et al. postulated multiple-SNP effects of genetic panels on gene expression, among them were variants from the *TNFRSF11B* superfamily [24]. The identification of a correlation between DF and CN with rs1872426 may prove useful in eQTL panels.

In the presented analysis, we showed that allele C of rs6993813 was more frequent in females with DF of neuro-pathic origin with type 1 DM as well as sex-specificity of rs6993813 in CN females with type 1 DM. Both of these findings are in line with other analyses. [25, 26]. A study conducted by Yoshida et al. indicated the correlation of rs6993813 with femoral neck fractures in rheumatoid arthritis patients in a Japanese population [27].

In the rs1485286 variant, we showed several differences in allele distribution and sex-specificity for males. Li et al. showed that genotype TT of rs1485286 was more frequent in patients who underwent alcohol-induced femoral neck fractures [28]. Beyens et al. proved its correlation (similarly to rs11573871 and rs6415470) with Paget's disease of the bone in females in a Belgian population [29]. Demonstration of sex-specificity of rs1485286 may be crucial in genetic panel design, however rs1485286 is localized in the *TNFRSF11B* intron and results in C/T nucleotide transition with no effect on amino acids.

We showed a greater frequency of allele C of rs7464496 in DF males and type 2 DM and DF of neuroischemic origin patients. In the Dong et al. study, rs7464496, along with 36 other variants correlated to RANKL/RANK/OPG, were assessed in the context of a femoral neck compression strength index (fCSI) [30]. The study showed no correlation between this SNP and fCSI value, however, other RANKL variants had a positive correlation (i.e., rs12585014, rs7988338 and rs2148073). In conclusion, an investigation for the correlation of genetic predisposition to DF development should not be limited to OPG but expanded to other components of RANKL/RANK/OPG. The variant rs7464496 is localized in the *TNFRSF11B* intron and results in C/T nucleotide transition with no effect on amino acids, however, a possible impact on protein activity may be explored.

We showed differences in rs11573829 allele distribution only in haplotype analysis. In the Genome-Wide Association Study (GWAS) database, there is a meta-analysis (HGVST680) showing a correlation of this SNP with Crohn's disease [31]. In this context, there is possibly no correlation with DF or DM, however, further investigations may be useful.

The RANK variant rs9533156 has not been previously assessed in the DF population. We showed a series of tendencies of allele C frequency in CN patients and a correlation with LLA in males and diabetic nephropathy in females. Biscetti et al. found a correlation of rs9533156 with a higher incidence of ischemic stroke in an Italian population [32]. In the same population, the correlation of rs2073618, rs2073617 and rs3134069 with cerebrovascular diseases was identified. In a recent Tunisian study published in 2017 by Sassi et al., allele TT rs9533156 was related with a risk of osteoporosis in postmenopausal women [33]. These findings are in line with the presented study in the aspect of sex-specificity of this SNP.

Altogether, the present data suggest that further investigations should include the genetic background of other RANKL/RANK/OPG system components and their correlation with DF risk.

The increased percentage of males with DF was also observed in a study by Simon et al. which calculated the associated risk as 1.4-fold compared to females. The impact of height on DF development was also shown by Sosenko et al. [34]. It is suspected that this is due to a higher risk of demyelination in tall patients.

Conclusions

Our findings suggest that the variants *TNFRSF11B* (rs2073618, rs2073617, rs1872426, rs1032128, rs7464496, rs11573829 and rs1485286), *COLEC10* (rs6993813, rs3134069) and *TNFSF11* (rs9533156) influence DF occurrence. This correlation is specific for gender, diabetes type and DF etiology. These findings contribute towards the use of these RANKL/RANK/OPG variants in genetic panels for assessing DF risk in the diabetic population alongside adequate correlation with clinical data, such as diabetes type, gender, age and other features.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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