ORIGINAL ARTICLE

Role of the rs2274907 allelic variant of the *ITLN1* gene in patients with diabetic foot

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KEY WORDS

diabetic foot, *ITLN1* gene, rs2274907 allelic variant, type 2 diabetes

ABSTRACT

INTRODUCTION Diabetic foot (DF) is a serious complication of diabetes mellitus (DM) that occurs due to neuropathy or atherosclerosis of the lower limbs. Omentin (encoded by the *ITLN1* gene) has been implicated as a protective factor in vascular complications of diabetes, likely due to its endothelial vasodilator activity and its anti-inflammatory actions. However, susceptibility to DF with respect to the allelic variants of the *ITLN1* gene has not been studied so far.

OBJECTIVES This study aimed to evaluate the association between the rs2274907 allelic variant of the *ITLN1* gene and the occurrence of DF in patients with type 2 diabetes mellitus (T2DM).

PATIENTS AND METHODS The study included 670 individuals: 204 with T2DM and DF (DF group), 299 with T2DM without DF (T2DM group), and 167 healthy controls.

RESULTS Ischemic heart disease, retinopathy, nephropathy, neuropathy, obesity, hyperlipidemia, and active smoking were more frequent in the DF group than in the T2DM group. Allele A of the rs2274907 variant was observed more frequently in the DF group compared with healthy controls in an additive model (odds ratio [OR] = 0.7, $P = 0.034$). This effect was also sex-specific for males in both the additive and recessive models (OR = 0.6, $P = 0.015$ and OR = 0.52, $P = 0.0017$, respectively). However, no differences in the distribution of alleles was observed between the DF and T2DM groups.

CONCLUSIONS The rs2274907 variant of the *ITLN1* gene is associated with increased prevalence of DF.

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INTRODUCTION Diabetic foot (DF) affects approximately 10% to 15% of the diabetic population.[1] DF is typically observed in patients in late stages of type 2 diabetes mellitus (T2DM); however, recent studies have shown that it is also increasingly recognized in younger age groups, which, owing to the recurrent nature of the disease, can generate a higher risk of lower limb amputation. Indeed, our observations, and those of others, show that DF is mainly observed in patients in their fourth and fifth decades of life, indicating a relatively early onset.[2-5] This early onset is likely due to the influence of environmental factors, such as a contemporary lifestyle,[6-7] and their modifying effects on the early disclosure of genetic factors. Therefore, genetic testing could potentially be used to identify patients more vulnerable to early development of DF.

Excessive distribution of visceral adipose tissue promotes the development of etiological factors for DF (ie, neuropathy and atherosclerotic changes in lower limb arteries).[8-10] Hypertrophy and hyperplasia of visceral adipocytes can cause local ischemia,[11-12] which disrupts the homeostasis of proinflammatory and anti-inflammatory cytokine secretion, resulting in, for example, insulin resistance.[13] In turn, ischemic adipocytes undergo apoptosis, which stimulates the influx of

macrophages into the adipose tissue.[14] These macrophages secrete cytokines such as tumor necrosis factor α (TNF-α), which augments the impairment of the endocrine and paracrine function of adipocytes (eg, secretion of visfatin, leptin, resistin, and others).[15-17] This enhances the intensity of inflammatory response, which accelerates the progression of atherosclerosis. The extent of this response (concentration of inflammatory markers such as C-reactive protein or procalcitonin) is a result of interaction between environmental and genetic factors.[18]

One of the cytokines involved in the endocrine homeostasis of body fat is omentin, which is synthesized by adipocytes in visceral and subcutaneous fat, as well as by endothelial cells.[19-20] Omentin, also known as intelectin 1, is encoded by the *ITLN1* gene located on the long arm of chromosome 1 (1q21.3).[21,22] Splichal et al[23] previously showed that the rs2274907 allelic variant of *ITLN1* affects the volume of supply of nutritional substances in a population of patients from central Europe. AA allele carriers were characterized by a higher energy supply (8764 [2467] J/d) compared to TT allele carriers (7977 [2780] J/d). A study of another allelic variant of the *ITLN1* gene, rs1333062, showed the strongest association of the variant in a body mass index-stratified analysis of Indians with T2DM. Considering the linkage disequilibrium analysis of rs2274907, it could suggest that these associations concern also the rs2274907 variant.[24] Furthermore, excess visceral adipose tissue decreases the plasma concentration of omentin, and administration of recombinant omentin was shown to increase insulin-dependent glucose uptake by activating the AKT protein kinase signaling pathway.[19] Yörük et al[25] investigated the association of the rs2274907 allelic variant with coronary artery disease (CAD). Although no significant difference was found regarding this omentin variant, the Val/Val (A/A) genotype was more frequent in patients with CAD (odds ratio [OR], 3.46). In a study of Jamshidi et al,[26] the T allele of rs2274907 was a risk factor for CAD in Iranian population, but only in men ($P = 0.031$).[26] Moreover, Zhong et al[27] found lower serum levels of omentin 1 in patients with CAD compared with controls ($P < 0.01$).[27] It is well known that T2DM and CAD are closely related.

It remains unclear whether the allelic variance of the *ITLN1* gene affects the early development of diabetes and its complications, particularly the etiological factors leading to the development of DF. Therefore, the aim of our study was to evaluate the association between this variance and the occurrence of DF in patients with T2DM. Based on the HapMap database and available literature, we chose to investigate this association using a single nucleotide variant (SNV) of the *ITLN1* gene: rs2274907.

PATIENTS AND METHODS The clinical part of the study was conducted in the Department of Internal Diseases and Diabetology, Medical University of Warsaw, Warsaw, Poland, and the Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion, Institute of Cardiology, Warsaw, Poland. The laboratory part of the study was conducted in the Laboratory of Experimental Pharmacogenetics, Department of the Clinical Pharmacy and Biopharmacy, Poznań University of Medical Sciences, Poznań, Poland, and the Department of General Biology and Parasitology and Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland.

The study included 670 individuals: 204 patients with T2DM and DF (DF group), 299 with T2DM without DF (T2DM group), and 167 healthy individuals (control group). Patients from the T2DM group and the DF group were treated in the Department of Diabetology and Internal Diseases and Diabetic Foot Outpatient Clinic in the years from 2009 to 2012. The control group consisted of healthy individuals who participated in the WOBASZ I project, a Polish national multicenter, cross-sectional study on the prevalence of risk factors for cardiovascular disease conducted in the years from 2003 to 2005 by the Institute of Cardiology in Warsaw.

DF was diagnosed according to the International Consensus on the Diabetic Foot and Practical Guidelines on the Management and Prevention of the Diabetic Foot 2007, which defined DF as "ulceration, infection, or destruction of deep tissues located in the lower limbs below the ankles in patients with diabetes and neuropathy and/or peripheral arterial disease."[28] All patients underwent physical examination and their medical history was taken. The physical examination included assessment of the foot ulceration and deformation, reflexes of the knee and Achilles tendon, and pulses on the posterior and dorsal tibial arteries. The presence of neuropathy was assessed using monofilament (sense of touch), tip-therm type device (temperature discrimination), neurotips (discrimination of pain), and Semmes–Weinstein tunnel fork (discrimination of vibration). Painless ulcerations after debridement were also assessed as neuropathic DF. The neuropathy was identified according to the Toronto Clinical Neuropathy Scoring System.[29] Diabetic polyneuropathy was diagnosed if 2 or more of the 5 abnormalities were observed: presence of symptoms, lack of ankle reflexes, impaired sensation of touch, temperature, and/or vibration. The presence of an ischemic component was diagnosed when there was no pulse detected on foot arteries (posterior tibial artery and/or dorsal artery), as confirmed by mini Doppler ultrasound. Patients with a lack of pulse detected on foot arteries were referred to a vascular surgeon for further diagnostic procedures.

Patients were compared with respect to several comorbidities. Obesity was defined as a body mass index (BMI) exceeding 29.9 kg/m^2. The criteria for hyperlipidemia were hypercholesterolemia (total cholesterol ≥5.0 mmol/l and/or low-density

lipoprotein cholesterol ≥3.0 mmol/l) or intake of lipid-lowering medications within the previous 2 weeks. Hypertension was diagnosed if the blood pressure exceeded 140/90 mm Hg according to the 2011 Guidelines of the European Society of Cardiology. The definition of ischemic heart disease included patients with clinically diagnosed ischemic heart disease who had no coronary event and patients with coronary artery disease, that is, with a history of percutaneous transluminal coronary angioplasty, coronary artery bypass grafting, and ST-elevation myocardial infarction (MI) or non-ST-elevation MI. Given the clinical relevance of MI, a qualitative predictor (ie, history of MI) was added as a separate variable in the analyses. Diabetic retinopathy was diagnosed by the ophthalmologist with a slit lamp and Haag Streit noncontact lens examinations. Diabetic retinopathy was staged according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification as either nonproliferative diabetic retinopathy or proliferative diabetic retinopathy. The nonproliferative type included background retinopathy and preproliferative retinopathy. Owing to the relatively small size of the study group, patients were not subdivided according to the stage of diabetic retinopathy. Nephropathy was classified by having a glomerular filtration rate below 60 ml/min/1.73 m^2 on the basis of the body surface area (ie, at least stage 3 chronic kidney disease based on the World Health Organization classification) and microalbuminuria (ie, results above 30 mg/dl in at least 3 urine samples). Each patient underwent assessment of blood glucose levels, glomerular filtration rate (estimated using the Modification of Diet in Renal Disease formula), and urine examination with assessment of protein concentrations. Active smokers were defined as patients currently smoking tobacco.

The genetic material was isolated from the whole blood samples collected in EDTA probes using the salting-out method.

Analysis of the allelic variant of the *ITLN1* gene (rs2 274907) was performed by real-time polymerase chain reaction (PCR) with the use of LightCycler® 480 (ROCHE, Pleasanton, California, United States) designed to ensure quick and accurate PCR. For such procedures, HybProbe hybridization probes were used along with LightCycler® 480 Basic to evaluate the results. For genotyping, that is, detection of the SNVs of the evaluated genes, we used fluorescent measurements taken during the analysis of the melting curve following PCR. LightSNiP (TIB MOLBIOL GmbH, Berlin, Germany) set with phials containing the proper concentration of starters and probes specific for the amplified fragment, was used to test alteration of the *ITLN1* gene. LightSNiP used for real-time PCR was prepared according to the manufacturer's instructions.

The reactions were performed respectively throughout 45 cycles for the evaluated gene, covering particular stages of amplification, which was followed by melting of the reaction's products once the temperature reached 95°C. The genotype was analyzed on the basis of the melting curve.

The rs2274907 is a missense variant A/T located in exon 4 of the *ITLN1* gene. It causes replacement of valine with asparagine at position 109 in *ITLN1*. In silico analysis (MutationTaster,[30] PolyPhen-2,[31] SIFT[32]) predicted a benign effect from this allelic variant (Supplementary material online, *Table S1*).

All genotyping results were tested for deviation from HWE by the Fisher exact test. The frequencies of alleles in cases and controls were compared using the χ^2 test. The effective models were defined as dominant AT+AA vs TT, additive AA vs AT vs TT, and recessive AA vs TT+AT. Associations between genotypes and clinical variables were tested by logistic regression. Normal distribution of the data was examined using the Kolmogorov–Smirnov test.

As none of the assessed quantitative variables met the criteria of normal distribution, the Mann–Whitney test or Kruskal–Wallis analysis of variance were used, as appropriate. For qualitative variables, the χ^2 test was used. A P value of less than 0.05 was considered statistically significant.

The obtained distribution of the genotypes was statistically analyzed with the online program Web-Assotest (available at: http://www.ekstroem.com/assotest/assotest.html). Other calculations were performed using the STATISTICA 12PL software (StatSoft, Inc. 2014, Tulsa, Oklahoma, United States).

RESULTS Patients in the DF group were significantly younger (mean [SD], 64.45 [9.67] years vs 67.14 [11.62] years, P = 0.001; OR, 0.98; 95% CI, 0.96–0.99). The DF group had significantly higher weight, height, and BMI than the T2DM group and healthy controls (all, P = 0.0001). Compared with the T2DM group, each additional kilogram of weight increased the risk of DF by 3.4% (OR, 1.034; 95% CI, 1.025–1.049), and each additional centimeter of height increased the risk of DF by 5.4% (OR, 1.054; 95% CI, 1.032–1.076). Moreover, patients in the DF group had a significantly higher hip circumference (P = 0.02) and longer history of diabetes (P = 0.002) than the T2DM group. Compared with the T2DM group, a higher hip circumference and mean diabetes duration increased the risk of DF by 2.6% (OR, 1.026; 95% CI, 1.003–1.050) and 3.3% (OR, 1.033; 95% CI, 1.013–1.054), respectively. There were no significant differences in sex distribution between the combined DF and T2DM groups compared with controls. The characteristics of the study groups are presented in TABLE 1.

A higher number of men was found in the DF group compared with both the T2DM and control groups (P = 0.00001). Ischemic heart disease, retinopathy, nephropathy, neuropathy, obesity, hyperlipidemia, and active smoking were significantly more common in the DF group compared with the T2DM group (TABLE 2). Hypertension was more frequent in the DF group than in the T2DM group,

TABLE 1 Characteristics of the study groups

Parameter		Diabetic foot (n = 204)	Type 2 diabetes mellitus (n = 299)	Healthy controls (n = 167)	*P* value
Sex, female/male, n (%)		73/131 (35.78/64.22)	166/133 (55.52/44.48)	69/98 (41.32/58.68)	0.00003
Age, y	Men	63.00 (57.50–67.50)	67.00 (55.00–74.25)	57.95 (53.65–63.425)	0.00001
	Women	67.00 (61.25–77.75)	70.00 (62.00–77.00)	56.30 (52.35–61.50)	0.00001
Diabetes duration, y		18.82 (12.00–25.00)	14.00 (9.00–22.00)	NA	0.0018
Weight, kg		91.00 (80.00–102.75)	80.00 (71.00–90.95)	72.60 (63.30–80.50)	0.00001
Height, cm		171.00 (1.64–1.76)	165.00 (1.58–1.74)	166.90 (160.0–172.5)	0.00001
BMI, kg/m^2		31.63 (27.77–34.94)	29.02 (25.55–32.86)	26.04 (23.63–28.89)	0.00001
Waist circumference, cm		107.00 (97.00–117.00)	102.00 (95.00–113.00)	92.00 (84.00–100.00)	0.00001
Hip circumference, cm		110.00 (102.00–120.00)	107.38 (100.00–114.50)	104.00 (99.00–110.00)	0.0003

Data are presented as median (interquartile range) unless otherwise indicated. A *P* value of less than 0.05 is considered statistically significant.

Abbreviations: BMI, body mass index; NA, not applicable

TABLE 2 Characteristics of clinical complications and other qualitative variables in the study groups

Variable	Diabetic foot, no. of patients, yes/no	Type 2 diabetes mellitus, no. of patients, yes/no	*P* value
Obesity, BMI >29.9 kg/m^2	112/68	125/161	0.0001
Hyperlipidemia	109/47	76/135	0.00001
Hypertension	167/33	189/59	0.06
Ischemic heart disease	99/88	110/163	0.01
Myocardial infarction	51/136	81/199	>0.05
Neuropathy	141/35	41/245	0.00001
Presence of pulse on foot arteries	141/35	240/46	0.30
Retinopathy	110/87	61/234	0.00001
Nephropathy	68/125	21/272	0.00001
Active smoking	79/92	17/100	0.00001

A *P* value of less than 0.05 is considered statistically significant.

Abbreviations: see TABLE 2

but the difference was not significant (TABLE 2). We also found no significant differences between the DF and T2DM groups in the incidence of MI (TABLE 2). Furthermore, the rs2274907 allelic variant had no effect on the incidence of MI in the DF and T2DM groups. DF was associated with ischemic heart disease, retinopathy, nephropathy, neuropathy, obesity, hyperlipidemia, and active smoking (TABLE 3). We observed no effect of obesity on the rs2274907 allele distribution in any of the groups.

We analyzed the distribution of the rs2274907 allelic variant in DF, T2DM, and control groups and found that allele A was more frequent in patients with DF compared with controls in an additive model (TABLE 4). This effect was also sex-specific for men in both the additive and recessive models. In contrast, we found no differences in allele distribution between the DF and T2DM groups (TABLE 4). Our results indicate that allele A of rs2274907 is associated with a sex-specific prevalence of DF. A logistic regression analysis adjusted for BMI, age, and sex did not affect the statistical significance.

DISCUSSION DF may be caused by excessive and abnormal distribution of visceral adipose tissue due to endocrinological disorders, including dysregulation of omentin metabolism. In an in vitro study, Yang et al[19] showed that omentin enhances

TABLE 3 Results of univariate logistic regression analysis of the association between qualitative variables and diabetic foot

Predictors	OR	95% CI	*P* value
Ischemic heart disease	1.67	1.15–2.43	0.01
Retinopathy	4.85	3.26–7.22	0.00001
Nephropathy	7.05	4.13–12.01	0.00001
Neuropathy	24.07	14.65–39.55	0.00001
Dyslipidemia	4.12	2.65–6.42	0.00001
Obesity	2.09	1.43–3.06	0.0001
Active smoking	5.05	2.78–9.16	0.00001

A *P* value of less than 0.05 is considered statistically significant.

Abbreviations: OR, odds ratio

the response to insulin in subcutaneous and visceral adipocytes by stimulating insulin-dependent glucose uptake. Numerous other studies indicated a direct correlation between omentin concentrations and various metabolic disorders; for example, Shibata et al[33] demonstrated a relationship between the plasma concentration of omentin and waist circumference, presence of dyslipidemia, elevated blood pressure, and glucose intolerance. Similar observations were reported in the largest study on omentin to date, KORA F4, where an inverse relationship was found between omentin concentrations and both BMI and systolic blood pressure.[34] Results from the KORA F4 study also demonstrated a proportional relationship between omentin and high-density lipoprotein concentration or insulin sensitivity. Moreno-Navarrete et al[35] showed that the omentin concentration correlates closely not only with the above factors, but also with endothelium-dependent and endothelium-independent vasodilatation, as well as C-reactive protein and interleukin-6 concentrations.[35] Therefore, in this study, we investigated whether single nucleotide variance in the omentin gene *ITLN1* was associated with the prevalence of DF.

Unlike previous studies, omentin concentrations were not investigated in this study; instead, we assessed the impact of genetic diversity by examining the effects of an *ITLN1* allelic variant on the development of DF. To the best of our knowledge, no published study has evaluated DF with respect to the *ITLN1* allelic variant. Compared with T2DM patients without DF, we found that patients T2DM and DF were characterized by a higher incidence of ischemic heart disease, retinopathy, nephropathy, neuropathy, obesity, and lipid disorders. However, there were no significant differences between the frequency of MI in DF and T2DM groups. This means that patients with DF more often present microvascular changes in the heart muscle or other conditions leading to ischemic heart disease, not necessarily to MI (eg, anemia). In addition, the group of patients with T2DM and DF included significantly more

TABLE 4 Associations of the single nucleotide variant rs2274907 in the study groups

Study group	Genotype % (n/N)			Model OR (95% CI); *P* value			HWE statistics
	TT	AT	AA	Dominant AT/AA vs TT	Additive AA vs AT = AT vs TT	Recessive AA vs TT/AT	χ^2; *P* value
DF group (md: n = 9)	11.28 (23/204)	45.59 (93/204)	38.37 (79/204)	0.54[a] (0.26–1.15); *P* = 0.104	0.70[a] (0.51–0.98); *P* = 0.034	0.68[a] (0.45–1.04); *P* = 0.073	0.306; *P* = 0.580
T2DM group (md: n = 36)	9.70 (29/299)	38.80 (118/299	39.47 (116/299)	0.59[b] (0.29–1.21); *P* = 0.138	0.87[b] (0.58–1.06); *P* = 0.116	0.79[b] (0.53–1.17); *P* = 0.237	0.015; *P* = 0.902
Control group (md: n=5)	6.59 (11/167)	41.92 (70/167)	48.50 (81/167)	–	–	–	0.635; *P* = 0.426
DF vs T2DM group	–	–	–	0.93 (0.52–1.66); *P* = 0.798	0.91 (0.69–1.20); *P* = 0.488	0.86 (0.59–1.26); *P* = 0.442	–
DF group (men) vs control group (men)	12.70 (16/126) vs 7.14 (7/98)	4.52 (57/126) vs 34.69 (34/98)	42.06 (53/126) vs 58.16 (57/98)	0.53 (0.21–1.34); *P* = 0.167	0.60 (0.40–0.91); *P* = 0.015	0.52 (0.31–0.89); *P* = 0.0017	–
DF group (women) vs control group (women)	6.06 (4/66) vs 10.29 (7/68)	54.55 (36/66) vs 51.47 (35/68)	39.39 (26/66) vs 38.24 (26/68)	0.56 (0.16–2.02); *P* = 0.369	0.87 (0.50–1.51); *P* = 0.611	0.95 (0.48–1.91); *P* = 0.891	–

a DF group vs control group

b T2DM group vs control group

A *P* value of less than 0.05 is considered statistically significant.

Abbreviations: DF, diabetic foot; HWE, Hardy–Weinberg equilibrium; n, number of alleles; N, total number of alleles; T2DM, type 2 diabetes mellitus; md, missing data; others, see TABLE 3

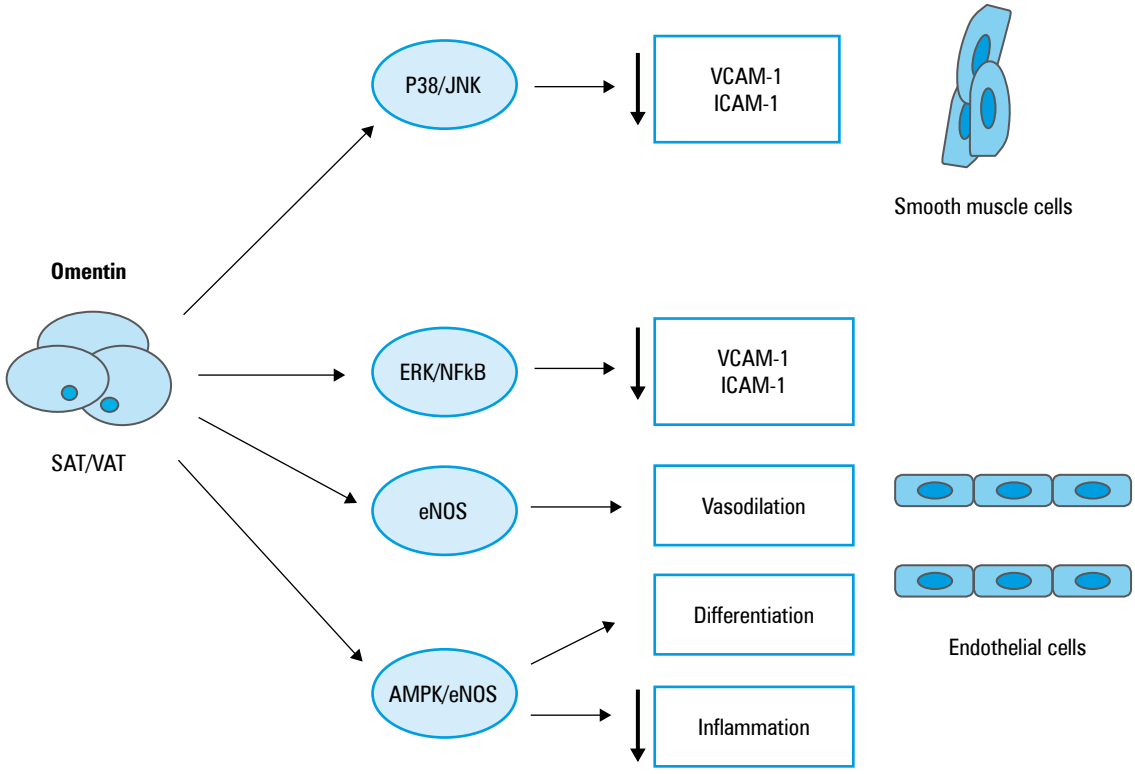


FIGURE 1 Protective role of omentin on endothelium and smooth muscle cells

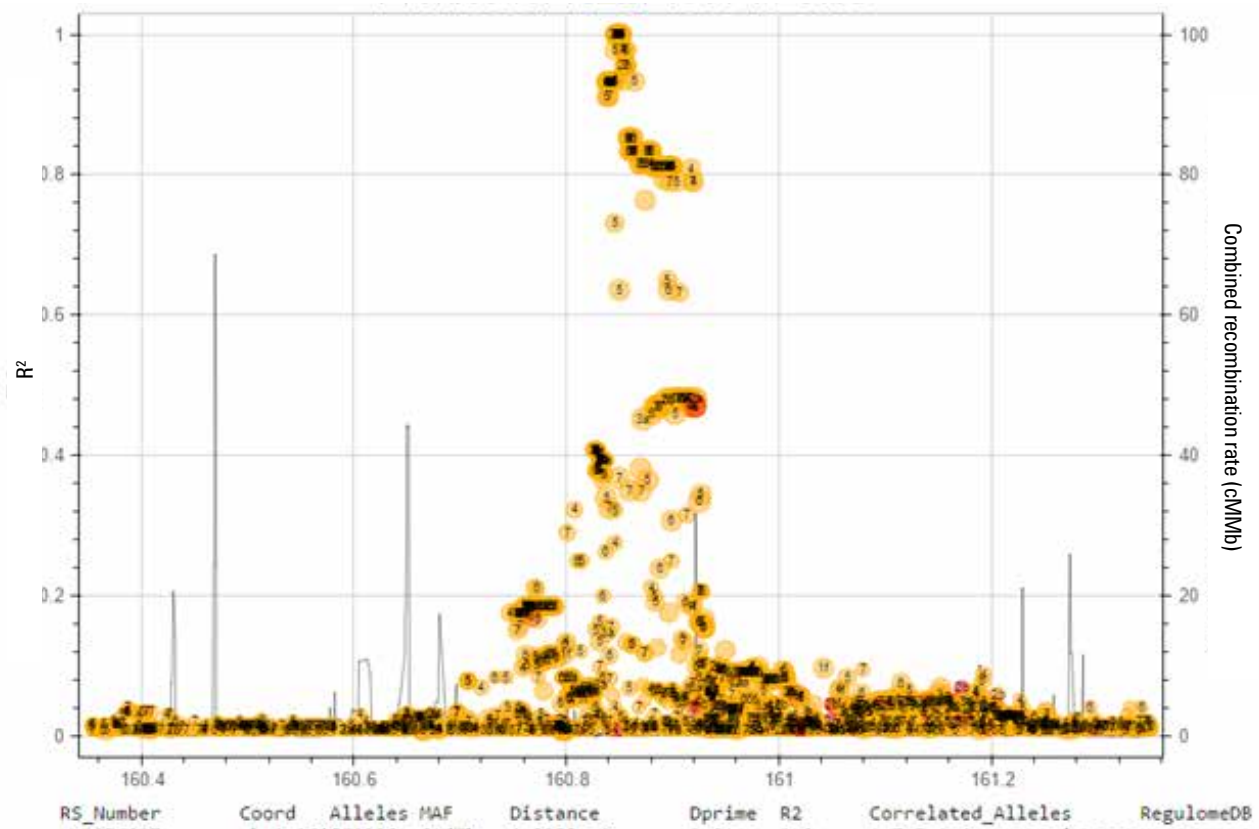
Abbreviations: ICAM-1, intercellular adhesion molecule 1; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; VCAM-1, vascular cell adhesion protein 1

smokers, which confirms the association between smoking and the development of neuropathy and peripheral artery disease.[36,37] The study group consisted of patients reporting at random to the Department of Internal Diseases and Diabetology and Diabetic Foot Clinic. Therefore, patients were not included in the study on the basis of selected inclusion and exclusion criteria, but represented a natural biological distribution of genetic factors in population with diverse phenotypes.

Our results indicate that allele A of *ITLN1* rs2274907 is more frequent in patients with DF compared with healthy controls, when using an additive model. However, we must consider whether this relationship is affected by the ischemic or neuropathic background of DF. Indeed, patients with DF are currently divided (according to the presence of etiological factors) into patients with neuropathic foot and neuropathic-ischemic foot.[38] In most patients with DF, neuropathy is present but atherosclerosis occurs much later. Therefore, whether omentin induces or is involved in the development of vascular lesions leading to DF, and whether it is concentration-dependent or affected by *ITLN1* allelic variance, are important considerations.

Unfortunately, the DF group was not subdivided into patients with neuropathic foot or neuropathic-ischemic foot in our study due to insufficient numbers that would allow us to statistically analyze the genetic factors. However, previous studies have shown associations between omentin concentrations and the development of both ischemic and neuropathic complications. Yamawaki et al[39] demonstrated that omentin has a protective effect on the endothelium due to its ability to stimulate nitric oxide production, resulting in endothelium-dependent vasodilation. Omentin can also inhibit the inflammatory response in endothelial cells by suppressing the activation of JNK via the AMPK/eNOS signaling pathway.[40] Another study showed that omentin reduces vascular cell adhesion protein-1 expression on the surface of monocytes and also reduces intercellular adhesion molecule-1 expression (via suppression of extracellular ERK/NF-κB), which results in reduced adhesion of monocytes to endothelial cells.[41] Omentin has also shown an inhibitory effect on TNF–α-induced adhesion of U937 monocytes in rat vascular smooth muscle cells.[42] Considering the aforementioned properties of omentin, many scientists conclude that it may be a good marker for evaluating vascular endothelial function when assessing ischemic complications in patients with diabetes.[35] However, whether these effects are related to the concentration of omentin or to the *ITLN1* allelic variant itself remains unclear.

FIGURE 1 summarizes our current understanding of the pathways leading to the protective effect of omentin on endothelium and smooth muscle cells. Our study showed no differences in the incidence of ischemic complications between patients with DF and T2DM ($P = 0.30$). This indicates that



RS_Number	Coord	Alleles	MAF	Distance	Dprime	R2	Correlated_Alleles	RegulomeDB
rs2274907	chr1:160851826	(A/T)	0.3333	0	1.0	1.0	A=T,T=A 4	missense
rs2274908	chr1:160851894	(G/A)	0.3333	68	1.0	1.0	A=A,T=G 4	synonymous
rs2274909	chr1:160852022	(C/T)	0.3333	196	1.0	1.0	A=T,T=C 4	NA
rs10908808	chr1:160851619	(A/G)	0.3333	-207	1.0	1.0	A=G,T=A 6	NA
rs2274910	chr1:160852046	(T/C)	0.3333	220	1.0	1.0	A=C,T=T 4	NA
rs6427551	chr1:160851564	(C/G)	0.3333	-262	1.0	1.0	A=G,T=C 7	NA
rs7532133	chr1:160851534	(A/G)	0.3333	-292	1.0	1.0	A=G,T=A 6	NA
rs6427552	chr1:160852167	(C/T)	0.3333	341	1.0	1.0	A=T,T=C 5	NA
rs2236515	chr1:160850936	(T/C)	0.3333	-890	1.0	1.0	A=C,T=T 5	NA
rs4656957	chr1:160850280	(G/A)	0.3333	-1546	1.0	1.0	A=A,T=G 4	NA
rs2274905	chr1:160849367	(G/T)	0.3333	-2459	1.0	1.0	A=T,T=G 7	NA
rs3820094	chr1:160849082	(A/G)	0.3333	-2744	1.0	1.0	A=G,T=A 5	NA
rs3766359	chr1:160849060	(G/A)	0.3333	-2766	1.0	1.0	A=A,T=G 5	NA
rs3766358	chr1:160849030	(T/A)	0.3333	-2796	1.0	1.0	A=A,T=T 5	NA
rs3766357	chr1:160848993	(G/A)	0.3333	-2833	1.0	1.0	A=A,T=G 5	NA
rs3766356	chr1:160848937	(T/C)	0.3333	-2889	1.0	1.0	A=C,T=T 5	NA
rs35214362	chr1:160848508	(T/-)	0.3333	-3318	1.0	1.0	A=-,T=T 5	NA
rs6667400	chr1:160848195	(A/G)	0.3333	-3631	1.0	1.0	A=G,T=A 6	NA
rs6703406	chr1:160848107	(C/T)	0.3333	-3719	1.0	1.0	A=T,T=C 7	NA
rs7524351	chr1:160847898	(T/C)	0.3333	-3928	1.0	1.0	A=C,T=T 7	NA
rs6427550	chr1:160847893	(A/G)	0.3333	-3933	1.0	1.0	A=G,T=A 7	NA
rs4656956	chr1:160847617	(C/T)	0.3333	-4209	1.0	1.0	A=T,T=C 3a	NA
rs4656955	chr1:160847410	(A/G)	0.3333	-4416	1.0	1.0	A=G,T=A 5	NA
rs17387157	chr1:160847064	(G/A)	0.3333	-4762	1.0	1.0	A=A,T=G 3a	NA
rs34067571	chr1:160846934	(G/A)	0.3333	-4892	1.0	1.0	A=A,T=G 7	NA
rs1333062	chr1:160846284	(T/G)	0.3333	-5542	1.0	1.0	A=G,T=T 4	NA
rs12409609	chr1:160845996	(T/C)	0.3333	-5830	1.0	1.0	A=C,T=T 4	NA
rs4656953	chr1:160845842	(C/T)	0.3333	-5984	1.0	1.0	A=T,T=C 5	NA

FIGURE 2 Analysis of linkage disequilibrium for proxies for rs2274907 in Northern Europeans from Utah (CEU)

the examined allelic variant may play a more important role for vasodilation within the vasa nervorum, whereas for large vessels, many genetic alternations could be affecting blood circulation and the resulting ischemia in the foot in diabetic patients.

When examining the risk of DF, we must also consider the presence of neuropathy. Herder et al[43] suggested that omentin protects against diabetic polyneuropathy. They showed an inverse association between omentin concentration and neuropathy, supported by the thesis that omentin has anti-inflammatory effects by inhibiting TNF–α-dependent inflammation. However, Jung et al[44] reported a different result, demonstrating a direct relationship between omentin concentrations and the incidence and severity of autonomic neuropathy of the cardiovascular system. Furthermore, contrary to previous reports, Jung et al[44] found no relationship between omentin concentrations and peripheral polyneuropathy or other microangiopathies including nephropathy and retinopathy. Therefore, these pathologies may not only depend on

the concentration of omentin, but also on its genetic diversity. However, further more detailed population-based studies are needed to confirm this hypothesis.

The recent immunocompromised district (ICD) theory suggests a relationship between impaired neurotransmission at the microvascular level and the occurrence of locally recurrent inflammation, a concept that can also be applied to DF.[45-48] According to the ICD theory, the normal transport of immune cells becomes compromised, and the neurotransmitters and neuropeptides released from peripheral nerves that send signal to these immune cells are affected. This creates a zone of reduced immunity, with increased susceptibility to secondary illnesses, including infections.[45] Since omentin modulates the local inflammatory response, ICD theory could also be used to explain the pathogenetic mechanism of DF.

Finally, we demonstrated that the presence of the A allele of *ITLN1* rs2274907 is specific to the male sex both in the additive and recessive models. Indeed, in the KORA F4 study, men were found to have a significantly lower plasma omentin concentration than women.[26] This observation may be related to the *ITLN1* allelic variant, which, in turn, increases the prevalence of DF in a sex-specific manner. However, as the gene for omentin is not located on the sex chromosome, other factors must be causing the phenotypic diversity, such as sex hormones. Indeed, this concept was proposed by Laque-Ramirez et al,[49] who found an inverse correlation between the concentrations of omentin and free testosterone, which suggests that sex hormones may modulate the serum concentration of omentin.

There are many SNVs in 1q23.3 in the neighborhood of rs2274907. Therefore, we checked whether any SNP that is in a linkage disequilibrium (LD, $r^2>0.8$) with rs2274907 was previously found to be associated with any disease in a genome-wide association study (GWAS). Only the rs2274910 SNV, which is located at a distance of 220 bp from the variant evaluated in this study, has been considered as a risk factor for Crohn disease[50] and asthma.[51] Based on these findings, it seems that omentin may affect an inflammatory process. We also looked for proxies of rs2274907 ($r^2 = 1$) associated with cardiovascular diseases in GWAS (Supplementary material online, *Table S1*) but none were identified.

Our study has some limitations. Similarly to other authors, we did not consider statistics presented in this paper for multiple testing because we investigated the already known association in general diabetic population.[52] Therefore, in this paper, we only stratified diabetic population for the presence of its complication (diabetic foot). Moreover, our study population is much smaller than the that in large consortia-based GWASs.

In summary, our analysis of the rs2274907 allelic variant indicates that the A allele of *ITLN1* may predispose patients with T2DM to the development of DF. Moreover, men with this nucleotide variant are particularly vulnerable to this complication. This association between *ITLN1* allelic variants and DF increases our understanding of the impact of gene variants on the final clinical phenotype. However, further research is needed to determine whether this genetic allelic variant affects vasodilation at the microcirculation level and initiates secondary neuropathy, or whether it can damage the walls of large blood vessels due to chronic inflammation generated by visceral fat tissue.

Supplementary material online Supplementary material online is available with the online version of the paper at www.pamw.pl.

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