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SEPP1 gene variants and abdominal aortic aneurysm: gene association in relation to metabolic risk factors and peripheral arterial disease coexistence

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An inadequate selenium level is supposed to be a risk factor for cardiovascular diseases. However little is known about variation of the genes encoding selenium-containing proteins that would confirm the causality in these diseases. The aim of this study was to analyze the relationships between two functional variants of selenoprotein P gene (*SEPP1* *rs3877899G>A*, *rs7579G>A*) and the occurrence of abdominal aortic aneurysm (AAA) and aortoiliac occlusive disease (AIOD), as well as their metabolic risk factors. In AAA, the *rs3877899A* allele was associated with higher systolic blood ($P < .003$) and pulse pressure ($P < .003$) values (recessive model), and with coexistence of peripheral arterial disease (PAD; carriers: $P = .033$). The other *SEPP1* variants were associated with BMI values and influenced the risk of aortic diseases, depending on body weight. The strongest associations in the case-control analysis was found between the presence of the *rs3877899G-rs7579G* haplotype and development of AAA in overweight and obese subjects (OR = 1.80, 95%CI = 1.16–2.79, $P = .008$). The higher BMI values were correlated with lower age of AAA patients and larger size of aneurysm. Our results suggests the potential role of the selenoprotein P in pathogenesis of AAA. Future studies should consider the role of the *rs3877899G-rs7579G* haplotype as a risk factor for aggressive-growing AAAs.

Abdominal aortic aneurysm (AAA) is a progressive dilatation of the abdominal aorta with a diameter of at least 3.0 cm¹. AAAs are often asymptomatic until they rupture. The disease affects ~9% of adults older than 65 years, causing almost 2% of male deaths in industrialized countries. AAA has a complex pathophysiology, in which both environmental and genetic factors play important roles². The mechanisms of AAA initiation and rupture are still not fully elucidated, partially because of the coexistence of atherosclerotic occlusive disease in the majority of patients, which has hindered the designation of risk factors specific to aneurysm formation.

Increasing evidence indicates that oxidative stress in the aorta may contribute to aortic wall weakening in AAA^{3,4} whereas factors with antioxidant capacity, such as resveratrol⁵, fruit consumption⁶, and increased levels of high-density lipoproteins⁷, could prevent its development or decrease the risk of rupture. The role of the antioxidant trace element selenium deficiency in AAA pathogenesis could also be postulated in countries with a relatively low dietary selenium intake⁸. European soil, particularly in Central Europe, is relatively low in this micronutrient, which results in low selenium content in food products. A decreased plasma selenium levels has been associated in European populations with development of different cardiovascular diseases⁸, including AAA in the Polish population⁹. In addition to nutritional selenium deficiency, cigarette smoking, the most important risk factor for AAA¹⁰, may substantially contribute to the reduction of selenium levels in patients¹¹. What is more, it was found that the levels of thioredoxin, a component of the selenium-dependent redox signaling system, correlate with the size and expansion rate of AAAs¹².

Selenium exerts its biological role as a selenocysteine residue in a family of 25 selenoproteins. The tissue distribution and availability of selenium depend on the status of selenoprotein P, a major selenoprotein in plasma with both transport and antioxidant activity. This protein might also act as a heavy-metal chelator¹³ and an anti-angiogenic agent¹⁴.



Selenoprotein P has two major domains: N-terminal, which contain 1 selenocysteine residue in a redox motif and several potential heparin-binding, and C-terminal with up to 9 selenocysteine residues. The heparin-binding domain facilitate binding of protein to proteoglycans on the vascular endothelium, and this may contribute to the protection against oxidants¹⁵. In humans, the full length protein (60-kDa) contain 10 selenocysteine residues, while the truncated isoform (50-kDa) only one¹⁶.

The liver produces most plasma selenoprotein P, although this protein is also expressed in other tissues and is presumably secreted by them¹⁷. The expression of *SEPP1* gene encoding selenoprotein P is upregulated in abdominal aorta of AAA patients (the data available in the NCBI's GEO database <http://www.ncbi.nlm.nih.gov/geo/>)¹⁸, most probably to protect the tissue from the effects of oxidative stress and inflammation¹⁹. Upregulation of this gene was also observed in a subclass of anti-inflammatory macrophages present in atherosclerotic plaques²⁰, and in vessels stimulated to remodel inwards by reduced blood flow, the process that mimics the effect of arterial hypertension on small arteries²¹. On the contrary, in dissected regions of ascending aorta a downregulation of *SEPP1* gene was observed²², which demonstrates a difference in the pathobiology between dissected thoracoabdominal aortic aneurysms and AAA.

The two studied *SEPP1* single nucleotide polymorphisms (SNPs) have been reported to have a functional effect: *rs3877899G>A* (Ala234Thr) in exon 5 and *rs7579G>A* in the 3' untranslated region^{23,24}. Both of these variants influence the proportion of the two protein isoforms; the *rs3877899G* and *rs7579A* alleles favor the production of the 60-kDa isoform, whereas the *rs3877899A* and *rs7579G* alleles - the production of the 50-kDa isoform²⁵. These SNPs affect selenium's bioavailability for the synthesis of all other selenoproteins (e.g. proteins that are involved in regulation of the extra- and intracellular redox environment, and protection against endoplasmic reticulum stress) by influencing the body's selenium status²³, effectiveness of supplementation²³, and selenium supply to target tissues²⁶. In consequence, they may influence cardiovascular outcomes independently of dietary selenium intake. Determination of the frequency of the *SEPP1* variants in AAA could be crucial for further studies of serum biomarkers that improve clinical prediction in this disease, because markers of inflammation and endothelium injury were previously found to be useful for indicating the development of life-threatening complications of repair surgery for AAA²⁷.

In search for the associations specific for the development of abdominal aortic aneurysms, the present study analyzed the *SEPP1 rs3877899* and *rs7579* polymorphisms in three groups: patients with AAA, patients suffering from aortoiliac occlusive disease (AIOD), an atherosclerotic disease partially sharing its location with AAAs, and controls. There are no previous reports on the distribution of *SEPP1* SNPs in these aortic diseases.

Results

Traditional risk factors distribution. Smoking was the most prevalent and the most important risk factor for AAA (80.0%, $P < .0001$) and AIOD (91.3%, $P < .0001$, Table 1). The other risk factors for both aortic diseases were: arterial hypertension and low high-density lipoprotein cholesterol level. Advanced age, male sex, overweight and obesity constituted risk factors that were more common in AAA. In AIOD, an increased proportion of underweight subjects (7.8%), as compared to both controls (1.8%) and AAA patients (1.8%, $P < .0001$) were observed. The AAA patients with concomitant PAD, compared with those without, had a significantly higher frequency of smokers (88.2% vs 77.3%; $P = .02$) and smaller aortic diameters (median [interquartile range], 57.5 [48, 69] mm vs 62.5 [55, 72] mm; $P < .01$).

Genotype distribution. The frequency of genotypes did not deviate significantly from the Hardy-Weinberg equilibrium in either the

patients or the controls (Tables 2 and 3). Because of the small distance between the two *SEPP1* variants (460 bp), resulting in the complete linkage disequilibrium between them ($D' = 1.0$), only the three common *rs3877899-rs7579* haplotypes: G-G, G-A, and A-G were observed in the our population. As a consequence, the effects of the *rs3877899A* and *rs7579A* alleles correspond to the impacts of the *rs3877899A-rs7579G* and *rs3877899G-rs7579A* haplotypes, respectively. The estimated pairwise r^2 value for studied SNPs (.129) was similar to that observed in other populations of European ancestry (range between .122 and .221, according to the Ensembl database).

The distribution of polymorphisms differed between the subgroups of AAA patients stratified by PAD coexistence. There were fewer carriers of the *SEPP1 rs3877899A* (*rs3877899A-rs7579G*) allele among patients without concomitant PAD than among those with PAD; respectively: 37.1% and 44.1% (OR = 0.61, 95%CI: 0.38–0.96, $P = .033$, Table 2 and 4). A 40% reduction in the risk of AAA without coexisting PAD was observed for the carriers of this allele (OR = 0.60 95%CI: 0.39–0.91, $P = .017$). These effects remained significant after adjusting for age and sex (Table 4). The studied *SEPP1* polymorphisms were not significantly correlated with the aortic diameter of AAA patients.

Effects of the polymorphisms on the metabolic risk factors for cardiovascular disease. The relations between *SEPP1 rs3877899-rs7579* haplotypes and age, sex, smoking status, blood pressure parameters, arterial hypertension, glucose levels, diabetes, BMI values, overweight, obesity, blood lipid, and lipoproteins level in AAA and AIOD were investigated. The associations of the specific *SEPP1* haplotypes with blood pressure parameters, BMI values and occurrence of overweight and obesity (BMI > 25) were found (Figure 1, Tables 3 and 4). In AAA, carriers of two copies of the A-G haplotype had significantly higher systolic blood pressure (SBP) and pulse pressure (PP) values, as compared to subjects with one copy of this haplotype and to non-carriers (these effects remained also significant after adjusting for other potential confounders: age, sex, hypertension and PAD). The G-G haplotype was associated with higher, while G-A - with lower BMI values in this patient group (Figure 1). Carriers of the G-G haplotype had more frequently BMI values > 25 in the whole studied population (two copies: OR = 1.51, 95%CI = 1.11–2.06, $P = .007$), and display a significant 1.80-fold increase in risk for the development of AAA in overweight and obese subjects (dominant model, 95%CI: 1.16–2.79, $P = .008$). In patients group (AAA plus AIOD), carriers of the G-A haplotype had less frequently BMI values > 25 (OR = 0.69, 95% CI = 0.50–0.93, $P = 0.016$) and display a 1.54-fold increase in the risk of studied aortic diseases in non-overweight subjects (95%CI: 1.01–2.351, $P = .045$). These relationships remained significant after adjusting for age and sex. The associations of the A-G haplotype with aortic diseases in overweight and obese subjects were significant only in univariate analysis.

BMI values and other risk factors for cardiovascular disease and aortic diameter. In both patients group, BMI values were positively correlated with TG levels (AAA: $r = .231$, $P < .0001$, AIOD: $r = .338$, $P < .0001$), and negatively with the HDLC concentration (AAA: $r = -.183$, $P = .001$, AIOD: $r = -.286$, $P = .001$). Positive associations between increased BMI values and hypertension (AAA: $P = .004$, AIOD: $P = .007$) or diabetes (AAA and AIOD: $P < .0001$) were also noted. In AAA, BMI values were also positively correlated with the aortic diameter ($r = .130$, $P = .023$), while negatively with the age of patients ($r = -.156$, $P = .005$). All these associations were significant after adjusting for sex or sex and age.

Discussion

The results of our study provide preliminary evidence of the role of *SEPP1* polymorphisms in AAA and AIOD pathogenesis and their


Table 1 | Demographic and clinical characteristics of patients with abdominal aortic aneurysm (AAA), patients with aortoiliac occlusive disease (AIOD), and controls

Variable	Controls N = 336	AAA N = 334	AIOD N = 333	P value
Demographic parameters				
Median age [y]	62 (56, 69) ^{a, b}	68 (61, 74) ^a	59 (54, 66) ^b	<.0001
Age range [y]	45–86	45–94	43–86	
Male sex	286 (86.1) ^c	288 (86.0)	240 (72.1) ^c	<.0001
Potentially modifiable vascular risk factors*				
Smoking (past or present)	121 (36.0) ^{d, e}	268 (80.0) ^d	304 (91.3) ^e	<.0001
Arterial hypertension	149 (44.3) ^{f, g}	228 (68.1) ^f	219 (66.4) ^g	<.0001
SBP	137 (122, 150)	135 (120, 145)	140 (120, 145)	.453
DBP	80 (80, 90) ^{h, i}	80 (75, 90) ^h	80 (70, 85) ⁱ	.007
PP	50 (41, 60)	55 (50, 65)	55 (50, 70)	.140
Diabetes	51 (15.2)	56 (16.7)	69 (20.7)	.199
Obesity (BMI ≥ 30.0)	81 (24.1) ^{j, k}	52 (15.5) ^j	38 (11.4) ^k	<.0001
Overweight (BMI = 25.0–29.9)	134 (39.9) ^l	156 (46.7) ^m	106 (31.8) ^{l, m}	<.001
Underweight (BMI < 18.5)	6 (1.8) ⁿ	6 (1.8) ^o	27 (8.1) ^{n, o}	<.0001
BMI	27.5 (25.1, 29.8) ^{p, r}	26.3 (23.6, 28.7) ^p	24.2 (21.3, 27.1) ^r	<.0001
Lipids and lipoproteins profile [mmol/L]				
TC	5.52 (4.57, 6.47)	5.27 (4.38, 6.18)	5.33 (4.50, 6.55)	.127
HDLc	1.35 (1.11, 1.63) ^{s, t}	1.14 (0.93, 1.40) ^s	1.15 (0.96, 1.40) ^t	<.0001
LDLc	3.36 (2.58, 4.33)	3.20 (2.40, 4.12)	3.29 (2.50, 4.30)	.298
TG	1.42 (1.08, 2.02)	1.51 (1.08, 2.05)	1.61 (1.18, 2.17)	.541

BMI, body mass index [kg/m²]; DBP, diastolic blood pressure; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; TC, total plasma cholesterol; TG, triglyceride; PP, pulse pressure; SBP, systolic blood pressure.

*P adjusted for age (in 5-year strata) and sex, using logistic or conditional regression analysis.

Significant differences between groups: a, c–g, i, k, m–o, r–t, P < .0001; p, P < .001; b, j, P < .010; h, l, P < .050.

association with the metabolic risk factors of these diseases and the coexistence of PAD in AAA. The main outcomes were: 1) the association of the *SEPP1* rs3877899A allele (the rs3877899A-rs7579G haplotype), that favors the production of the low-selenium isoform of selenoprotein P, with coexistence of PAD in AAA as well as with increased systolic blood pressure and pulse pressure parameters in these patients, and 2) the association of the *SEPP1* rs3877899G-rs7579G haplotype with increased BMI values in the studied

population and AAA formation in overweight and obese subjects. These associations were novel in the context of cardiovascular disease development.

The *SEPP1* rs3877899A allele, related to a more complex phenotype of AAA, was previously associated with reduced risk of gender-dependent cancers: breast²⁸ and prostate²⁹, while the opposite allele of this SNP has been shown to enhance the risk of prostate cancer in carriers of the variant of the mitochondrial superoxide dismutase

Table 2 | Distribution of genotypes, alleles, and haplotypes of the selenoprotein P (*SEPP1*) polymorphisms in patients with abdominal aortic aneurysms (AAAs) stratified according to peripheral arterial disease (PAD) coexistence, patients with aortoiliac occlusive disease (AIOD), and controls

SEPP1 genotype	Controls N = 336	AAA			AIOD N = 333
		All N = 334	Without PAD n = 130	With PAD n = 204	
<i>rs3877899G>A</i> P _{HWE}	.870	.551	.977	.418	.895
GG	186 (55.5)	201 (60.5)	88 (67.7)	113 (55.9)	198 (59.5)
GA	128 (38.2)	117 (39.1)	38 (29.2)	79 (39.1)	117 (35.1)
AA, MAF	21 (6.3), .254	14 (5.0), .218	4 (3.1), .177	10 (5.0), .245	18 (5.4), .230
<i>rs7579G>A</i> P _{HWE}	.066	.287	.614	.309	.361
GG	176 (52.5)	162 (48.5)	59 (45.4)	103 (49.5)	156 (46.8)
GA	124 (37.0)	147 (44.0)	59 (45.4)	88 (42.3)	149 (44.7)
AA, MAF	35 (10.4), .290	25 (7.5), .295	12 (9.2), .319	13 (6.3), .279	28 (8.4), .308
<i>rs3877899- rs7579</i>					
GG - GG	77 (23.0)	78 (23.6)	35 (27.1)	43 (21.3)	70 (21.0)
GG - GA	74 (22.1)	98 (29.6)	41 (31.8)	57 (28.2)	100 (30.0)
GG - AA	35 (10.4)	25 (7.6)	12 (9.3)	13 (6.4)	28 (8.4)
GA - GG	78 (23.3)	68 (20.5)	20 (15.5)	48 (23.8)	68 (20.4)
GA - AG	50 (14.9)	48 (14.5)	17 (13.2)	31 (15.3)	49 (14.7)
AA - GG	21 (6.3)	14 (4.2)	4 (3.1)	10 (5.0)	18 (5.4)
Haplotype frequency					
G - G	0.457	0.486	0.508	0.473	0.462
G - A	0.290	0.296	0.318	0.282	0.308
A - G	0.254	0.218	0.174	0.245	0.230

MAF, Minor allele frequency; P_{HWE}, P - value for deviation from Hardy-Weinberg equilibrium.



Table 3 | Distribution of haplotypes of the selenoprotein P (*SEPP1*) polymorphisms in patients with abdominal aortic aneurysms (AAAs), patients with aortoiliac occlusive disease (AIOD), and controls, stratified according to BMI values

SEPP1 haplotype <i>rs3877899</i> - <i>rs7579</i>	Controls (N = 335)		AAA (N = 331)		AIOD (N = 333)	
	BMI < 25 n = 120	BMI ≥ 25 n = 215	BMI < 25 n = 123	BMI ≥ 25 n = 208	BMI < 25 n = 189	BMI ≥ 25 n = 144
<i>G - G</i> P_{HWE}	.771	.082	.730	.830	.225	.334
0	36 (29.8)	70 (32.7)	43 (35.0)	44 (21.2)	52 (27.5)	43 (29.9)
1	58 (47.9)	94 (43.9)	61 (49.6)	105 (50.5)	102 (54.0)	66 (45.8)
2, haplotype frequency	26 (21.5), 0.458	51 (23.8), 0.456	19 (15.4), 0.402	59 (28.4), 0.536	35 (18.5), 0.455	35 (24.3), 0.472
<i>G - A</i> P_{HWE}	.287	.135	.419	.411	.228	.963
0	64 (52.9)	112 (52.3)	52 (42.3)	108 (51.9)	81 (42.9)	75 (52.1)
1	44 (36.4)	80 (37.4)	59 (48.0)	87 (41.8)	91 (48.1)	58 (40.3)
2, haplotype frequency	12 (9.9), 0.283	23 (10.7), 0.293	12 (9.8), 0.337	13 (6.3), 0.272	17 (9.0), 0.331	11 (7.6), 0.278
<i>A - G</i> P_{HWE}	.185	.431	.614	.695	.702	.575
0	64 (52.9)	122 (57.0)	67 (54.5)	134 (64.4)	115 (60.8)	83 (57.6)
1	51 (42.1)	77 (36.0)	49 (39.8)	67 (32.2)	66 (34.9)	51 (35.4)
2, haplotype frequency	5 (4.1), 0.256	16 (7.5), 0.253	7 (5.7), 0.256	7 (3.4), 0.195	8 (4.2), 0.217	10 (6.9), 0.247

P_{HWE} , P - value for deviation from Hardy-Weinberg equilibrium.

gene³⁰. Furthermore, the risk of colorectal cancer was influenced by the two-loci interactions between the *SEPP1* *rs3877899* alleles and polymorphisms in other selenoprotein encoding genes³¹. However, this relation, while observed in Europe, was not replicated in populations of United States³² and Korea³³, most probably because of population differences in selenium supplementation⁸ and allele frequencies. The analysis of data from the International HapMap Project, the 1000 Genomes Project, the NHLBI GO Exome Sequencing Project, and other previously published^{23,24,28–36} demonstrates significant population diversity in the distribution of the *SEPP1* SNPs (Supplementary Table), which may influence the impact of alleles on cardiovascular morbidity. The frequency of the *rs3877899A* allele ranged from 0.001 to 0.412 (from 0.213 to 0.360 in subjects with European ancestry), while the frequency of the *rs7579A* allele was more similar in ethnically different populations, and it ranged from 0.120 to 0.325 (from 0.248 to 0.337 in subjects with European ancestry).

Polymorphisms in several selenoprotein encoding genes have been previously found to significantly influence cardiovascular health. For example, a variant allele of glutathione peroxidase 1 was found to protect against thoracic aortic aneurysm development in hypertensive patients, while increasing the risk of coronary artery disease, PAD, and stroke³⁷. Other examples include a polymorphism

of the glutathione peroxidase 3, which has been associated with the occurrence of stroke and cerebral venous thrombosis³⁸, and the selenoprotein S gene variants which influence the risk of stroke and coronary artery disease³⁹. The genome-wide association study approach for AAAs has so far failed to find any statistically significant associations with *SEPP1*². However, atherosclerosis may be a potential confounder in these studies, since the polymorphisms identified in these reports have been shown to be also associated with other cardiovascular diseases. Similarly, shared disease pathways between AAA and atherosclerosis may contribute to failure of studies, that focus on AAA-specific biomarkers⁴⁰.

The link between the *SEPP1* *rs3877899A* allele and the presence of PAD in AAAs corroborates the reports of the protective role of selenoprotein P and selenium against atherosclerosis development⁸. Studies show that the initiation and acceleration of atherogenesis in AAA may be a consequence of a pro-inflammatory response induced by cigarette smoking⁴¹. In accordance with this observation, smoking in our study was more common in patients with concomitant PAD, than in those without. This environmental factor has been found to exert an adverse effect on both the severity of inflammation⁴² and the levels of selenium⁹ in AAA tissue. *SEPP1* gene expression is induced by interleukin-10¹⁹, thus the preferential expression of this cytokine in AAA tissue, as compared to occlusive atherosclerotic lesions⁴¹,

Table 4 | The odds ratios (ORs) with 95% confidence intervals (CIs) for the associations between the selenoprotein P (*SEPP1*) polymorphisms and development of abdominal aortic aneurysms (AAAs)

Compared groups	Effect of <i>SEPP1</i> haplotype <i>rs3877899</i> - <i>rs7579</i> ^a	OR _{crude} (95% CI)	P _{crude}	P _{adjusted} ^b
AAA without PAD vs controls	<u>A</u> - G (carriers)	0.60 (0.39–0.91)	.017	.012
AAA without PAD vs AAA with PAD	<u>A</u> - G (carriers)	0.61 (0.38–0.96)	.033	.034
AAA without PAD vs all other subjects	<u>A</u> - G (carriers)	0.64 (0.43–0.94)	.024	.025
BMI ≥ 25 vs BMI < 25				
All subjects	G - G (2 copies)	1.51 (1.11–2.06)	.008	.004
AAA + AIOD	G - G (2 copies)	1.74 (1.19–2.54)	.004	.002
AAA	G - G (2 copies)	2.17 (1.22–3.85)	.007	.009
AAA + AIOD	G - <u>A</u> (carriers)	0.69 (0.50–0.93)	.016	.020
AAA vs controls				
Subjects with BMI ≥ 25	G - G (carriers)	1.80 (1.16–2.79)	.008	.004
Subjects with BMI ≥ 25	<u>A</u> - G (dose of haplotype)	0.70 (0.48–0.98)	.047	.060
AAA vs AIOD + controls				
Subjects with BMI ≥ 25	<u>A</u> - G (dose of haplotype)	0.71 (0.48–0.99)	.033	.050
AAA + AIOD vs controls				
Subjects with BMI < 25	G - <u>A</u> (carriers)	1.54 (1.01–2.35)	.045	.049

PAD, Peripheral arterial disease, BMI, body mass index [kg/m²].

a - The effects of the *rs3877899A*-*rs7579G* and *rs3877899G*-*rs7579A* haplotypes correspond to the impacts of the *rs3877899A* and *rs7579A* alleles, respectively (lower frequency alleles underlined), b - Adjusted for age (in 5-year strata) and sex using logistic regression analysis.

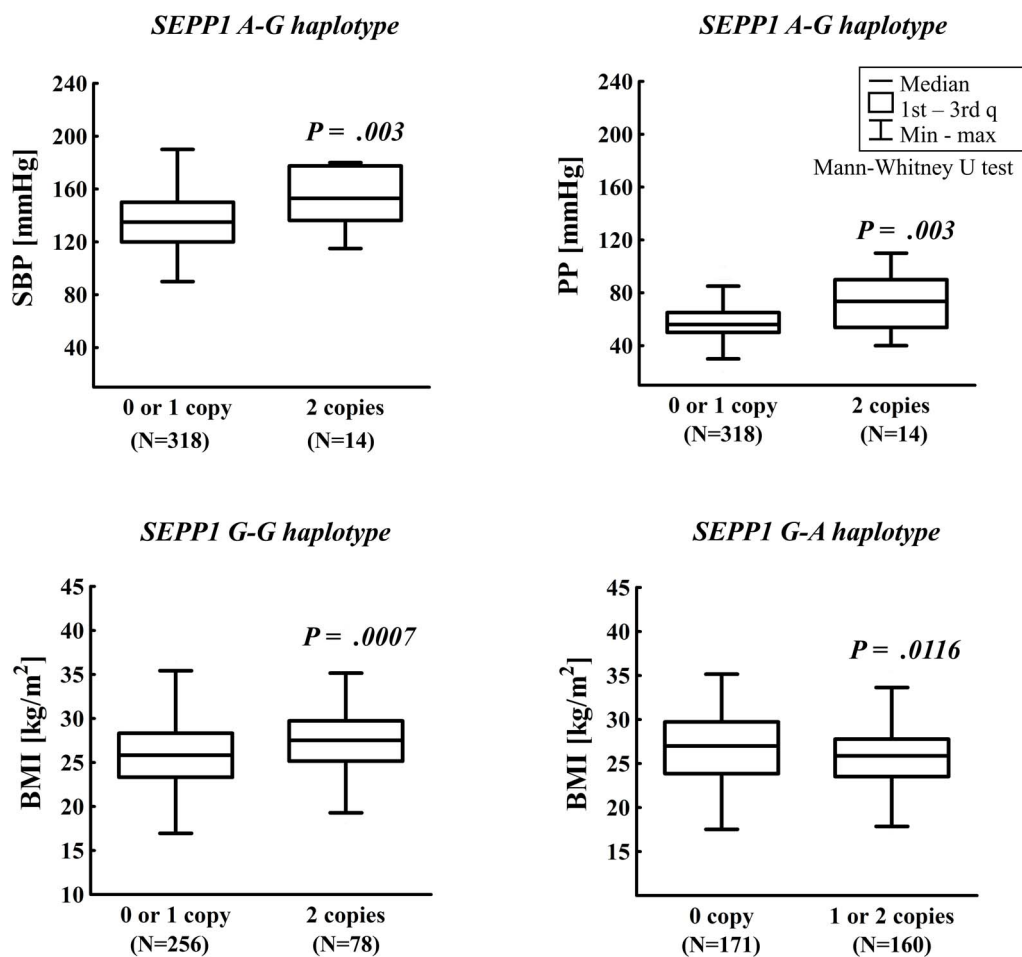


Figure 1 | Influence of the *SEPP1* rs3877899-rs7579 haplotypes on systolic blood pressure (SBP), pulse pressure (PP) parameters and body mass index (BMI) values in patients with abdominal aortic aneurysm (AAA). The x-axis indicates the number of haplotypes in patients.

may partially explain the observed lack of effects of this gene variant on AIOD development. The relative decrease in the prevalence of the rs3877899A allele in AAA without concomitant PAD, as compared to controls seems to be a consequence of subgroup selection. Nevertheless, this association confirms the heterogeneity of AAA pathogenesis, which may result in different mechanisms of disease initiation in unrelated cases.

Our data indicate that the specific *SEPP1* variants may affect the risk of AAA by enhancing the adverse effects of other risk factors: isolated systolic hypertension (the rs3877899A allele) and high BMI values (the rs3877899G- rs7579G haplotype). This type of hypertension is characterized by increased systolic and normal diastolic blood pressure values, resulting in an increase in pulse pressure values. It is primarily a consequence of a reduction in the elasticity of the large arteries, and progresses with age. Clinical studies show increased prevalence of AAA in systolic hypertension and an independent association between high pulse pressure values and the occurrence of the disease⁴³. Thus, genetically determined reduced activity of selenoprotein P resulting in the promotion of elastin degradation and endothelial dysfunction⁴⁴ under oxidative-stress conditions and may be the common mechanisms for both AAA and systolic hypertension development. Experiments involving the induction of aortic aneurysms by angiotensin II infusions in hyperlipidemic mouse strains¹ confirmed the important role of blood pressure in AAA pathogenesis, whereas recent functional studies in spontaneously hypertensive rats demonstrated the link between hypertension, low selenium diet, endothelial injury, and extensive degeneration of the aortic wall⁴⁵.

The presence of lower extremity arterial occlusion in AIOD could alter the effects of genotype, since PAD itself was found to be an independent predictor of isolated systolic hypertension in the elderly⁴⁶. Accordingly, we observed slightly higher prevalence of this type of hypertension in AIOD than in AAA (22% vs 18%, $P = .080$, data not presented). Isolated systolic hypertension is a strong predictor of cardiovascular mortality, whereas diet modification, smoking cessation and supplementation with antioxidants may serve as a therapeutic strategy for the improvement of endothelial dysfunction; therefore, further studies on selenoprotein variants in hypertension seem advisable.

In clinical studies, the two functional *SEPP1* polymorphisms, together with the values of BMI, have been shown to predict baseline plasma selenium content in the general population²³, but there are no previous reports on the role of these variants in human obesity. Notwithstanding, this function may be postulated from studies conducted on mice without the selenoprotein P gene, as these animals lost weight when fed with low-selenium diet, and did not gain it significantly when fed with a high-fat, high-sucrose diet⁸. Moreover, studies in diabetics show correlation between the levels of selenoprotein P and adiponectin⁴⁷, an adipose tissue hormone with protective function in cardiovascular diseases. The associations between the *SEPP1* polymorphisms and several metabolic phenotypes related to diabetes have also been documented; for example, the rs7579 variant was previously associated with insulin sensitivity, fasting insulin, and fibrinogen levels³⁴. Because BMI values in AAA were associated with the aorta diameter and the age of patients, evaluation of the clinical utility of *SEPP1* polymorphisms for



stratification of AAA risk and growth rate should be considered in future studies.

There are several limitations to our study; the statistical power of study is restricted by the sample size, then a future replication study should be drawn to confirm the findings. However, the low P-values for most analyzes indicate that the results are not random. The second limitation is the absence of the ankle-brachial index (ABI) measurements for PAD diagnosis. We had to use a more precise method, because ABIs could not be reliably obtained in many cases. The use of BMI for defining the risk of cardiovascular diseases has also numerous limitations; abdominal adiposity, which correlates with inflammation and systemic oxidative stress, would be a better predictor for cardiovascular risk. However, the correlation of BMI values with the size of aneurysm, age of patients and modifiable risk factors confirm the usefulness of this parameter for estimating the AAA risk. On the other hand, the symptomatic and chronic course of AIOD may weaken the strength of the relationship between the *SEPP1* polymorphisms and BMI values. As the severe symptoms of lower extremity ischemia lead to physical exhaustion in a high number of patients, an increased frequency of underweight individuals was observed among examined AIOD cases. The fourth limitation is that this was only a clinical association study, and thus selenium content, and other confounding factors that might impair the selenoprotein P production: deficiency of other nutrients, exposition to heavy-metals and food additive carrageenan, insulin resistance, visceral obesity, nonalcoholic fatty liver disease, were not examined; therefore, potential mechanisms were not fully elucidated. Also the influence of cigarette smoking could not be reliably evaluated, because of underrepresentation of nonsmokers in studied diseases (statistical analyzes indicated no effect of smoking; data not presented). Nevertheless, it should be stressed that genetic research is highly useful in delineating causal disease pathways, because it represents lifelong exposure and is not affected by reverse causality.

In conclusion, this study provide preliminary genetic evidence that the *SEPP1* functional variants contribute to the AAA risk. The results suggests that the *rs3877899A* allele may increase the susceptibility or promote AAA progression through influencing the risk of peripheral atherosclerosis and isolated systolic hypertension. The *SEPP1 rs3877899G-rs7579G* haplotype seems to be a factor that increases predisposition to AAA in overweight and obese subjects and a potential marker of aggressive-growing AAAs. Further work is required to confirm the findings in other populations and to study the functional role of these variants in AAA.

Methods

Study population. The study enrolled 335 AAA patients and 333 AIOD patients scheduled for surgery at the Department of General and Vascular Surgery of the Poznan University of Medical Sciences in the years 1999–2011. The control group, consisting of 336 subjects matched for age (± 5 years) and sex to the AAA patients, was selected during the same time from the Poznan district. The patients and controls included in this study were previously investigated for polymorphisms involved in homocysteine metabolism⁴⁸ and the hypoxia signaling pathway⁴⁹. Detailed definitions and clinical characteristics were presented in previous publications^{48,49}. In short, the exclusion criteria for the controls included known aneurysms and peripheral arterial disease (PAD), whereas for AIOD – the coexistence of AAA. The diagnosis of AAA was evaluated by computed tomography angiography or magnetic resonance angiography, while the diagnosis of AIOD – by computed tomography angiography alone. Based on physical examination supplemented with ultrasound duplex color scanning, the coexistence of PAD was recognized in 60.3% of the AAA patients. All patients were treated pharmacologically with statins, antiplatelet drugs, and other drugs (antihypertensive or antidiabetic), depending on their clinical condition.

Questionnaires were used to obtain information concerning cigarette smoking history, and standardized methods were employed for the evaluation of the remaining known risk factors for cardiovascular diseases. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg, as well as in the case of antihypertensive therapy. Diabetes was determined by fasting plasma glucose levels (>6.8 mmol/L) and antidiabetic medication status. Obesity was recognized at a body mass index (BMI) ≥ 30.0 kg/m² overweight at BMI = 25.0–29.9 kg/m², whereas underweight at BMI < 18.5 kg/m². The study was conducted in accordance with the Declaration of Helsinki. The study protocol was

approved by the Bioethical Committee of the Poznan University of Medical Sciences, Poland (decision no 628/10), and all subjects provided their informed and written consent to participate in this study.

Genotyping. Genomic DNA was extracted from circulating blood lymphocytes by a chemical method (using the acid guanidinium thiocyanate and phenol/chloroform extraction). Polymorphisms were ascertained with the use of the TaqMan Pre-Designed SNP Genotyping Assays (Life Technologies) employing the ABI 7900HT Fast Real-Time PCR System (Life Technologies).

Data analysis. Genotype frequencies were tested for Hardy-Weinberg equilibrium using the χ^2 test (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>). Haploview was used for *SEPP1* haplotype analysis, and QUANTO software (<http://hydra.usc.edu/GxE/>) was employed for sample size calculations. For genotypes, alleles, and haplotypes, the values of odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. The post-hoc analysis revealed that the statistical power of our study for analyses of the differences in genotype distribution between subgroups of patients and controls ranged between 50%–70%. In univariate analyses, the Mann-Whitney U test, the Kruskal-Wallis or ANOVA tests were performed for quantitative variables and the χ^2 test was employed for qualitative variables. Logistic and conditional regression analysis or MANOVA tests were used to adjust the effects of modifiable risk factors of AAA and AIOD and genotype for age, sex and other potential confounders. In parametric analyses, continuous variables which deviate in distribution from the normal curve were analyzed after logarithmic transformation. Pearson's coefficient of correlation, were calculated (r) to assess the strength of the association between BMI values and other risk factors (when appropriate). These analyses were performed using STATISTICA version 10.0 software. The observed differences were considered significant at $P < .05$, with the exception of the effects of the *rs3877899G-rs7579G* haplotype, which were considered significant at $P < .025$ (the Bonferroni correction for multiple comparisons was applied).

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Author contributions

E.S. designed the study, analyzed and interpreted the data, performed genotyping and statistical analysis, drafted the manuscript and prepared figure. E.S. and G.O. participated in data acquisition. G.O. and R.S. took part in patients selection and examination, and performed critical revision of the article. E.S., R.S. and G.O. participated in obtaining funding. All authors read and approved the final manuscript. Overall responsibility of E.S.

Additional information

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