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ORIGINAL RESEARCH

Relationships between *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 polymorphisms and the occurrence of selected metabolic and hormonal disorders in middle-aged and elderly men – a preliminary study

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Purpose: Metabolic disorders, including MetS, obesity, and lipid disorders, may be related to genetic factors. Metabolic disorders are associated with decreased TS levels in aging men. The aim of this study was to evaluate the relationship between *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 polymorphisms and the presence of MetS and its components, the concurrent lipid disorders, as well as sex hormone concentrations.

Subjects and methods: This study involved 272 men of Caucasian descent aged 50–75 years. Lipid profile, including TCh, LDL, HDL, and TG, was evaluated by spectrophotometric method. Anthropometric measurements concerned WC and blood pressure. MetS was diagnosed according to the criteria of the IDF. Sex hormone profile, including TST, FTS, E_2, DHEAS, and SHBG, was examined using enzyme-linked immunosorbent assay. Polymorphisms within *FTO*, *MC4R*, and *PPARγ* genes were identified using polymerase chain reaction-restriction fragments length polymorphism.

Results: This study did not show links between the analyzed genetic polymorphisms and the presence of MetS, T2DM, HT, and obesity. However, higher concentrations of TCh and LDL were found in men with the *FTO* rs9939609 polymorphism in the recessive mode of inheritance ($P=0.03$ and $P=0.05$, respectively). Lower WC was found to be associated with *MC4R* rs17782313 gene inherited in the same model ($P=0.005$).

Conclusion: *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 polymorphisms seem to have little effect on the incidence of metabolic malfunctions and no effect on androgen-related disorders in the examined middle-aged and elderly men.

Keywords: aging men, metabolic syndrome, sex hormones, genes, *FTO*, *MC4R*, *PPARγ*

Introduction

Although the incidence of MetS is known to depend on lifestyle, diet, and physical activity,[1,2] it is also suggested that genetic factors may play an important role. For example, the risk for MetS may be increased by the presence of common SNPs within the *FTO*-associated gene, the *MC4R* gene, and the *PPAR* gene. As the expression profile of the corresponding proteins is strictly dependent on the genetic information within their genes, functional implications of these three polymorphisms seem to be significant. However, their exact mechanisms of action are still not clear.

The *FTO* gene is located on chromosome 16 and encodes 2-oxoglutarate-dependent nucleic acid demethylase. The gene is expressed in many tissues, especially in

the hypothalamus, responsible for the control of energy balance.[3,4] Increases in the hypothalamic expression of *FTO* are associated with the regulation of energy intake but not with the feeding reward.[5] Few SNPs within *FTO* gene were found to play a role in obesity and T2DM.[6–9] To date, three SNPs (rs9939609, rs1121980, and rs1558902) have been associated with obesity and BMI in Caucasian and Hispanic Americans.[3,6,7] The association of rs9939609 (A>T variant) with obesity was found to be the strongest, especially in the dominant model of inheritance.[10] In addition, a link between the *FTO* rs9939609 polymorphism and obesity and MetS has been confirmed in many studied populations, including in Europe.[11–16]

The *MC4R* and leptin genes are essential in the hypothalamic regulation of appetite.[17,18] The *MC4R* gene plays an essential role in the maintenance of energy balance and is stimulated by endogenous melanocortins.[19] Its mutations account for 2.4%–4% of morbidly obese people; the polymorphisms of this gene have also been associated with obesity.[20] Common variants within the *MC4R* gene have been reported as the second strongest association signal for common obesity in the GWAS.[21] In adult Europeans, the *MC4R* rs17782313 (C>T variant) polymorphism has been widely studied and found to predispose to obesity.[21] With regard to MetS, the studied association with the *MC4R* rs17782313 polymorphism disappears after adjusting for WC, indicating that the association with MetS is driven by the association with this factor.[22] Moreover, studies on males proved that the rs17782313 polymorphism is associated with lower HDL concentration[23] and elevated DBP.[14,24]

PPARs are nuclear receptors, participate in adipogenesis and lipid metabolism, regulate insulin sensitivity, and participate in transformations in the energy system.[25] Of the three types of PPARs (α, β, and γ), the best known is PPARγ, with two isoforms – PPARγ1, which is expressed in many tissues, and PPARγ2, which is expressed almost exclusively in adipocytes.[26] The PPARγ activation plays an important role in adipocyte differentiation and maturation, lipid metabolism and transport, and improving insulin sensitivity.[27,28] The *PPARγ* gene is located on the short arm of chromosome 3 at 3p25 band. The most common *PPARγ* gene polymorphism is rs1801282 (C>G variant), resulting from proline being replaced with alanine.[29] Some literature data indicate a relationship between the rs1801282 polymorphism and metabolic disorders.[30] Healthy nonsmoking men carrying the mutant allele of rs1801282 polymorphism are at a high risk for MetS and insulin resistance.[30] In a large study of the Caucasians, the rs1801282 was linked to WC in patients with T2DM.[31] However, it must be

remembered that other researchers found no such associations.[32] In addition, PPARγ is involved in the activation of androgen receptor (AR), which may affect the action of androgens.[33]

Literature studies on men show significant relationships between MetS and hormonal changes. It is emphasized that a decrease in FTS and TST promotes the development of metabolic disorders, while disorders of carbohydrate metabolism, lipid metabolism and obesity contribute to hypogonadism.[34–37] This indicates that these pathologies are interrelated.

Both cross-sectional and longitudinal epidemiological studies indicate that the level of SHBG is lower in men with MetS.[38–40] Other researchers emphasize, however, that the relationships between MetS and either TS or SHBG are stronger in younger men.[34,35] Dehydroepiandrosterone does not seem to play a significant role in the development of MetS in men.[34,37,41] Finally, the conclusions of the EMAS support the supposition that E_2 levels in aging men are not related to the development of MetS.[42]

Despite some knowledge on the interrelationships between metabolic and hormonal disorders in men, we still do not know which factors trigger them. It remains to be solved whether they are environmental or lifestyle factors, or genetic factors, and what comes first – metabolic or hormonal changes. Regarding the genetic factors that with a high probability are responsible for MetS and associated disorders, it seems important to find any potential links with hormonal disturbances.

We hypothesized that the selected genetic polymorphisms (*FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282) may contribute to the occurrence and increase in metabolic and androgen disorders in aging men. We aimed to examine the relationships between the *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 and the prevalence of MetS and related disorders, such as HT, T2DM, and obesity. In addition, we aimed to analyze the relationships between selected polymorphisms and the parameters of lipid profile, including TCh, LDL, HDL, TG, sex hormones, including TST, FTS, E_2, DHEAS, SHBG, and anthropometric parameters, including BMI, WC, and ABP in middle-aged and elderly men, which may contribute to increased knowledge about the mutual relationships between metabolic and sex hormone disorders in aging men.

Subjects and methods
Study population
This study involved 272 Caucasian men aged 50–75 years (mean age 62±6.4 years) who voluntarily signed up after receiving information about the study from their doctors at

primary health-care centers in the city of Szczecin (Poland). The exclusion criteria in this study included cancer treatment, receiving steroids (including T and dehydroepiandrosterone), thyroid disease, and receiving neuroleptics or antidepressants. The medical questionnaire showed that none of the participants exceeded a daily alcohol intake of 40 g. We excluded patients on slimming diets or showing above-average physical activity. The research material was collected from June 2013 to July 2014. Patients participating in the study filled in an original questionnaire (questions about lifestyle including nutritional habits, smoking, alcohol intake, demographics status, physical activity, diseases, and drug intake). The study was performed in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0012/159/12). The men in the study were informed about the details of the research project and expressed their written consent to participate in the study. They also signed a separate agreement for storing their genetic material.

Among the respondents, we conducted a survey on demographic data and the presence of chronic diseases. Surveys revealed that 150 people suffered from HT (55.1%), 50 people had T2DM (18.4%), and statins were taken by 30 of the men (11.0%). None of the patients received fibrates.

MetS was diagnosed according to the 2005 IDF criteria for European men (WC ≥94 cm and at least two of the following: FPG ≥100 mg/dL or T2DM treatment; ABP ≥130/85 mmHg or HT treatment; HDL <40 mg/dL or dyslipidemia treatment; and TG ≥150 mg/dL or dyslipidemia treatment).

Anthropometric and blood pressure (BP) measurements

In order to accurately measure WC, a physician located patients' upper hip bone and then placed a tape measure around bare stomach just above the hip bone. Each time, the measuring tape was parallel to the floor and adjacent to the patient's body. During measurement, all the patients were asked to relax the abdomen and exhale. The measurements were performed under identical environmental conditions and by one person. BMI was calculated; we assumed that BMI in the range of 18.5–24.99 denotes normal weight, of 25–29.99 indicates overweight, and of ≥30 means obesity.

To assess the SBP and DBP, the sphygmomanometer was used. The smallest cuff size covering ~2/3 of the right upper arm and encircling the entire arm completely, was selected. BP was measured in a supine position after 15 minutes rest, and only one of the physicians performed all of the BP measurements using a standardized protocol.

Blood sampling

Blood was taken from the tested men on an empty stomach from an ulnar vein, between 7.30 am and 9.00 am. For the biochemical and hormonal assays, blood was drawn into a tube with a coagulator and gel separator and then centrifuged. For the genetic assays, blood was collected into tubes with ethylenediaminetetraacetic acid (anticoagulant). The sera were stored at −70°C.

In the blood serum, we determined TCh, LDL, HDL, TG, and FPG by spectrophotometric method, with the use of reagent kits (BIOLABO; Aqua-Med, Lodz, Poland). FPG was determined only in the nondiabetic men. MetS was diagnosed according to the criteria of the IDF from 2005.[43] Enzyme-linked immunosorbent assay was used to determine serum concentrations of DHEAS, TST, FTS, E₂, and SHBG with the use of reagent kits (DRG Medtek, Warsaw, Poland). Measurements were performed in triplets, and arithmetic mean (AM) was calculated. The measurements were rejected when the coefficient of variation was >10%.

Genotyping of the *FTO, MC4R,* and *PPARγ*

Genetic investigations were carried out in a laboratory of the Department of Gerontobiology, Pomeranian Medical University, Szczecin, Poland. Genomic DNA from peripheral blood leukocytes was extracted using an extraction kit (High Pure PCR Template preparation Kit; Roche, Mannheim, Germany) according to the manufacturer's instructions. DNA samples were stored at 4°C for further analyses. Protocols for polymorphisms *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 followed previously published polymerase chain reaction-restriction fragments length polymorphism techniques,[44–46] which were performed in a Hightech Thermocycler Cycler-Technology for Life (SensoQuest, Gottingen, Germany). The genotypes were determined by 2%–3% agarose gel electrophoresis (Agarose; Sigma-Aldrich, Munich, Germany) stained with DNA-star dye (Lonza, Inc, Rockland, ME, USA). The correctness of results was verified by repeating each mutated sample from PCR onward. All results were found to be reliable. For each of the loci, we performed analyses in overdominant models of inheritance followed by the adopted recessive models of inheritance (*FTO*: AA + TA vs TT; *MC4R*: CC + CT vs TT; and *PPARγ*: GG + CG vs CC).

Statistical analysis

Statistical analysis was performed using the StatView software, Version 5.0 (SAS Institute Inc., Cary, NC, USA). Continuous variables (ie, BMI, ABP, TST, FTS, SHBG, E₂, DHEAS, TG, TCh, LDL, HDL, and FPG) were described by AM with standard deviation, median, and range. In the

description of qualitative variables (ie, genotype, T2DM, HT, MetS, overweight and obesity, and statin treatment), we presented the number (n), which is also expressed as a percentage.

First, χ^2 test was used to verify whether genotype frequencies fit to the Hardy–Weinberg (H–W) equilibrium. Then, an analysis of variance test was used to assess the associations between the genotypes *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 polymorphisms and the anthropometric indicators, hormonal and metabolic parameters. In the next step, the assessment of relationships between genotypes and the qualitative variables was performed using a χ^2 test of independence. To evaluate whether the studied polymorphisms determined any of the anthropometric and metabolic indices, which were found to be significant in previous analyses, we used the logistic regression analysis for the determination of odds ratio (OR) and 95% confidence intervals. The level of significance was set at $P \leq 0.05$.

Results

The characteristics of the study group and descriptive analysis of the metabolic and hormonal parameters are listed

in Table 1. Normal body weight was found in 65 patients (23.9%), overweight was found in 130 patients (47.8%), and obesity was found in 77 (28.3%). MetS was diagnosed in 144 patients (52.9%).

Genotype and allele distribution is listed in Table 2. They did not differ from H–W distribution ($P > 0.05$).

We found no associations between *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 polymorphisms and the qualitative variables (MetS, T2DM, HT, obesity, and overweight), and also in the overdominant and recessive models of inheritance (Table 3).

In the case of continuous variables, we found that *FTO* rs9939609 polymorphism inherited only in the recessive pattern, which was associated with LDL concentration; the difference in LDL concentration was statistically significant (AA + AT: 136.18±51.57 mg/dL vs TT: 153.04±62.48 mg/dL; P=0.03). We also found that the presence of two mutant alleles was associated with higher TCh level (TT: 223.07±64.94 mg/dL and AA + AT: 207.3±54.9 mg/dL; P=0.05). There was no other evidence of links between the analyzed continuous variables and the *FTO* rs9939609 polymorphism in any of the analyzed models of inheritance. However, the association between the recessive genotype of rs9939609 polymorphism and SBP was close to statistical significance (P=0.07).

Analyzing the relationship between the continuous variables and the *MC4R* rs17782313 polymorphism, we demonstrated that men with the CC and CT genotypes had a significantly greater WC (CC + CT: 103.91±12.61 cm vs TT: 99.77±10.88 cm; P=0.005) (Table 4). For other continuous variables, we found no statistically significant relationships

Table 1 Characteristics of the study group (n=272)

Parameter	AM ± SD	Med	Range
Continuous variables			
BMI	28.31±4.46	27.68	18.72–46.7
SBP (mmHg)	130.85±19.13	130.0	90.0–220.0
DBP diastolic (mmHg)	82.43±11.57	80.0	50.0–140.0
TST (ng/mL)	3.68±1.64	3.35	0.9–9.83
FTS (pg/mL)	108.03±61.19	98.48	8.87–423.67
SHBG (nmol/L)	44.57±23.46	41.66	2.74–192.0
E_2 (pg/mL)	36.67±22.94	33.85	5.61–120.27
DHEAS (µg/mL)	1.40±0.78	1.32	0.01–3.85
TG (mg/dL)	144.18±80.82	129.50	37.00–609.0
TCh (mg/dL)	211.36±57.99	204.00	95.00–416.0
LDL (mg/dL)	140.52±54.96	134.50	18.00–342.00
HDL (mg/dL)	42.54±12.53	41.0	19.00–82.00
FPG (mg/dL)	106.33±29.44	102.0	63.75–215.0
		n	**%**
Categorical variables			
T2DM		50	18.4
HT		150	55.1
MetS		144	52.9
Overweight		130	47.8
Obesity		77	28.3

Abbreviations: AM, arithmetic mean; SD, standard deviation; Med, median; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TST, total testosterone; FTS, free testosterone; SHBG, sex hormone binding globulin; E_2, estradiol; DHEAS, dehydroepiandrosterone sulfate; TG, triglyceride; TCh, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; T2DM, type 2 diabetes mellitus; HT, hypertension; MetS, metabolic syndrome.

Table 2 Genotype and allele distribution in the study group (n=272)

SNP	Genotype/allele	n=272/544 (%)	P-value
FTO rs9939609	AA	60 (22.06)	0.45
	TA	142 (52.21)	
	TT	70 (25.73)	
	A	262 (48.16)	
	T	282 (51.84)	
MC4R rs17782313	CC	8 (2.94)	0.33
	CT	91 (33.46)	
	TT	173 (63.60)	
	C	107 (19.67)	
	T	437 (80.33)	
PPARγ rs1801282	CC	186 (68.38)	0.13
	CG	82 (30.15)	
	GG	4 (1.47)	
	C	454 (83.46)	
	G	90 (16.54)	

Abbreviations: SNP, single-nucleotide polymorphism; *FTO*, fat mass and obesity; *MC4R*, melanocortin 4 receptor; *PPARγ*, peroxisome proliferator-activated receptor.

Table 3 Relations between T2DM, HT, MetS, BMI, and the presence of *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 gene polymorphisms evaluated by means of χ^2 tests

Variable	FTO			MC4R			PPARγ		
	AA + TA, n (%)	TT, n (%)	P-value	CC + CT, n (%)	TT, n (%)	P-value	CG + GG, n (%)	CC, n (%)	P-value
T2DM (n=50)	38 (76)	12 (24)	0.75	23 (46)	27 (54)	0.11	15 (30)	35 (70)	0.78
Without T2DM (n=222)	164 (73.87)	58 (26.13)		76 (34.23)	146 (65.77)		71 (31.98)	151 (68.02)	
HT (n=150)	113 (75.33)	37 (24.67)	0.65	55 (36.67)	95 (63.33)	0.92	45 (30.00)	105 (70.00)	0.52
Without HT (n=122)	89 (72.95)	33 (27.05)		44 (36.07)	78 (63.93)		41 (33.61)	81 (66.39)	
MetS (n=144)	109 (75.69)	35 (24.31)	0.57	52 (36.11)	92 (63.89)	0.92	48 (33.33)	96 (66.67)	0.52
Without MetS (n=128)	93 (72.65)	35 (27.35)		47 (36.72)	81 (63.28)		38 (29.69)	90 (70.31)	
Normal BMI (n=65)	44 (67.69)	21 (32.31)	0.37	21 (32.31)	44 (67.69)	0.36	19 (29.23)	46 (70.77)	0.43
Overweight (n=130)	100 (76.92)	30 (23.08)		45 (34.62)	85 (65.38)		46 (35.39)	84 (64.61)	
Obesity (n=77)	58 (75.32)	19 (24.68)		33 (42.86)	44 (57.14)		21 (27.28)	56 (72.72)	

Abbreviations: T2DM, type 2 diabetes mellitus; HT, hypertension; MetS, metabolic syndrome; BMI, body mass index; *FTO*, fat mass and obesity; *MC4R*, melanocortin 4 receptor; *PPARγ*, peroxisome proliferator-activated receptor; A, adenine; T, thymine; C, cytosine; G, guanine.

with the *MC4R* rs17782313 polymorphism in both models of inheritance.

This study showed no connection between the *PPARγ* (rs1801282) polymorphism and the studied continuous variables.

In the final step of our experiments, we aimed to establish whether recessive genotypes of the polymorphisms determined any of the clinical conditions (T2DM, MetS, HT, overweight, and obesity), or continuous variables – anthropometric, hormonal, and metabolic indices – by means of logistic regression. We found no statistical significance with regard to the relationship between *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* (rs1801282) polymorphisms

and any of the clinical conditions – MetS, T2DM, HT, overweight, and obesity ($P>0.05$). On the other hand, two mutant alleles of *FTO* rs9939609 polymorphism were found to be related to a minimal elevation of LDL, and the recessive genotype (TT homozygotes) of *MC4R* rs17782313 polymorphism was associated with a reduction in WC. The remaining anthropometric and biochemical parameters showed no statistical significance in the logistic regression model. The results are presented in Table 5.

Discussion

In this study, we found no relationship between the presence of *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282

Table 4 Relations between the anthropometric parameters: WC, BMI, and ABP; biochemical parameters: TST, FTS, SHBG, E$_2$, TG, TCh, LDL, HDL, WC, and the *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 gene polymorphisms, evaluated by ANOVA (n=272)

Variable	FTO rs9939609			MC4R rs17782313			PPARγ rs1801282		
	AA + TA	TT	P-value	CC + CT	TT	P-value	CG + GG	CC	P-value
BMI	27.99±4.25	28.30±4.94	0.61	28.62±4.66	27.75±4.32	0.12	27.67±4.09	28.25±4.16	0.32
SBP (mmHg)	130.33±18.99	132.37±19.57	0.44	133.58±20.22	129.29±18.36	0.07	131.45±20.10	130.57±18.71	0.72
DBP (mmHg)	82.08±11.24	83.43±12.53	0.40	83.48±11.61	81.82±11.54	0.25	82.44±11.11	82.42±11.8	0.98
TST (ng/mL)	3.66±1.65	3.73±1.61	0.78	3.72±1.79	3.66±1.55	0.77	3.72±1.79	3.65±1.68	0.77
FTS (pg/mL)	105.60±59.10	115.05±66.59	0.26	114.05±62.52	104.59±60.33	0.22	114.05±62.52	106.22±61.22	0.22
SHBG (nmol/L)	43.50±23.19	47.54±24.15	0.22	44.13±27.87	44.83±20.61	0.81	46.18±21.47	43.83±24.35	0.45
E$_2$ (pg/mL)	35.56±22.82	39.88±23.14	0.17	37.23±22.16	36.36±23.43	0.76	34.66±20.79	37.60±23.86	0.33
DHEAS (µg/mL)	1.37±0.78	1.52±0.78	0.17	1.44±0.84	1.38±0.75	0.59	1.43±0.78	1.39±0.78	0.70
TG (mg/dL)	143.89±77.53	145.00±90.27	0.92	146.95±75.57	142.59±83.86	0.67	142.73±77.39	144.85±82.56	0.84
TCh (mg/dL)	207.30±54.97	223.07±57.14	0.05*	211.57±59.93	211.24±57.03	0.96	215.28±54.86	209.55±59.44	0.45
LDL (mg/dL)	136.18±51.57	153.04±62.48	0.03*	140.93±57.36	140.29±53.71	0.93	144.32±49.37	138.76±57.41	0.44
HDL (mg/dL)	42.95±12.55	41.37±12.47	0.36	42.42±13.17	42.61±12.9	0.91	42.43±12.84	42.58±12.42	0.92
WC (cm)	100.85±11.15	102.53±13.09	0.3	103.91±12.61	99.77±10.86	0.005*	100.5±11.11	101.64±11.95	0.45

Notes: *Statistically significant. Data presented as AM ± SD.
Abbreviations: WC, waist circumference; BMI, body mass index; ABP, arterial blood pressure; TST, total testosterone; FTS, free testosterone; SHBG, sex hormone-binding globulin; E$_2$, estradiol; TG, triglyceride; TCh, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; *FTO*, fat mass and obesity; *MC4R*, melanocortin 4 receptor; *PPARγ*, peroxisome proliferator-activated receptor; ANOVA, analysis of variance; SBP, systolic blood pressure; DBP, diastolic blood pressure; DHEAS, dehydroepiandrosterone sulfate; A, adenine; T, thymine; C, cytosine; G, guanine.

Table 5 The influence of recessive *FTO* rs9939609, *MC4R* rs17782313, and *PPARγ* rs1801282 genotypes on metabolic, hormonal, and anthropometric indices by means of logistic regression

Variable	*FTO* rs9939609, TT vs AA + TA			*MC4R* rs17782313, TT vs CC + CT			*PPARγ* rs1801282, CC vs CG + GG		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
BMI	1.016	0.956–1.079	0.61	0.957	0.906–1.012	0.12	1.031	0.972–1.094	0.31
SBP (mmHg)	1.006	0.992–1.02	0.44	0.988	0.976–1.001	0.07	0.998	0.984–1.011	0.72
DBP (mmHg)	1.01	0.987–1.034	0.49	0.988	0.967–1.009	0.25	1.00	0.978–1.022	0.99
TST (ng/mL)	1.024	0.868–1.207	0.78	0.978	0.842–1.137	0.77	0.972	0.832–1.135	0.72
FTS (pg/mL)	1.002	0.998–1.007	0.27	0.998	0.994–1.002	0.22	0.998	0.994–1.003	0.47
SHBG (nmol/L)	1.007	0.996–1.018	0.22	1.001	0.991–1.012	0.81	0.996	0.985–1.007	0.44
E_2 (pg/mL)	1.008	0.996–1.02	0.18	0.998	0.988–1.009	0.76	1.006	0.994–1.017	0.33
DHEAS (µg/mL)	1.268	0.9–1.786	0.17	0.917	0.669–1.256	0.59	0.936	0.676–1.297	0.69
TG (mg/dL)	1.00	0.997–1.004	0.92	0.99	0.996–1.002	0.66	1.00	0.997–1.004	0.84
TCh (mg/dL)	1.005	1.00–1.009	0.05*	1.00	0.996–1.004	0.96	0.998	0.994–1.003	0.45
LDL (mg/dL)	1.006	1.001–1.011	0.03*	1.00	0.995–1.004	0.93	0.998	0.994–1.003	0.44
HDL (mg/dL)	0.99	0.968–1.012	0.36	1.001	0.982–1.021	0.91	1.001	0.981–1.022	0.92
WC (cm)	1.012	0.989–1.036	0.29	0.97	0.949–0.991	0.006*	1.009	0.986–1.031	0.45

Note: *Statistically significant.
Abbreviations: *FTO*, fat mass and obesity; *MC4R*, melanocortin 4 receptor; *PPARγ*, peroxisome proliferator-activated receptor; OR, odds ratio; CI, confidence interval; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TST, total testosterone; FTS, free testosterone; SHBG, sex hormone-binding globulin; E_2, estradiol; DHEAS, dehydroepiandrosterone sulfate; TG, triglyceride; TCh, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; WC, waist circumference; A, adenine; T, thymine; C, cytosine; G, guanine.

polymorphisms and the incidence of T2DM, HT, MetS, and obesity in Caucasian men aged 50–75 years, living in the city of Szczecin (Poland). We also found no links between the presence of these polymorphisms and the levels of the tested hormones (TST, FTS, E₂, and DHEAS) and SHBG. With regard to biochemical parameters, we found a statistically significant association between the levels of TCh and LDL and the *FTO* rs9939609 polymorphism in the recessive model of inheritance. We proved that mutant homozygotes had higher TCh and LDL concentrations in comparison to other genotypes. In the group of anthropometric parameters, WC was statistically significantly associated with the *MC4R* rs17782313 polymorphism. Possessing two mutant alleles was found to be associated with lower WC in comparison to wild-type homozygotes and heterozygotes for the analyzed SNP.

Our results on the *FTO* rs9939609 polymorphism stand in contrast to a study in which non-Caucasian subjects demonstrated links between the *FTO* rs9939609 polymorphism and the occurrence of MetS.[47] Moreover, that study also reported that an increased risk for MetS, especially in men, is associated with the presence of at least one wild-type allele. This regularity is also mentioned in a meta-analysis by Freathy et al,[48] where allele A frequency correlated with lower HDL and higher TG concentrations and other components of MetS, but after adjusting for BMI, this relationship became statistically insignificant. In this study, we found no statistically significant relationships between either HDL or TG level and the *FTO* rs9939609 polymorphism. However, we did observe a relationship between the presence of the *FTO* rs9939609 polymorphism and both LDL and TCh concentrations. Our results indicated that the men with at least one allele of wild type had significantly lower levels of TCh and LDL compared to the men with the TT (mutant) genotype. Probably, lipid concentrations result primarily from the lifestyle of middle-aged and older men, including a high-calorie diet and low physical activity.

Carlos et al[15] concluded that rs9939609 variant predisposes to obesity in Portuguese population aged 18–50 years. Woehning et al[16] found that the polymorphism is linked to initial body weight, based on the results of their cross-sectional study in adults aged 18–72 years. Liguori et al[49] proved that in a population of obese subjects of Italian origin, TA heterozygotes of rs9939609 are more prone to develop MetS (OR 2.53). A study by Jacobsson et al[50] finds no relationship between the *FTO* rs9939609 polymorphism and BMI in elderly men from Sweden, indicating that its contribution to the incidence of obesity decreases with age. Zavatarri et al[51] showed no association between the *FTO* rs9939609 polymorphism and the parameters of lipid metabolism and stressed that AA homozygotes are particularly predisposed to obesity. Population studies conducted by Gustavsson et al[52] on Gothenburg residents confirmed that AA and TA genotypes have a greater WC and a higher BMI compared to TT genotype.

All the aforementioned results stand in contrast to our findings, showing the lack of relationship between metabolic parameters and the *FTO* rs9939609 polymorphism. It may

have been due to the relatively small size of the group and the differences in selection of the participants in terms of age and gender. Our male population was aged between 50 and 75 years, while there is evidence that in people aged >65 years, the relationship between the *FTO* rs9939609 polymorphism and obesity is reduced, especially in men.[53]

With respect to the *MC4R* rs17782313 polymorphism, some researchers emphasize the role of C (wild-type) allele being associated with a higher risk for insulin resistance, T2DM, and HT incidence, independently of BMI.[54] The relation between the *MC4R* rs17782313 polymorphism and obesity was also described by Xi et al.[55] With regard to MetS, the association with the *MC4R* rs17782313 polymorphism disappears after adjusting for WC, indicating that the association with MetS is driven by the link with this factor,[13] which shows that C allele may predispose to abdominal obesity. This is confirmed by our results where men with at least one C allele had a significantly higher WC, in addition to a higher BMI, although that difference was not statistically significant. Therefore, it can be assumed that the wild-type homozygous and heterozygous genotypes of *MC4R* rs17782313 polymorphism promote the accumulation of visceral fat. On the other hand, Marcadenti et al[14] found that *MC4R* rs17782313 is positively associated with neck circumference and BMI in women and negatively associated with BP in men. However, the study was conducted in non-Caucasians, which makes the comparisons complicated.

In this study, we found no statistically significant associations between the *PPARγ* rs1801282 polymorphism and MetS, T2DM, HT, overweight, obesity, and anthropometric and biochemical parameters in men aged between 50 and 75 years. On the other hand, Tellechea et al[30] found that in men from Argentina, with the exception of Caucasians, the rs1801282 polymorphism showed a relation with the occurrence of MetS diagnosed using the criteria of the National Cholesterol Education Program/Adult Treatment Panel III and with insulin resistance, especially in nonsmokers. However, those studies were conducted among younger men, so it can be assumed that environmental influences were lower. As in this study, Milewicz et al[56] did not observe any association between the studied *PPARγ* rs1801282 polymorphism and MetS and metabolic parameters in postmenopausal women from Poland. Also studies conducted on an ethnically diverse group of people (Caucasian, South Asian, and African American) did not show any association between the *PPARγ* rs1801282 polymorphism and MetS.[32] Dytfeld and Horst-Sikorska[29] highlighted the role of diet in metabolic disorders in patients with the *PPARγ* rs1801282 polymorphism and

modification of the genotype by environmental factors, which can lead to diverse phenotypes. On the other hand, Kruzliak et al[31] conducted analyses in a group of >1000 Caucasians with T2DM and proved that the homozygous Slovene females with wild-type alleles had lower WC in comparison with homozygous females with mutated alleles. All in all, these findings were not replicated in this study, probably due to gender and age differences.

In this study on men, we found no relationship between the *FTO* rs9939609 polymorphism and the concentration of hormones DHEAS, E_2, TST, FTS, and SHBG. In the available literature, we found no reports on the relationships between the *FTO* rs9939609 and *MC4R* rs17782313 polymorphisms and the levels of sex hormones in men. In this study, we also found no such relations.

Hainerová et al,[57] based on the results of studies in young obese men with hypogonadism and G181D mutation in the *MC4R* gene, reported that obesity caused by the mutation in the *MC4R* rs17782313 polymorphism indirectly contributes to hormonal changes, as the adipose tissue is an active endocrine organ. In this study, we found no association between the *PPARγ* rs1801282 polymorphism and TST, FTS, E_2, DHEAS, and SHBG concentrations, but the study included men aged >50 years, in whom metabolic disorders are mainly the result of lifestyle. Studies of Park et al[58] in men have shown that the *PPARγ* polymorphisms play a significant role in tumorigenesis as a tumor suppressor and PPARγ agonists may have a beneficial effect in the treatment of prostate cancer. It is, therefore, necessary to conduct further studies on the possible associations between the *PPARγ* rs1801282 polymorphism and hypogonadism, prostatic hyperplasia, and prostate cancer.

Some studies[59,60] have demonstrated the importance of dietary factors and physical activity level for the development of MetS. Lifestyle modification is effective in resolving MetS and reducing the severity of related abnormalities (FPG, WC, SBP, DBP, and TG) in patients with MetS.[59] Although it does not necessarily influence any given risk factor as much as dedicated drugs, its benefit lies in a moderate reduction in all the metabolic risk factors.

Genetic factors in MetS are still under investigation. Our results and other researchers' findings show that metabolic disorders in middle-aged and elderly people, including MetS and its components, are mainly the effect of lifestyle, including many years of improper eating habits and low physical activity, while genetic predisposition plays a much less significant role. Also in case of a singular SNP within the gene, it may be difficult to fully explain the risk for

metabolic disorders and related traits.[61] This phenomenon may be linked to potential gene–gene interactions. In case of singular SNP, other modest-risk variants may be the origin of the risk or control the expression of the particular risk factor.[62]

On the other hand, if an SNP may be an independent risk factor for a given clinical event, its effect can be observed with the passage of time. In complex diseases such as metabolic disorders, the effect of biallelic polymorphisms is most visible in the elderly, as the majority of independent environmental factors (eg, eating habits) remain at relatively stable levels. That is why this period of life is the most appropriate to observe the direct influence of genetic variation on the value of a given clinical parameter. Patients aged >50 years, ie, those we studied in this research, are therefore the optimal group in this context. However, it must be remembered that this study had significant limitations, as we included patients who reported voluntarily and we did not exclude patients with extreme obesity.

Due to the multitude of statistically insignificant results, we performed a post hoc power analysis using the G* Power software.[63] The power of tests, which showed no statistical significance, was below the recommended level of 0.8.[64] On the other hand, tests showing statistical significance had an acceptable power, reaching as high as 0.84. In our opinion, the small power of tests may be responsible for the great number of statistically insignificant results of the analysis. In order to increase the power of the tests, the sizes of the study groups would have to increase significantly. Besides, in a genetic association study, a reliable assessment of the prevalence of alleles increases with sample size (which means a greater number of alleles at a given locus). One should also take into account additional factors, such as the adopted model of inheritance, and especially the frequency of risk alleles at a given locus and the risk for the disease in a given population. In conclusion, a limited number of statistically significant results in this study may have been caused by the insufficient number of patients in the study, and thus the low power of statistical tests.

Conclusion
The *FTO* rs9939609 and *MC4R* rs17782313 gene polymorphisms have little significant relationships with metabolic health problems (T2DM, HT, overweight and obesity, and lipid disorders) and do not result in androgen disorders in aging men. However, our results extend the knowledge on genotype susceptibility for metabolic disturbances in relation to a specific geographical area of residence.

Abbreviations
MetS, metabolic syndrome; SNP, single-nucleotide polymorphism; FTO, fat mass and obesity-associated gene; MC4R, melanocortin 4 receptor gene; PPARγ, peroxisome proliferator-activated receptor gene; GWAS, Genome-Wide Association Study; T2DM, type 2 diabetes mellitus; HT, hypertension; TS, testosterone; TST, total testosterone; FTS, free testosterone; E_2, estradiol; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TCh, total cholesterol; ABP, arterial blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; WC, waist circumference; BMI, body mass index; EMAS, European Male Aging Study; IDF, International Diabetes Federation.

Author contributions
IR designed the study and collected the data. IR, KS-Z, GA, AR, and ML conducted the data analysis. KS-Z conducted the statistical analysis. IR, KS-Z, and DK-B drafted the article. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work. All authors have read and approved the final version submitted for publication.

Disclosure
The authors report no conflicts of interest in this work.

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