

Genetic variability in the cholecystokinin A receptor affects lipid profile and glucose tolerance in patients with polycystic ovary syndrome

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Abstract

Introduction: Cholecystokinin (CCK) is involved in several metabolic pathways, and CCK agonists are considered as a potential novel treatment option in populations with increased metabolic risk, including polycystic ovary syndrome (PCOS). As genetic variability of cholecystokinin A and B receptor genes (*CCKAR* and *CCKBR*, respectively) may modify their biological actions, we investigated the impact of *CCKAR* and *CCKBR* genetic variability on anthropometric and metabolic parameters in patients with PCOS.

Material and methods: Our cross-sectional study included 168 patients with PCOS and 82 healthy female controls genotyped for polymorphisms in *CCKAR* (rs6448456 and rs1800857) and *CCKBR* (rs2929180, rs1800843, rs1042047 and rs1042048) genes.

Results: The investigated polymorphisms were not associated with anthropometric characteristics of patients with PCOS. However, among healthy controls, carriers of at least one polymorphic *CCKBR* rs1800843 allele had a larger waist circumference ($p = 0.027$) and more visceral fat ($p = 0.046$). Among PCOS patients, carriers of at least one polymorphic *CCKAR* rs6448456 C allele had significantly higher total blood cholesterol and LDL, and significantly lower blood glucose levels after 30, 60 and 90 min of the oral glucose tolerance test (all $p < 0.05$). Healthy controls with at least one polymorphic *CCKAR* rs1800857 C allele were less likely to have a high metabolic syndrome burden ($p = 0.029$).

Conclusions: Genetic variability in *CCKAR* affects lipid profile and post-load glucose levels in patients with PCOS and is associated with metabolic syndrome burden in healthy young women. Further investigation of the role of genetic variability in *CCKAR* and *CCKBR* could contribute to development of individually tailored treatment strategies with CCK receptor agonists.

Key words: cholecystokinin, *CCKAR*, *CCKBR*, PCOS, polymorphism.

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine, reproductive and metabolic disorders in women of childbearing age [1]. The prevalence of PCOS in Europe varies widely, which could be attributable to different environmental and genetic factors [2]. Lifestyle modification in combination with metformin is considered as the main treatment strategy for the metabolic phenotype of the syndrome. However, treatment goals including weight loss and normalization of glycaemic and lipid profile often remain unmet with the established approach, and the need for novel treatment options is growing [1, 3].

Cholecystokinin (CCK) is a digestive hormone, neuromodulator and neurotransmitter. Its biological actions are mediated by its binding and activation of cholecystokinin A receptor (CCKAR) and cholecystokinin B receptor (CCKBR) [4]. Binding of CCK via CCKAR increases postprandial satiety, increases secretion of digestive enzymes and endocrine hormones in the pancreas, acts as a growth factor on pancreatic β cells, increases postprandial gallbladder emptying and delays gastric emptying. Binding of CCK or gastrin via CCKBR regulates pancreatic growth [4]. Furthermore, studies in mice with a homozygous *Cckbr* deletion suggest that CCKBR could also increase postprandial satiety and improve glucose tolerance [5]. Therefore, CCK receptor agonists have a potential for the treatment of obesity, type 2 diabetes and also PCOS with high metabolic risk [6].

Many common functional polymorphisms are present in *CCKAR* and *CCKBR* genes that might affect signalling of endogenous CCK. Only a few studies addressing their genetic variability have been conducted to date [7, 8]. A study of middle-aged and elderly adults showed that the homozygous carriers of two *CCKAR* promoter polymorphisms had increased fat content, higher insulin and higher leptin levels than wild-type and heterozygous individuals [7]. Another study reported an association between *CCKBR* polymorphisms and antipsychotic-induced weight gain in patients with schizophrenia [8].

According to our knowledge, the role of the *CCKAR* and *CCKBR* polymorphisms in patients with PCOS has not been addressed yet. We aimed to investigate genetic variability of *CCKAR* and *CCKBR* and their relationship with anthropometric and metabolic parameters in patients with PCOS.

Material and methods

Our cross-sectional study included 168 patients with PCOS treated at the outpatient clinics of Department of Endocrinology, Diabetes and Metabolic Diseases at the University Medical Centre (UMC),

Ljubljana. Women were eligible for enrolment if they were more than 18 years old, premenopausal and diagnosed with phenotype A PCOS according to Rotterdam criteria. Pregnant women and women who had diabetes were excluded. The study was approved by the Republic of Slovenia National Medical Ethics Committee and was carried out according to the Helsinki Declaration.

Anthropometric characteristics included were height, weight, waist circumference, body mass index (BMI) and visceral adipose tissue (VAT) area. Waist circumference was measured in a standing position midway between the lower costal margin and the iliac crest. BMI was calculated as the weight in kilograms divided by square of height in meters. Whole-body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Discovery A; Hologic, Waltham, MA, USA) with the software provided by the manufacturer (QDR for Windows Version 12.5). Metabolic characteristics were obtained by drawing fasting blood and determining fasting blood glucose and insulin. Afterwards patients underwent a standard 75 g oral glucose tolerance test (OGTT). Glucose levels were determined using a standard glucose oxidase method (Beckman Coulter Glucose Analyzer, Beckman Coulter Inc., Brea, CA). Insulin levels were determined by immunoradiometric assay (Biosource Europe S.A., Nivelles, Belgium). Homeostasis model assessment ($HOMA_{IR}$) score calculation was applied as a measure for insulin resistance (IR) [9]. Values greater than 2.0 were considered as indicative of the presence of IR [10]. The World Health Organization diagnostic criteria of 2006 were used to determine basal baseline glycemia, impaired glucose tolerance and type 2 diabetes [11].

In addition, the healthy control group included 82 first year female students of the Nursing programme at the University of Ljubljana, Faculty of Health Sciences. The data on anthropometric characteristics included weight, BMI, waist circumference, visceral fat, fat mass and fat free mass, and the metabolic syndrome burden was defined as described by Šoštarič *et al.* [12].

With regards to molecular genetic analysis, genomic DNA was extracted from venous blood using a FlexiGene DNA kit (Qiagen, Hilden, Germany). Polymorphisms in *CCKAR* and *CCKBR* were selected using the LD TAG SNP Selection tool [13]. We considered only polymorphisms with a minor allele frequency (MAF) equal to or greater than 0.05 in the European population and a putative functional effect predicted using the SNP function prediction [13]. Linkage disequilibrium was checked using the LDlink program for final selection of tag polymorphisms [14]. In total, two *CCKAR* tag polymorphisms (rs6448456, rs1800857)

and four *CCKBR* tag polymorphisms (rs2929180, rs1800843, rs1042047, rs1042048) were genotyped using competitive allele specific KASPar assays according to the manufacturer's instructions (LGC, England). The selected polymorphisms, their predicted effect and genotype frequencies in our sample are shown in Supplementary Table S1. The genotype frequencies of the *CCKBR* rs2929180 and the *CCKBR* rs1042047 polymorphisms were not in Hardy-Weinberg equilibrium (HWE) in PCOS patients; thus, *CCKBR* rs1042047 was excluded from further statistical analysis.

Statistical analysis

In the statistical analysis, the median and interquartile range were used to describe the central tendency and variability of continuous variables. Frequencies were used to describe the distribution of categorical variables. Deviation from HWE was evaluated using a standard χ^2 test. If the genotype frequencies for a polymorphism were not in HWE, we used Fisher's exact test to compare the distribution of genotype frequencies of our sample with the expected distribution in the European population. In the subsequent analyses, a dominant genetic model was used. A nonparametric Mann-Whitney test was used to assess the associations of polymorphisms with continuous variables. Spearman's rho (ρ) was used to evaluate correlations between continuous variables. A p -value of < 0.05 was considered statistically significant. All statistical analyses were performed with IBM SPSS Statistics, version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

Anthropometric and metabolic characteristics of PCOS patients ($N = 168$) are presented in Table I. The median total cholesterol and TAG were within the reference interval, while the median HDL was lower, and LDL was higher than the reference value. Insulin resistance was present in 71 out of 98 (72.4%) patients as assessed by HOMA_{IR}. Median fasting glucose and glucose concentration after 120 min and fasting insulin and insulin concentration after 120 min were within the reference interval. Nevertheless, 17 out of 142 patients (12.0%) had impaired fasting glucose and 37 out of 140 patients (26.4%) had impaired glucose tolerance. Several biochemical parameters were correlated with both increased BMI and increased waist circumference: TAG ($\rho = 0.211$, $p = 0.015$ and $\rho = 0.285$, $p = 0.002$, respectively), HOMA_{IR} ($\rho = 0.357$, $p < 0.001$ and $\rho = 0.261$, $p = 0.011$, respectively), fasting glucose ($\rho = 0.232$, $p = 0.006$ and $\rho = 0.187$, $p = 0.034$, respectively), glucose concentration after 120 min ($\rho = 0.291$, $p = 0.001$

Table I. Characteristics of patients with polycystic ovary syndrome ($N = 168$)

Characteristic	Median (25–75%)
Age [years]	30 (25–35.8)
Body weight [kg]	100 (85–111.3) [5]
BMI [kg/m ²]	35.8 (31.8–39.9) [6]
Waist circumference [cm]	112 (102–121.3) [31]
VAT area [cm ²]	156.5 (116.8–199.5) [74]
Total cholesterol [mmol/l]	4.7 (4.2–5.3) [33]
HDL [mmol/l]	1.2 (1–1.4) [33]
LDL [mmol/l]	2.9 (2.4–3.4) [34]
TAG [mmol/l]	1.4 (1–1.8) [33]
HOMA _{IR} [I]	2.8 (1.8–5) [70]
Glucose 0 min OGTT [mmol/l]	5.2 (4.8–5.6) [26]
Glucose 30 min OGTT [mmol/l]	8.3 (6.9–9.4) [79]
Glucose 60 min OGTT [mmol/l]	8.3 (6.8–9.8) [80]
Glucose 90 min OGTT [mmol/l]	7.6 (5.9–9) [82]
Glucose 120 min OGTT [mmol/l]	6.6 (5.5–7.9) [28]
Insulin 0 min OGTT [mU/l]	12.4 (7.5–20) [70]
Insulin 30 min OGTT [mU/l]	71.3 (45.1–104.8) [84]
Insulin 60 min OGTT [mU/l]	97.5 (63.6–132.3) [84]
Insulin 90 min OGTT [mU/l]	87.9 (61.9–121.5) [84]
Insulin 120 min OGTT [mU/l]	78.5 (52.8–124.3) [76]

BMI – body mass index, VAT – visceral adipose tissue, HDL – high-density lipoproteins, LDL – low-density lipoproteins, TAG – triacylglycerols, HOMA_{IR} – homeostatic model assessment for insulin resistance, OGTT – oral glucose tolerance test, [I] – missing data.

Table II. Characteristics of healthy controls ($N = 82$)

Characteristic	Median (25–75%)
Age [years]	20 (20–22)
Body weight [kg]	62.2 (55.5–72.2)
BMI [kg/m ²]	22.4 (20.2–25.0)
Waist circumference [cm]	73 (66.8–78)
Visceral fat (arbitrary units)	3 (3–4)
Fat mass (%)	32.3 (26.3–37.1)
Fat free mass (%)	28.5 (27–30.6)

BMI – body mass index.

and $\rho = 0.295$, $p = 0.001$, respectively) and fasting insulin ($\rho = 0.326$, $p = 0.001$ and $\rho = 0.219$, $p = 0.033$, respectively).

Anthropometric and metabolic characteristics of healthy controls ($N = 82$) are presented in Table II. Controls were significantly younger compared to PCOS patients ($p < 0.001$), and had lower body weight, BMI and waist circumference (all $p < 0.001$). Among healthy controls, only 2 (2.4%) had dyslipidaemia and 29 (35.4%) had a higher burden of metabolic syndrome.

Genotype distribution of the investigated polymorphisms is presented in Supplementary Table S1.

None of the investigated polymorphisms was associated with PCOS susceptibility in univariable analysis or after adjustment for age (Supplementary Table SII).

None of the investigated polymorphisms in either *CCKAR* or *CCKBR* was associated with anthropometric characteristics of patients with PCOS, as shown in Supplementary Tables SIII and SIV, respectively. *CCKAR* polymorphisms were also not associated with anthropometric characteristics of healthy controls (Supplementary Table SV). On the other hand, carriers of at least one polymorphic *CCKBR* rs1800843 allele among healthy controls had a larger waist circumference ($p = 0.027$) and tended to have more visceral fat ($p = 0.046$).

Weight and BMI were also higher in these subjects, but the difference did not reach statistical significance (Supplementary Table SVI).

The impact of the two *CCKAR* polymorphisms on metabolic characteristics of patients with PCOS is shown in Table III. Carriers of at least one polymorphic *CCKAR* rs6448456 C allele had significantly higher levels of total cholesterol ($p = 0.034$) and LDL ($p = 0.036$) compared to the wild-type genotype. Although there was no significant difference in fasting glucose concentration ($p = 0.253$), carriers of at least one polymorphic *CCKAR* rs6448456 C allele had significantly lower blood glucose after 30 ($p = 0.022$), 60 ($p = 0.001$), and 90 ($p = 0.010$) min of OGTT (Figure 1). After 120

Table III. *CCKAR* polymorphisms and metabolic characteristics of patients with polycystic ovary syndrome ($N = 168$)

Characteristic	Genotype*	<i>CCKAR</i> rs6448456		<i>CCKAR</i> rs1800857	
		Median (25–75%)	<i>P</i> -value	Median (25–75%)	<i>P</i> -value
Total cholesterol [mmol/l] [33]	X/X	4.6 (4.2–5.1)	0.034	4.7 (4.2–5.3)	0.880
	X/x+x/x	5.1 (4.4–5.6)		4.8 (4.2–5.3)	
HDL [mmol/l] [33]	X/X	1.2 (1–1.4)	0.749	1.2 (1.1–1.4)	0.053
	X/x+x/x	1.2 (1–1.4)		1.2 (1–1.3)	
LDL [mmol/l] [34]	X/X	2.8 (2.3–3.4)	0.036	2.9 (2.4–3.4)	0.944
	X/x+x/x	3.2 (2.5–3.6)		2.9 (2.4–3.4)	
TAG [mmol/l] [33]	X/X	1.4 (1.1–2)	0.373	1.3 (1–1.9)	0.282
	X/x+x/x	1.4 (1–1.7)		1.6 (1.2–1.8)	
HOMA _{IR} [I] [70]	X/X	2.7 (1.7–4.6)	0.180	2.7 (1.7–5.1)	0.503
	X/x+x/x	3.1 (2.5–5.6)		3.2 (2–4.9)	
Glucose 0 min OGTT [mmol/l] [26]	X/X	5.2 (4.8–5.7)	0.253	5.1 (4.8–5.5)	0.219
	X/x+x/x	5.1 (4.9–5.4)		5.2 (4.9–5.7)	
Glucose 30 min OGTT [mmol/l] [79]	X/X	8.7 (7.3–9.5)	0.022	8 (6.8–9.3)	0.236
	X/x+x/x	7.7 (6.5–8.5)		8.7 (7.1–10.3)	
Glucose 60 min OGTT [mmol/l] [80]	X/X	9.3 (7.4–10.7)	0.001	7.8 (6.6–9.6)	0.059
	X/x+x/x	7.1 (5.9–8.7)		9 (7.3–11.2)	
Glucose 90 min OGTT [mmol/l] [82]	X/X	7.9 (6.3–9.5)	0.010	7.4 (5.9–8.8)	0.204
	X/x+x/x	6.7 (5.6–8.1)		7.9 (6.4–9.6)	
Glucose 120 min OGTT [mmol/l] [28]	X/X	6.9 (5.6–8.2)	0.092	6.6 (5.4–7.8)	0.488
	X/x+x/x	6.3 (5.4–7.4)		6.5 (5.8–8)	
Insulin 0 min OGTT [mU/l] [70]	X/X	11.5 (7.4–19.7)	0.141	12.4 (7.1–20.2)	0.839
	X/x+x/x	15.1 (11–21.8)		13 (8.8–19.5)	
Insulin 30 min OGTT [mU/l] [84]	X/X	70.3 (43.2–106.3)	0.468	77.4 (50.6–110.8)	0.324
	X/x+x/x	78.5 (52.3–102.7)		65.7 (40.9–102.9)	
Insulin 60 min OGTT [mU/l] [84]	X/X	98 (63.5–136.3)	0.681	86.4 (63.2–120.5)	0.302
	X/x+x/x	89.5 (63.9–127.3)		100.7 (67.3–146)	
Insulin 90 min OGTT [mU/l] [84]	X/X	82.3 (60.5–145)	0.873	88.2 (58.3–125)	0.730
	X/x+x/x	93.6 (67.7–118)		87.9 (69.6–119.5)	
Insulin 120 min OGTT [mU/l] [76]	X/X	79.3 (53.3–127)	0.730	78 (51.8–125)	0.981
	X/x+x/x	74.9 (52.3–118.3)		79.5 (56.1–121.5)	

*X – major (common) allele, x – minor (variant) allele. HDL – high-density lipoproteins, LDL – low-density lipoproteins, TAG – triacylglycerols, HOMA_{IR} – homeostatic model assessment for insulin resistance, OGTT – oral glucose tolerance test, [I] – number of missing data. Statistically significant *p*-values are shown in bold.

min of OGTT, carriers of at least one polymorphic *CCKAR* rs6448456 C allele still had a lower median blood glucose, but the difference was no longer statistically significant ($p = 0.092$). No other significant associations between *CCKAR* polymorphisms and metabolic characteristics of patients with PCOS were observed.

The impact of *CCKBR* polymorphisms on metabolic characteristics of patients with PCOS is shown in Table IV. Carriers of at least one polymorphic *CCKBR* rs2929180 T allele had a higher insulin concentration after 90 ($p = 0.004$) minutes of OGTT. However, *CCKBR* rs2929180 T genotype was not associated with insulin concentration after 60 ($p = 0.152$) or 120 ($p = 0.634$) min of OGTT. No other significant associations between *CCKBR* polymorphisms and metabolic characteristics of patients with PCOS were detected.

Among healthy controls, only the association with metabolic syndrome burden could be assessed (Table V). Carriers of at least one polymorphic *CCKAR* rs1800857 C allele were less likely to have a high metabolic syndrome burden ($p = 0.029$). No other significant associations between *CCKAR* or *CCKBR* polymorphisms and metabolic characteristics of healthy controls were observed.

Discussion

We report for the first time on the impact of genetic variability in *CCKAR* and *CCKBR* on metabolic parameters in patients with PCOS. The main finding of our study was that the carriers of at least one polymorphic *CCKAR* rs6448456 C allele had a significantly higher total cholesterol and LDL and significantly lower blood glucose levels after 30, 60, and 90 min of OGTT.

First, we assessed whether the investigated *CCKAR* and *CCKBR* polymorphisms influence lipid profile in our group of patients with PCOS. A meta-analysis in 2011 showed that patients with PCOS have low HDL, increased triglycerides, increased LDL and increased non-HDL. Furthermore, these lipid parameters were found to be worse in women with PCOS than in healthy women regardless of BMI or ethnicity [15]. The guidelines from 2018 recommend that overweight and obese patients with PCOS, regardless of age, should have a fasting lipid profile at diagnosis and, thereafter, repeated measurements based on the presence of hyperlipidaemia and global cardiovascular disease risk [16]. In our study, carriers of at least one polymorphic *CCKAR* rs6448456 C allele had significantly higher total blood cholesterol and LDL than non-carriers. We have not found functional studies to explain our observations directly. The observed effect might be caused by an increased contraction of the gallbladder [4]. Our results could be in line with preclinical models,

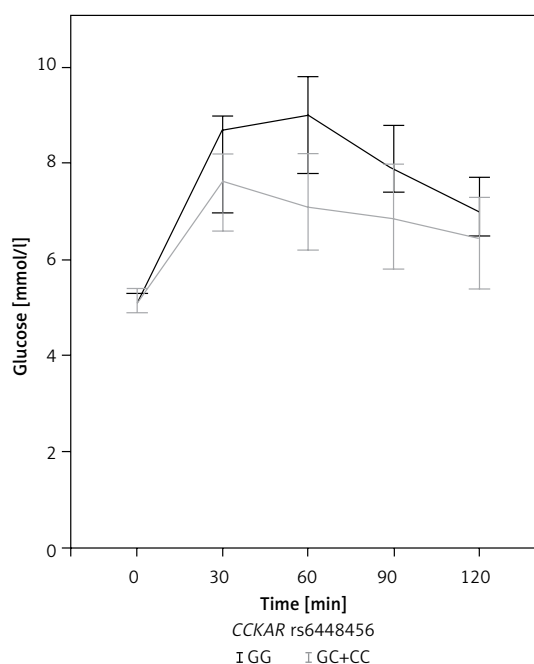


Figure 1. Graph of blood glucose as a function of time (median with 95% confidence interval) in 83 PCOS patients with complete oral glucose tolerance test data. The grey curve indicates carriers of at least one polymorphic *CCKAR* rs6448456 allele. The black curve indicates carriers of two normal alleles

where intravenous administration of CCK in mice resulted in an increase in blood cholesterol and TAG due to increased bile secretion mediated by CCK via CCK receptors and subsequent reabsorption of the biliary lipids [17]. On the other hand, studies examining the effect of *Cckar* gene deletion on cholesterol metabolism in animal models report conflicting data [17–19]. Another possible explanation for higher total blood cholesterol and LDL in carriers of at least one polymorphic *CCKAR* rs6448456 C allele may be the effect of CCK on secretion of pancreatic lipase [4]. It was observed that inhibition of lipase led to decreased digestion of lipids, resulting in lower total cholesterol and LDL levels [20]. Although we cannot unequivocally explain the mechanisms leading to higher total blood cholesterol and LDL levels in carriers of at least one polymorphic *CCKAR* rs6448456 C allele, our findings may be of clinical importance. Since CCK levels increase with aging [21], the risk for hypercholesterolemia in these patients is expected to increase over time and appropriate medical surveillance may be needed to prevent the related late complications.

Next, we assessed whether the investigated *CCKAR* and *CCKBR* polymorphisms influence glucose tolerance in our group of patients with PCOS. We observed that carriers of at least one polymorphic *CCKAR* rs6448456 C allele had significantly

Table IV. CCKBR polymorphisms and metabolic characteristics of patients with polycystic ovary syndrome (N = 168)

Characteristic	Genotype*	CCKBR rs2929180			CCKBR rs1800843			CCKBR rs1042048		
		Median (25–75%)	P-value	Median (25–75%)	P-value	Median (25–75%)	P-value	Median (25–75%)	P-value	
Total cholesterol [mmol/l] [33]	X/X	4.8 (4.3–5.4)	0.226	4.7 (4.2–5.5)	0.805	4.7 (4.2–5.4)	0.679	4.7 (4.2–5.4)	0.679	
	X/x+x/x	4.6 (4.1–5.3)		4.7 (4.2–5.2)		4.7 (4.2–5.3)		4.7 (4.2–5.3)		
HDL [mmol/l] [33]	X/X	1.2 (1–1.4)	0.358	1.2 (1.1–1.4)	0.998	1.2 (1–1.3)	0.332	1.2 (1–1.3)	0.332	
	X/x+x/x	1.2 (1–1.3)		1.2 (1–1.4)		1.2 (1–1.4)		1.2 (1–1.4)		
LDL [mmol/l] [34]	X/X	2.9 (2.4–3.4)	0.471	2.9 (2.4–3.5)	0.962	2.9 (2.4–3.5)	0.484	2.9 (2.4–3.5)	0.484	
	X/x+x/x	2.8 (2.3–3.6)		2.8 (2.4–3.4)		2.8 (2.3–3.4)		2.8 (2.3–3.4)		
TAG [mmol/l] [33]	X/X	1.5 (1.1–2)	0.083	1.5 (1.1–1.8)	0.331	1.4 (1.2–1.8)	0.979	1.4 (1.2–1.8)	0.979	
	X/x+x/x	1.2 (0.9–1.7)		1.4 (0.9–1.9)		1.5 (1–2)		1.5 (1–2)		
HOMA _{ir} [1] [70]	X/X	3.1 (1.9–5.1)	0.171	2.8 (1.8–4.6)	0.838	3.2 (2.3–5.1)	0.290	3.2 (2.3–5.1)	0.290	
	X/x+x/x	2.4 (1.5–4.6)		2.9 (1.7–5.6)		2.7 (1.7–4.9)		2.7 (1.7–4.9)		
Glucose 0 min OGTT [mmol/l] [26]	X/X	5.1 (4.8–5.6)	0.980	5.2 (4.8–5.5)	0.784	5.2 (4.8–5.6)	0.917	5.2 (4.8–5.6)	0.917	
	X/x+x/x	5.2 (4.9–5.6)		5.2 (4.9–5.6)		5.2 (4.9–5.6)		5.2 (4.9–5.6)		
Glucose 30 min OGTT [mmol/l] [79]	X/X	8.1 (6.9–9.4)	0.542	8.2 (6.8–9.4)	0.513	8.3 (6.8–10)	0.990	8.3 (6.8–10)	0.990	
	X/x+x/x	8.7 (7–9.4)		8.4 (7.3–9.4)		8.1 (7.1–9.3)		8.1 (7.1–9.3)		
Glucose 60 min OGTT [mmol/l] [80]	X/X	8.1 (6.6–9.8)	0.199	8.3 (6.6–9.8)	0.909	8.6 (6.2–11.1)	0.571	8.6 (6.2–11.1)	0.571	
	X/x+x/x	9.2 (7.3–10.7)		8.7 (7.1–9.8)		7.8 (7–9.5)		7.8 (7–9.5)		
Glucose 90 min OGTT [mmol/l] [82]	X/X	7.1 (5.8–8.8)	0.106	7.4 (5.8–9.1)	0.704	7.7 (5.7–9.6)	0.826	7.7 (5.7–9.6)	0.826	
	X/x+x/x	8.1 (6.8–9.5)		7.9 (6.4–8.9)		7.6 (6.5–8.7)		7.6 (6.5–8.7)		
Glucose 120 min OGTT [mmol/l] [28]	X/X	6.6 (5.6–7.9)	0.661	6.6 (5.4–7.8)	0.695	6.3 (5.4–7.9)	0.594	6.3 (5.4–7.9)	0.594	
	X/x+x/x	6.4 (5.3–7.8)		6.6 (5.6–7.9)		6.8 (5.6–7.8)		6.8 (5.6–7.8)		
Insulin 0 min OGTT [mU/l] [70]	X/X	13 (8.3–20.2)	0.185	12.4 (7.6–19.1)	0.655	13.3 (9.6–21.1)	0.321	13.3 (9.6–21.1)	0.321	
	X/x+x/x	10.4 (6.3–19.8)		13.5 (7.3–23)		11.8 (6.8–19.7)		11.8 (6.8–19.7)		
Insulin 30 min OGTT [mU/l] [84]	X/X	71.2 (45.9–99.4)	0.831	69.3 (42.7–98.3)	0.067	72.2 (44.8–106.3)	0.834	72.2 (44.8–106.3)	0.834	
	X/x+x/x	80.5 (38.7–115.5)		87.7 (53–124.5)		71.4 (44.4–105.5)		71.4 (44.4–105.5)		
Insulin 60 min OGTT [mU/l] [84]	X/X	87.7 (62–115.5)	0.152	90.7 (68.4–129)	0.670	99.2 (66.8–133.8)	0.397	99.2 (66.8–133.8)	0.397	
	X/x+x/x	117.5 (64.7–145.5)		97.9 (58.1–145)		88.2 (52.3–131)		88.2 (52.3–131)		
Insulin 90 min OGTT [mU/l] [84]	X/X	80.1 (58.3–113.5)	0.004	82.5 (60.6–117.5)	0.145	92.3 (70.6–123)	0.461	92.3 (70.6–123)	0.461	
	X/x+x/x	118 (83.4–168.8)		104 (66.4–183.5)		81.8 (57.8–124)		81.8 (57.8–124)		
Insulin 120 min OGTT [mU/l] [76]	X/X	79.5 (51.4–119.5)	0.634	74.9 (51.7–107)	0.121	86.7 (65.3–128)	0.096	86.7 (65.3–128)	0.096	
	X/x+x/x	77.8 (57.8–142)		88.7 (61.5–148)		66.9 (46–118)		66.9 (46–118)		

*X – major (common) allele, x – minor (variant) allele. HDL – high-density lipoproteins, LDL – low-density lipoproteins, TAG – triacylglycerols, HOMA_{ir} – homeostatic model assessment for insulin resistance, OGTT – oral glucose tolerance test, [] – number of missing data. Statistically significant p-values are shown in bold.

Table V. Association of selected *CCKAR* and *CCKBR* polymorphisms with metabolic syndrome burden in healthy controls ($N = 82$)

SNP	Genotype	Low burden N (%)	High burden N (%)	OR (95% CI)	P-value
<i>CCKAR</i> rs6448456	G/G	38 (71.7)	16 (55.2)	Reference	
	G/C+C/C	15 (28.3)	13 (44.8)	2.06 (0.80–5.30)	0.134
<i>CCKAR</i> rs1800857	T/T	33 (62.3)	25 (86.2)	Reference	
	T/C+C/C	20 (37.7)	4 (13.8)	0.26 (0.08–0.87)	0.029
<i>CCKBR</i> rs2929180	G/G	37 (69.8)	18 (62.1)	Reference	
	G/T+T/T	16 (30.2)	11 (37.9)	1.41 (0.55–3.66)	0.477
<i>CCKBR</i> rs1800843	C/C	36 (67.9)	19 (65.5)	Reference	
	C/A+A/A	17 (32.1)	10 (34.5)	1.12 (0.43–2.91)	0.825
<i>CCKBR</i> rs1042048	A/A	24 (45.3)	9 (31.0)	Reference	
	A/G+G/G	29 (54.7)	20 (69.0)	1.84 (0.71–4.78)	0.211

OR – odds ratio, CI – confidence interval. Statistically significant p-values are shown in bold.

lower blood glucose after 30, 60, and 90 min of OGTT. Since blood glucose is regulated by two processes, there are two possible explanations. The first explanation may be that delayed gastric emptying causes slower passage of glucose into the duodenum, leading to slower absorption and lower postprandial glucose. Our observation is in agreement with data reported in a study of eight healthy men who underwent OGTT, showing delayed gastric emptying, a lower glucose peak and a lower insulin peak when they were administered CCK as compared to when administered saline. It is less likely that the observed effect might be due to faster glucose uptake into tissues in carriers of at least one polymorphic *CCKAR* rs6448456 C allele [22]. Namely, CCK secretion is mainly stimulated by proteins and fats, whereas glucose causes a significant but smaller rise of plasma CCK [23]. Moreover, a study on five healthy men showed that physiological concentrations of CCK potentiate amino acid-induced insulin secretion but not glucose-induced insulin secretion [24].

All investigated *CCKAR* and *CCKBR* polymorphisms are also common in the general population (MAF between 9.5% and 38.2% according to the dbSNP Allele Frequency Aggregator (ALFA) project). In our study, none of the investigated polymorphisms was associated with PCOS susceptibility. However, in young healthy women, *CCKBR* rs1800843 was associated with anthropometric characteristics and *CCKAR* rs1800857 with metabolic syndrome burden. Therefore, further studies are needed to assess the association of these polymorphisms with metabolic parameters also in other, non-PCOS patients.

One of the limitations of our study was that the genotype distributions were not in HWE for two out of six polymorphisms, namely *CCKBR* rs2929180 and *CCKBR* rs1042047. We decided to include the *CCKBR* rs2929180 polymorphism in

further analysis because the distribution of genotype frequencies was not significantly different from the distribution reported for the European population, and there were no other polymorphisms in the respective gene region that could affect PCR amplification. However, we did not include the *CCKBR* rs1042047 polymorphism. Although the distribution of *CCKBR* rs1042047 genotype frequencies was not significantly different from the distribution in the dbSNP database, we found a common rs8192471 polymorphism only three nucleotides away from rs1042047 at the 5' end that could affect annealing and genotyping results. A similar conclusion was reported by other researchers [25].

Another limitation was that all the clinical data were not available for all patients, although they were managed in the same department at the UMC Ljubljana. Additionally, for healthy controls, not all anthropometric or metabolic parameters were measured. The association with dyslipidaemia could not be evaluated among healthy controls, as only two subjects had altered lipid levels. Therefore, only the association with metabolic syndrome burden was evaluated. As all the patients and controls were young adults, the risk that our data could be affected by treatments of other conditions was minimized. Furthermore, our study was not biased by genetic heterogeneity, as all subjects included in the study belonged to the Slovenian population, which is ethnically and genetically homogeneous [26].

In conclusion, our data indicate interesting associations between the *CCKAR* rs6448456 polymorphism and the metabolic characteristics of patients with PCOS. Better understanding of the role of genetic variability in *CCKAR* and *CCKBR* may be of clinical importance for future development of treatment strategies with CCK receptor agonists, which have already been shown to en-

hance the weight-lowering, appetite-suppressing, and positive β -cell actions of GLP-1-based drugs in preclinical models [27–29]. It needs to be pointed out that the investigated receptor polymorphisms could also play an important role in response to treatment with potential CCK receptor agonists as previously observed for other receptor polymorphisms, for example in liraglutide treatment in obese women with PCOS [30] as well as in other therapeutic fields [31].

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Ethical approval

Approval number: KME 0120-180/2019/11.

Conflict of interest

The authors declare no conflict of interest.

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