CLINICAL STUDY

Polymorphisms of *TCF7L2* gene in South Brazilian women with polycystic ovary syndrome: a cross-sectional study

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Abstract

Objective: To assess whether *TCF7L2* single nucleotide polymorphisms rs7903146 C/T and rs11196236 C/T are associated with polycystic ovary syndrome (PCOS) in South Brazilian women.

Design: Cross-sectional study.

Methods: Two hundred PCOS patients and 102 non-hirsute, ovulatory controls were genotyped by real-time PCR. Haplotypes were constructed from the combination of both polymorphisms. Frequencies were inferred using the PHASE 2.1.1 software.

Results and conclusions: The distribution of rs7903146 (PCOS, 54.4% CC; 28.5% CT; 17.1% TT; controls, 51.0% CC; 37.0% CT; 12.0% TT) and rs11196236 (PCOS, 4.3% CC; 33.5% CT; 62.2% TT; controls, 3.2% CC; 35.5% CT; 61.3% TT) was similar between the groups. rs7903146 and rs11196236 were not in linkage disequilibrium ($|D'|=0.34$; $r^2=0.07$). PCOS participants were younger, with higher age-adjusted BMI, waist circumference, blood pressure, triglycerides, insulin, homeostasis model assessment index to estimate insulin resistance and total testosterone, and lower HDL-C and sex hormone binding globulin vs controls. In PCOS, no differences between genotypes and haplotypes were found for clinical and metabolic variables. However, for each T (rs7903146) and T (rs11196236) allele added to the haplotypes, a variation of 5.87 cm in waist (P trend=0.01), 10.7 mg/dl in total cholesterol (P trend=0.03), and 10.3 mg/dl in LDL-C (P trend=0.01) was recorded. *TCF7L2* variants are probably not implicated in PCOS development in South Brazilian women.

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Introduction

Polycystic ovary syndrome (PCOS) is a complex endocrine disease characterized by hyperandrogenism and chronic anovulation. It is associated with metabolic disturbances such as central obesity, insulin resistance, dyslipidemia, and increased risk of diabetes and hypertension (1, 2, 3, 4, 5, 6).

Current evidence indicates that PCOS is a multifactorial polygenic disorder, whose pathogenesis and clinical presentation are influenced by both genetic susceptibility and environmental exposure (5, 7). To date, a number of genes have been reported to be associated with PCOS. Most of them are presumed to be relevant to the pathogenesis of PCOS, such as the genes encoding steroid biosynthesis enzymes and androgen and insulin receptors. However, a variant contributing substantially to the development of PCOS has not been identified (8, 9).

Recently, common variants of the transcription factor 7-like 2 (*TCF7L2*) gene on chromosome 10q25.2 have been found to contribute to the risk of

type 2 diabetes (T2DM) in various ethnic groups (10, 11, 12, 13). Association studies on the single nucleotide polymorphisms (SNPs) of the *TCF7L2* gene in PCOS women have produced controversial data, with some studies showing association between SNPs rs7903146 and rs11196236 with obesity-related traits (14) and peripheral insulin resistance (15) and others reporting no such association (16, 17).

Therefore, the aim of our study was to assess whether *TCF7L2* SNPs rs7903146 C/T and rs11196236 C/T or their haplotypes are associated with PCOS and to determine a possible impact of these polymorphisms on anthropometric and metabolic variables in PCOS women from Southern Brazil.

Subjects and methods

Patients

We studied 302 women, including 200 PCOS patients and 102 non-hirsute women with regular ovulatory

cycles (luteal phase progesterone >3.8 ng/ml). All participants were of reproductive age, consulting at a university hospital in Brazil or recruited by advertisement in local media between 2009 and 2012. PCOS was diagnosed according to Rotterdam criteria, in the presence of two of three signs: i) oligomenorrhea and/or chronic anovulation (≤ 9 cycles/year and/or luteal phase progesterone <3.8 ng/ml); ii) clinical and/or biochemical hyperandrogenism; and iii) polycystic ovaries. Diagnosis of PCOS also relied on exclusion of other hyperandrogenic disorders (18). None of the PCOS or control participants had received any drugs known to interfere with hormonal levels (such as OC pills, antiandrogens, metformin, fibrates, and statins) for at least 3 months before the study. Exclusion criteria were pregnancy and hepatic or renal diseases. Approval for this study was obtained from the institutional review board and the local ethics committee, functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London, and written informed consent was obtained from every subject.

Study protocol

Participants were evaluated according to a standard protocol, as previously reported (19, 20). Anthropometric measures included weight, height, and waist circumference (WC) (waist measured at the mid point between the lower rib margin and the iliac crest) (21). BMI was then calculated (current weight in kilograms divided by square of height in meters). Blood pressure was measured after a 10-min rest, in the sitting position, with feet on the floor and the arm supported at heart level. Two measurements were performed 10 min apart using automatic blood pressure monitor HEM-742INT OMRON (Rio de Janeiro, Brazil) with correct cuff size for the arm diameter. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or current use of antihypertensive drugs (22). Hormonal and metabolic assessments were made between days 2 and 10 of the menstrual cycle, or on any day if the patient was amenorrheic. All samples were obtained between 0800 and 1000 h. Blood samples were drawn after a 12-h overnight fast for determination of plasma cholesterol, HDL-C, triglycerides, total testosterone (TT), and sex hormone binding globulin (SHBG). Glucose was measured before and 2 h after the ingestion of a 75-g oral glucose load.

Assays

Total cholesterol, HDL-C, triglycerides, and glucose were determined by enzymatic colorimetric methods (Bayer 1650 Advia System). LDL-C was estimated indirectly with the Friedewald formula (23). The lipid

accumulation product (LAP) index was calculated using the formula (waist (cm) $- 58$) $\times$ triglyceride concentration (mmol/l) (24). Homeostasis model assessment index to estimate insulin resistance (HOMA-IR) was calculated by multiplying insulin (mIU/ml) by glucose (mmol/l) and dividing this product by 22.5 (25). Frequency of the metabolic syndrome was defined in accordance with the Joint Scientific Statement (26).

TT levels were measured by chemiluminescence (Siemens Advia Centaur XP, Mannheim, Germany), with a sensitivity of 0.10 ng/ml and intra-assay and interassay coefficient of variation (CV) of 3.3 and 7.5% respectively. SHBG was measured by chemiluminescence (Immulite 2000 Siemens), with a sensitivity of 0.02 nmol/l and intra-assay and interassay CV of 5.3 and 6.6% respectively. Plasma insulin levels were measured by electrochemiluminescence (Siemens Advia Centaur XP), with a sensitivity of 0.50 U/ml and intra-assay and interassay CV of 2.8 and 2.1% respectively.

Genotyping

In addition to serum samples, whole blood samples were collected from all participants. Genomic DNA was extracted from peripheral leukocytes using the technique described by Miller *et al.* (27). DNA samples were diluted to 2 ng/ml and genotyped for SNP 7903146 C $>$ T and rs11196236 C $>$ T of the *TCF7L2* gene by real-time PCR (7500 Fast; Applied Biosystems), with allelic discrimination assays (Taqman MGB Probes) following the manufacturer's instructions (Applied Biosystems). Reaction conditions for SNP rs7903146 were 10 min at 95 °C after 60 cycles of denaturation at 95 °C (15 s) and annealing at 62 °C (1 min). Reaction conditions for rs11196236 were 10 min at 95 °C after 50 denaturation cycles at 95 °C (15 s) and annealing at 62 °C (1 min). Endpoint fluorescent readings were performed by 7500 Fast System Sequence Detection Software version 1.4. In this sample, 10% were made in duplicate.

After frequency analyses, a dominant model was assumed and PCOS carriers of allele C were analyzed together in both polymorphisms. Therefore, Lewontin's D' ($|D'|$) r^2 was calculated for each pair of genetic markers for estimating linkage disequilibrium (28, 29). Haplotypes were constructed from the combination of the two *TCF7L2* polymorphisms (rs7903146 and rs11196236), and their frequencies were inferred using the PHASE 2.1.1 software (30). The first letter in each haplotype refers to the rs7903146 polymorphism and the second to the rs11196236 polymorphism.

Statistical analysis

Sample size estimation was based on a pilot study with PCOS participants, in which we found a non-significant

Table 1 Clinical, hormonal, and metabolic features of patients with PCOS. Values are expressed as mean $\pm$ s.d. or median (25–75 interquartile range). Total testosterone reference range (female): 0.2–0.8 ng/ml.

Variable	PCOS (n=200)	Controls (n=102)	P
Age	22.8 $\pm$ 6.6	25.2 $\pm$ 7.6	0.005
BMI (kg/m ²)	30.2 $\pm$ 7.1	27.0 $\pm$ 6.0	0.001
SBP (mmHg)	121.8 $\pm$ 16.2	109.6 $\pm$ 12.8	0.001
DBP (mmHg)	78.3 $\pm$ 11.6	70.8 $\pm$ 9.3	0.001
Waist circumference (cm)	90.3 $\pm$ 16.4	78.1 $\pm$ 11.5	0.001
Triglycerides (mg/dl)	91 (63–131)	66 (50–99)	0.001
Total cholesterol (mg/dl)	175 $\pm$ 38	170 $\pm$ 30	0.23
HDL-C (mg/dl)	48 $\pm$ 10	52 $\pm$ 12	0.003
LDL-C (mg/dl)	105 $\pm$ 32	101 $\pm$ 26	0.35
Fasting glucose (mg/dl)	89 $\pm$ 16	88 $\pm$ 7	0.46
Glucose at 120 min (mg/dl)	113 $\pm$ 40	95 $\pm$ 20	0.001
Fasting insulin (μ U/ml)	17.6 (9.9–28.1)	11.0 (7.3–15.4)	0.001
HOMA-IR	3.6 (1.9–6.5)	2.1 (1.3–3.1)	0.001
LAP	30.3 (14.2–60.5)	14.2 (6.9–24.4)	0.001
SHBG (nmol/l)	24.0 (15.0–37.2)	39.3 (29.3–56.5)	0.001
Total testosterone (ng/ml)	0.91 $\pm$ 0.4	0.53 $\pm$ 0.1	0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product index.

association between rs7903146 variants of the *TCF7L2* gene and WC. Therefore, considering a difference of 6 cm in WC between the genotypes, an α of 5% and a β of 80%, the sample size was estimated as 264 PCOS participants. A subsequent interim analysis showed that a difference of 6 cm in WC was actually obtained with the sample of 200 PCOS enrolled in the study.

Results are presented as mean $\pm$ s.d. for variables with normal distribution or as median and interquartile range for variables with non-Gaussian distribution. Non-Gaussian variables were log-transformed for statistical analysis with independent Student's *t*-test and one-way ANOVA and reported back-transformed in their original units. To account for multiple comparisons, Bonferroni correction procedure was applied and *P* values $\leq$ 0.002 were considered significant. A test for linear trend was used to test co-dominant effects of genotypes on dependent variables. All analyses were performed using the Statistical Package for the Social Sciences (SPSS version 20).

Results

Participants were mostly Caucasian (94.2%), with the remaining participants having mixed (African and European) ancestry. Mean age was 23.7 $\pm$ 7.1 years. In the PCOS group, 25.5% patients had metabolic syndrome and 25.3% had hypertension. In the control group, 2.3% participants had metabolic syndrome and 6.9% had hypertension.

Table 1 summarizes the clinical, metabolic, and hormonal profile of PCOS and control participants. Total cholesterol, LDL-C, and fasting glucose were similar between the groups. Women with PCOS were

significantly younger than controls. As expected, women with PCOS also had significantly higher BMI, SBP, DBP, WC, serum triglycerides, glucose at 120 min, fasting insulin, HOMA-IR, LAP, and TT and lower SHBG in comparison with the control group. These results remained significant even when adjusted for age.

For the 200 women in the PCOS group, the frequencies of genotypes for polymorphism rs7903146 were as follows: 54.4% for CC genotype, 28.5% for heterozygous CT, and 17.1% for TT genotype. These frequencies did not differ from those recorded for the control group: 51.0% for CC genotype, 37.0% for heterozygous CT, and 12.0% for homozygous TT genotype (Table 2). Of the attempted genotypes, 96.5% were successful for this SNP. Genotypic distributions of SNP rs11196236 in women with PCOS were 4.3% for CC genotype, 33.5% for heterozygous CT, and 62.2% for homozygous TT genotype. Similar frequencies were observed in controls: 3.2% for CC, 35.5% for heterozygous CT, and 61.3% for homozygous TT genotype (Table 2). Of the attempted genotypes, 93.0% were successful for this SNP. The genotype distributions for both SNPs were in Hardy–Weinberg equilibrium in controls.

The rs7903146 polymorphism was not in linkage disequilibrium with the rs11196236 polymorphism ($|D'| = 0.34$; $r^2 = 0.07$). Ten haplotypes were inferred in this sample (Ht1: CCCC, Ht2: CCCT, Ht3: CCTC, Ht4: CTCT, Ht5: CTTC, Ht6: CTTT, Ht7: TCTC, Ht8: TCTT, Ht9: TTCT, and Ht10: TTTT). Haplotype frequencies were 18.5% for Ht1/Ht2/Ht3, 69.5% for Ht4/Ht5/Ht6/Ht7/Ht8, and 12.0% for Ht9/Ht10.

Subsequent analyses were made specifically with the PCOS group. Table 3 shows clinical and metabolic variables according to SNP rs7903146 genotypes. There were no differences between genotypes for any of the studied variables.

Table 4 presents clinical and metabolic variables according to SNP rs11196236 genotypes in PCOS. No statistical differences were observed among the different genotypes for the studied variables.

Table 2 Genotype distribution of polymorphisms rs7903146 and rs11196236 in the *TCF7L2* gene in PCOS women. Data expressed as % (χ^2 test).

	Frequency (%)		P
	PCOS	Controls	
Rs7903146			
CC	54.4	51.0	0.25
CT	28.5	37.0	
TT	17.1	12.0	
Rs11196236			
CC	4.3	3.2	0.97
CT	33.5	35.5	
TT	62.2	61.3	

Table 3 Clinical and metabolic characteristics of PCOS women according to genotypes of polymorphism rs7903146 in the *TCF7L2* gene. Data are presented as mean $\pm$ s.d. or median (25–75 interquartile range).

	PCOS		P
	CC/CT	TT	
BMI (kg/m ²)	29.8 $\pm$ 7.1	32.8 $\pm$ 7.2	0.030
SBP (mmHg)	121.8 $\pm$ 17.4	123.8 $\pm$ 10.5	0.51
DBP (mmHg)	77.9 $\pm$ 12.1	81.3 $\pm$ 9.2	0.13
Waist circumference (cm)	89.3 $\pm$ 16.2	96.1 $\pm$ 16.7	0.034
Triglycerides (mg/dl)	90 (62–131)	89 (63–132)	0.76
Total cholesterol (mg/dl)	175 $\pm$ 40	172 $\pm$ 33	0.65
HDL-C (mg/dl)	49 $\pm$ 11	46 $\pm$ 10	0.30
LDL-C (mg/dl)	104 $\pm$ 33	105 $\pm$ 29	0.96
Fasting glucose (mg/dl)	89 $\pm$ 15	90 $\pm$ 18	0.70
Glucose at 120 min (mg/dl)	113 $\pm$ 40	115 $\pm$ 38	0.80
Fasting insulin (μ U/ml)	17.7 (9.8–28.5)	16.4 (11.8–25.6)	0.94
HOMA-IR	3.7 (1.9–6.6)	3.5 (1.9–5.8)	0.98
LAP	27.6 (13.1–60.3)	36.9 (21.2–74.0)	0.09
SHBG (mmol/l)	23.3 (14.8–38.3)	29.0 (17.4–41.4)	0.17

SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product index.

Table 5 shows clinical and metabolic variables according to haplotypes in PCOS. Individuals with the Ht9/Ht10 haplotype had a non-significant higher WC ($P=0.02$) and LDL-C ($P=0.03$) compared with Ht1/Ht2/Ht3. In addition, for each T (rs7903146) and T (rs11196236) allele added to the haplotypes, a variation of 5.87 cm in waist (P trend=0.01), 10.7 mg/dl in total cholesterol (P trend=0.03), and 10.3 mg/dl in LDL-C (P trend =0.01) was recorded (Fig. 1).

Discussion

In this study, SNPs rs7903146 and rs11196236 of the *TCF7L2* gene were not associated with the occurrence of PCOS in women from South Brazil. Previous genetic association studies with PCOS patients, although not entirely conclusive, have shown similar results. Barber *et al.* (31), studying 369 women with PCOS and 2574 controls of UK British or Irish origin, and 540 PCOS and 1083 controls from the Northern Finland Birth Cohort of 1966 found no association of polymorphism rs7903146 with PCOS. That was also the case with another large study analyzing 624 PCOS and 553 control women of European ancestry, which did not find an association between rs7903146 of *TCF7L2* and PCOS. However, the latter study reported that variation in a specific region of the gene, where SNP rs11196236 is located, was associated with reproductive and metabolic quantitative traits in PCOS (32). Studies focusing on SNP rs7903146 in Chinese (33) and Korean women (34) were also unable to detect an association with PCOS. The genotype distribution of the *TCF7L2* variants found in our PCOS

women and controls agree with these earlier findings (14, 31).

Interestingly, we found that the haplotypes Ht9/Ht10 were weakly associated with obesity-related traits and LDL-C. While very few studies are available in PCOS populations, studies with diabetic individuals have shown analogous associations of *TCF7L2* and obesity-related traits (14, 35, 36). In North India, Sanghera *et al.* (37) observed increased cholesterol and LDL-C levels in risk allele carriers of SNP rs7903146; SNP rs7903146 was associated with high triglyceride levels in Mexican and Finnish populations (38, 39). Moreover, a meta-analysis indicated an association between the T allele of rs7903146 and the metabolic syndrome (40). By contrast, other studies with diabetic populations did not find an influence on body weight (37, 41, 42). Our sample size of 200 PCOS may have been a limiting factor for these multiple analyses regarding associations between genotype and metabolic variables.

It is well established that common polymorphisms of the *TCF7L2* gene are associated with T2DM (10, 41, 43, 44). Grant *et al.* (10) have shown that microsatellite DG10S478 of the *TCF7L2* gene increases the risk of T2D by ~ 1.45 in heterozygotes and 2.41 in homozygotes. In that study, DG10S478 was in strong linkage disequilibrium with rs7903146. The TT genotype of rs7903146 also predicted the risk of diabetes in the Diabetes Prevention Program (DPP) (36) and the Finnish Diabetes Prevention Study (45).

In this sense, studies demonstrate that variation in the *TCF7L2* gene is associated with defects in insulin secretion (46, 47, 48). Greater proinsulin/insulin ratio has consistently been observed among rs7903146 T allele carriers; contrarily, associations with proinsulin level have been less consistent (42, 47, 49). In PCOS women with abnormal glucose tolerance,

Table 4 Clinical and metabolic characteristics of PCOS women according to genotypes of polymorphism rs11196236 in the *TCF7L2* gene. Data are presented as mean $\pm$ s.d. or median and interquartile range (25th–75th percentile).

	PCOS		P
	CC/CT (70)	TT (115)	
BMI (kg/m ²)	29.2 $\pm$ 6.8	30.7 $\pm$ 7.4	0.16
SBP (mmHg)	119.0 $\pm$ 12.4	123.4 $\pm$ 18.2	0.08
DBP (mmHg)	76.9 $\pm$ 11.2	78.6 $\pm$ 11.7	0.35
Waist circumference (cm)	86.8 $\pm$ 14.4	91.7 $\pm$ 17.3	0.05
Triglycerides (mg/dl)	88 (60–125)	93 (65–139)	0.44
Total cholesterol (mg/dl)	165 $\pm$ 34	180 $\pm$ 40	0.01
HDL-C (mg/dl)	48 $\pm$ 10	48 $\pm$ 10	0.89
LDL-C (mg/dl)	96 $\pm$ 27	109 $\pm$ 34	0.004
Fasting glucose (mg/dl)	90 $\pm$ 20	89 $\pm$ 13	0.59
Glucose at 120 min (mg/dl)	114 $\pm$ 51	113 $\pm$ 33	0.86
Fasting insulin (μ U/ml)	18.2 (9.8–26.3)	16.3 (9.5–28.8)	0.99
HOMA-IR	3.5 (1.9–6.2)	3.6 (2.0–6.7)	0.94
LAP	27.6 (12.9–49.6)	34.4 (14.4–68.0)	0.25
SHBG (mmol/l)	25.3 (15.2–38.8)	24.0 (16.0–36.5)	0.99

SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product.

Table 5 Clinical and metabolic characteristics of PCOS women according to haplotype of *TCF7L2* gene. Data are presented as mean $\pm$ s.d. or median (25–75 interquartile range).

	PCOS			P
	Ht1/Ht2/Ht3	Ht4/Ht5/Ht6/ Ht7/Ht8	Ht9/Ht10	
BMI (kg/m ²)	28.7 $\pm$ 6.7	30.2 $\pm$ 7.3	32.6 $\pm$ 5.8	0.11
SBP (mmHg)	117 $\pm$ 14	122 $\pm$ 17	122.3 $\pm$ 12.1	0.26
DBP (mmHg)	76 $\pm$ 13	78 $\pm$ 11	79 $\pm$ 10	0.48
Waist circumference (cm)	85.9 $\pm$ 15.4	90.0 $\pm$ 16.2	98.3 $\pm$ 16.8	0.02
Triglycerides (mg/dl)	89 (54–125)	91 (65–137)	93 (63–147)	0.60
Total cholesterol (mg/dl)	162 $\pm$ 31	177 $\pm$ 40	181 $\pm$ 34	0.06
HDL-C (mg/dl)	47 $\pm$ 11	49.2 $\pm$ 10	46.7 $\pm$ 10	0.47
LDL-C (mg/dl)	92 $\pm$ 26	107 $\pm$ 33	112 $\pm$ 30	0.03
Fasting glucose (mg/dl)	88 $\pm$ 17	89.5 $\pm$ 14	90.3 $\pm$ 20	0.86
Glucose at 120 min (mg/dl)	109 $\pm$ 49	114 $\pm$ 36	116 $\pm$ 44	0.81
Fasting insulin (μ U/ml)	18.5 (10.0–30.6)	16.9 (10.5–28.1)	16.8 (12.7–33)	0.29
HOMA-IR	3.4 (2.0–6.4)	3.6 (2.3–6.8)	3.6 (2.6–7.7)	0.41
LAP	22.4 (9.5–55.1)	30.0 (14.3–62.0)	40.1 (23.9–82)	0.13
SHBG (mmol/l)	24.0 (12.4–38.8)	23.2 (16.0–35.6)	28.8 (17.9–36)	0.45

SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; LAP, lipid accumulation product index.

TCF7L2 polymorphisms were associated with defects in insulin secretion (32). Another study with a small sample of 31 PCOS women has found that SNP rs11196236 was associated with peripheral insulin (15). By contrast, similar to other studies with PCOS populations (14, 33, 34), we did not find an association between insulin resistance and SNPs of the *TCF7L2*. It should be noted that differences in anthropometry, proportion of less severe phenotypes, and ethnicity among these PCOS populations could have, at least in part, masked the influence of *TCF7L2* SNPs on clinical expression of insulin resistance.

The mechanism linking the *TCF7L2* gene with obesity traits and metabolic disturbances in diabetes has not been established. *TCF7L2* encodes a transcriptional factor that mediates downstream Wnt signaling after binding with β -catenin. Some evidence indicates that *TCF7L2* is significantly expressed in human target tissues for glucose homeostasis, including visceral and subcutaneous fat (45, 50, 51), and that Wnt signaling could be a key regulator of adipogenesis through the β -catenin/*TCF7L2*-dependent pathway (52). Thus, overexpression of Wnt signaling might block adipogenesis, whereas inhibition of *TCF7L2* could stimulate adipogenesis, as observed *in vitro* (53). Such observations further support multiple effects of *TCF7L2* on homeostasis through modulation of adipogenesis. However, it is unclear how these intronic variants of the *TCF7L2* gene may influence phenotypes. It is likely that these intronic variants act by affecting the expression of *TCF7L2* rather than altering the structure of expressed protein (54). Wnt signaling could also affect insulin secretion (55). One hypothesis suggests

that genetic variation in the *TCF7L2* gene might impair the expression of glucagon-like peptide 1, possibly by Wnt/*TCF7L2* pathways, which influences insulin secretion from the pancreatic β cells (10, 56).

One limitation of this study was the relatively small sample size of 300 participants. However, the effect sizes

Figure 1 Association of haplotypes with waist circumference (A), total cholesterol (B), and LDL-C (C). $P=0.02$ (A); $P=0.06$ (B); $P=0.03$ (C).

observed in our sample are similar to those reported in other PCOS populations. Our data for haplotypes of the *TCF7L2* gene suggest a weak association with some quantitative traits may be present in women with PCOS from Southern Brazil. However, further studies are needed with different populations to confirm these data, to elucidate the mechanisms through which *TCF7L2* risk genotypes and haplotypes could influence obesity-related traits and metabolic components and to establish further consequences of the interactions between genetic and environmental factors.

In conclusion, the present results suggest that variants of the *TCF7L2* gene are not implicated in the development of PCOS in women from South Brazil.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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