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Common variants in SOCS7 gene predict obesity, disturbances in lipid metabolism and insulin resistance

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KEYWORDS
SOCS7 (suppressor of cytokine signaling, 7);
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Abstract *Background and aims:* Specific Suppressor of Cytokine Signaling (SOCS) members, such as SOCS7, may play a role in the development of insulin resistance (IR) owing to their ability to inhibit insulin signaling pathways. The objective was to explore the association between common variants and related haplotypes in SOCS7 gene and metabolic traits related to obesity, lipid metabolism and IR.
Methods and Results: 780 unrelated men were included in a cross-sectional study. We selected three tagged SNPs that capture 100% of SNPs with minor allele frequency ≥ 0.10. Analyses were done separately for each SNP and followed up by haplotype analysis. rs8074124C was associated with both obesity ($p = 0.005$) and abdominal obesity ($p = 0.002$) and allele C carriers showed, in comparison with TT carriers, lower BMI ($p = 0.001$) and waist circumference

Acronyms: SOCS, suppressor of cytokine signaling; IR, insulin resistance; DM2, type 2 diabetes; MS, metabolic syndrome; JAK, janus kinase; STAT, signal transducer and activator of transcription; CIS, cytokine-inducible src homology 2 domain-containing protein; IRS-1, receptor substrate-1; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; BMI, body mass index; WC, waist circumference; HTG, hypertriglyceridemia; HW, hypertriglyceridemic waist; IFG, impaired fasting glucose; AHA/NHLBI, American heart association/national heart, lung, and blood institute; HOMA, homeostasis model assessment; SNP, single nucleotide polymorphism; CEPH, caucasian european utah dataset; LD, linkage disequilibrium; MAF, minor allele frequency; RFLP, restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium; df, 2 degrees of freedom; OR, odds ratio; 95%CI, 95% confidence intervals; GWAS, genome-wide association studies.

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($p = 0.001$). rs8074124CC- carriers showed lower fasting insulin ($p = 0.017$) and HOMA-IR ($p = 0.018$) than allele T carriers. rs12051836C was associated with hypertriglyceridemia ($p = 0.009$) and hypertriglyceridemic waist ($p = 0.006$). rs12051836CC- carriers showed lower fasting insulin ($p = 0.043$) and HOMA-IR ($p = 0.042$). Haplotype-based association analysis (rs8074124 and rs12051836 in that order) showed associations with lipid and obesity -related phenotypes, consistent with single locus analysis. Haplotype analysis also revealed association between haplotype CT and both decreased HDL-C ($p = 0.026$) and HDL-C ($p = 0.014$) as a continuous variable.

Conclusions: We found, for the first time, significant associations between SOCS7 common variants and related haplotypes and obesity, IR and lipid metabolism disorders.

Introduction

Insulin resistance (IR) can occur with obesity, inflammation and ageing, being an important component leading to the development of type 2 diabetes (DM2) and metabolic syndrome (MS). Adiponectin and inflammatory markers can predict the course of MS [1]. The inflammatory state in adipose tissue can lead to IR first in adipose tissue, then in all insulin-sensitive tissues. The infiltrated macrophages release inflammatory proteins causing further recruitment of macrophages to adipose tissue and the release of inflammatory cytokines. Increased circulating free fatty acids released by insulin-resistant adipocytes, reduced circulating adiponectin levels and leptin resistance in turn lead to decreased lipid oxidation in non-adipose tissues, thereby triggering ectopic accumulation of lipids, lipotoxicity and insulin resistance [2].

One of the most important mechanisms by which many cytokines activate gene transcription is the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway [3]. The Suppressor of Cytokine Signaling (SOCS) proteins are known to act as negative regulators of cytokine action and are responsible for a complete negative feedback loop in the JAK/STAT pathway. There is now a growing appreciation of a role for SOCS proteins in the negative regulation of receptor tyrosine kinase signaling, such as the inhibition of insulin signaling [4] by multiple mechanisms.

The SOCS family consists of SOCS1 to SOCS7 and CIS (cytokine-inducible src Homology 2 domain-containing protein). SOCS1 and SOCS3 may play a crucial role in IR and in type 2 diabetes development. SOCS2 has been studied in the context of the growth hormone signaling pathway and appears to be a potent growth regulator in several tissues [5]. However, the physiological role of CIS and SOCS4 to SOCS7 has to be explored in more detail.

SOCS7 $-/-$ mouse [6] shows increased insulin sensitivity, possibly through a mechanism involving the regulation of stability and activity of Insulin Receptor Substrate-1 (IRS-1).

It is known that one mechanism potentially implicated in IRS-1 and IRS-2 level regulation is operated by SOCS proteins and the level of SOCS7 shown to be involved in IRS-1 ubiquitin-mediated degradation [6]. SOCS7 overexpression can lead to decreased protein levels of IRS-1, which is common in obesity and DM2. SOCS7 has been shown to interact with IRS-2 and IRS-4 [7], molecules of the signaling pathway of the insulin and leptin receptors. It is well documented the cross-talk between the insulin and leptin signal transduction pathways. Insulin carries out its biological effects through the phosphorylation of IRS-1 and -2. Leptin was reported to induce IRS-1 and IRS-2 phosphorylation [8]. It is well established that insulin stimulation leads to IRS-4 tyrosine phosphorylation in vitro [9,10] and, more recently, IRS-4 tyrosine phosphorylation upon leptin stimulation [11] was observed. The cytokine-like hormone leptin receptor (LEPR) is a member of the class I cytokine receptor family [12] and transduces signal mainly through the JAK2/STAT3 pathway. It was reported a SOCS7 dependent attenuation of leptin-mediated activity at low, most likely physiologically levels of SOCS-7 expression [13].

The aim of this study was to explore associations between tagged SNPs of SOCS7 and their predicted haplotypes and phenotypes and quantitative metabolic traits related to obesity, lipid metabolism and IR.

Methods

The total sample included 780 unrelated nondiabetic men, with normal examination findings, of self-reported European ancestry. Individuals were randomly recruited at the Department of Haemotherapy of the José de San Martín Hospital of the University of Buenos Aires in the context of a cross-sectional study conducted between April 2006 and April 2008. Ages ranged between 18 and 65 years. Clinical characteristics of the sample are shown in Table 1.

This work was carried out in accordance with the Declaration of Helsinki, and approved by the Ethic Committee of the same Hospital. All subjects gave their written consent.

Anthropometric measurements were obtained by a standardized protocol in every subject. Systolic (SBP) and diastolic (DBP) blood pressure were recorded using a standard mercury sphygmomanometer after at least 10 min of rest. After a 12-h overnight fast, fasting blood samples were drawn in every individual. Total cholesterol, triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and fasting plasma glucose (FPG) were determined by enzymatic methods (Roche Diagnostics, Mannheim, Germany). Fasting serum insulin was measured by radioimmunoassay (Human Insulin Specific RIA kit, Linco Research Inc., St. Louis, MO, USA).

Each subject was tested for [1] obesity according to body mass index (BMI) $\geq$30.0 kg/m^2 [2]; abdominal obesity according to WC $>$102 cm [3]; hypertriglyceridemia (HTG)

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Table 1 Clinical characteristics of the sample (n = 780).

Variable	Mean ± SD	Phenotype	Prevalence (%)
Age	37.11 ± 10.91	MS	24.1
TG	138.77 ± 95.97	HTG	31.1
TC	187.45 ± 40.75	HW	27.8
HDL-C	41.25 ± 10.66	Decreased HDL	45.6
WC	96.15 ± 12.26	Abdominal obesity	31.3
BMI	28.20 ± 4.40	Obesity	29.3
FPG	91.87 ± 12.23	IFG	18.0
SBP	126.30 ± 11.02		
DBP	80.11 ± 7.39		
Fasting insulin	16.18 ± 9.27		
HOMA-IR	2.07 ± 1.16		

TG = Triglycerides [mg/dL]; TC = Total cholesterol [mg/dL]; HDL-C [mg/dL]; WC = Waist circumference [cm]; BMI [kg/m2]; FPG = Fasting plasma glucose [mg/dL]; SBP = Systolic blood pressure [mmHg]; DBP = Diastolic blood pressure [mmHg]; Fasting insulin [μU/ml]; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance; MS = Metabolic syndrome; HTG = Hypertriglyceridemia; HW = Hypertriglyceridemic waist. IFG = Impaired FPG.

according to fasting TG ≥150 mg/dl or drug treatment [4]; hypertriglyceridemic waist (HW) according to TG ≥ 150 mg/dl + WC ≥90 cm [5]; decreased HDL-C according to fasting HDL-C <40 mg/dl or drug treatment [6]; Impaired Fasting Glucose (IFG) according to FPG ≥100 mg/dl; and [7] MS using the AHA/NHLBI (American Heart Association/National Heart, Lung, and Blood Institute) 2005 criteria [14].

Participants who had FPG ≥ 126 mg/dl were excluded from the study [15].

Insulin sensitivity was assessed with HOMA (Homeostasis Model Assessment) using the software HOMA Calculator v.2.2.2 for Windows [16].

Selection of tagSNPs

To select Single Nucleotide Polymorphism (SNP) for genotyping ("tagSNPs") in candidate gene SOCS7 (48.015 bp) we downloaded information from the International HapMap Project web site (http://www.hapmap.org/) (HapMap Data Rel 28 PhaseII + III, August10) from the Caucasian European Utah dataset (CEPH) and used Haploview 4.1 [17] to analyze the linkage disequilibrium (LD). Three SNPs, rs8068600, rs8074124 and rs12051836 capture eight of eight alleles [pairwise tagging algorithm, MAF = 0.10, minimum $r2$ ≥0.8 and minimum LOD = 3.0] (Supplemental material Figure 1). We used the program GLIDERS [18] to analyze long-range LD (MAF ≥0.05, r^2 ≥ 0.8, HapMap phase 3 CEU sample) and detected LD between rs12051836 and rs4359493, rs1045638, rs3748725, rs3748723, rs7405482, rs8070208 and rs6503581.

Genotyping

Genomic DNA was isolated according to standard procedures.

rs12051836 and rs8068600 genotypes were scored blindly using PCR-RFLP (Restriction Fragment Length Polymorphism) analysis. The sequences of primers and thermal cycling conditions were: for rs12051836 (NC_000017.10, chr17:36,549,172−36,549,839): 5′ TCAGTGGGCCTCAGTTTCTT3′ and 5′ TGACTCACTGCAAGCTCCAA 3′, initial denaturation step at 94 °C for 5 min followed by 35 cycles of 94 °C for 50 s, 64 °C for 50 s, 72 °C for 1 min and a final extension at 72 °C for 10 min; and for rs8068600 (NC_000017.10, chr17:36523895+36524594): 5′ AGGGTATCACCCACCACACT 3′ and 5′ GGCCAACAGAATAAAACACTG 3′, initial denaturation step at 94 °C for 5 min followed by 30 cycles of 94 °C for 50 s, 60 °C for 50 s, 72 °C for 1 min and a final extension at 72 °C for 10 min. The restriction digestion of rs12051836 and rs8068600 PCR products was carried out with MspI and HpyCH4IV (New England Biolabs Inc.), respectively, at 37 °C for about 12 hs. The digested products were loaded and visualized on 2% agarose gel after staining with ethidium bromide. Genotyping accuracy was assessed by inclusion of duplicates and negative controls. Genotyping success rate was 99.9% for rs12051836 and 99.8% rs8068600. The specificity of the reactions was confirmed by automatic sequencing. rs12051836 results were confirmed by restriction digestion of PCR products with BstNI (New England Biolabs Inc.) at 60 °C for about 12 hs. rs8068600 results were confirmed by automatic sequencing.

rs8074124 was genotyped by KBiosciences, Hoddesdon, Herts., UK, using the KASPar system (http://www.kbioscience.co.uk/chemistry/chemistry-intro.htm).

Statistical analysis

Deviation of the genotype distribution from the Hardy−Weinberg equilibrium (HWE) was tested using Chi square test. Analyses were done separately for each SNP and followed up by haplotype analysis. For individual SNP association analyses, we first performed a 2-df (2 degrees of freedom) overall test of genotypic association. If statistical significance was achieved, dominant and recessive genetic models (1-df) were tested. Differences between prevalence rates were assessed by Fisher exact test. We calculated odds ratio (OR) and 95% confidence intervals (95%CI). Quantitative data were expressed as means ± SD. For comparison of continuous variables we conducted one-way ANOVA with Levene's Test for equality of variances. If the data failed to meet the Levene's test criteria, we used the non-parametric Kruskal−Wallis procedure. Regression analysis was used to adjust for confounding variable age.

For each individual case−control study power estimations [19] were performed for single-point allelic effects, with an odds ratio of 1.5 at a nominal significance level of 0.05 for HapMap- CEPH -predicted MAF. The power estimation was found to be between 87 and 90% assuming a dominant model and less than 80% assuming a recessive model.

Haplotype frequencies for SOCS7 tagSNPs were estimated using the program PHASE 2.1 [20]. Fisher exact test was used to compare haplotype frequencies of case and control samples. The effects of a particular haplotype load (0: no copies of the particular haplotype; 1:1 copy; and 2:2

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copies) on continuous variables were tested using linear regression. A p-value less than 0.05 was considered statistically significant. In adjusting the p-value to account for multiple testing we follow the recommendations of van den Oord and Sullivan [21], who suggest that a level of significance of $p = 0.01$ on average control the false discovery rate at 0.10. It should be noted that, after applying the Bonferroni correction for multiple tests, the significance level was $p < 0.02$ (0.05/3 for 3 loci). The significance level was $p < 0.01$ (0.05/4) for correction for multiple tests in haplotype analyses (4 haplotypes for 2 loci).

Except for the estimations of haplotype frequency, statistical analyses were conducted using the program for Statistical Package for the Social Sciences, version 12.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Allele frequencies were as follow: rs8074124 T 0.831 and C 0.169; rs12051836 T 0.844 and C 0.156; and rs8068600 C 0.918 and G 0.088. rs8074124 ($p = 0.396$) and rs12051836 ($p = 0.393$) were in HWE, while rs8068600 showed a departure from HWE ($p = 0.007$). As departure from HWE could indicate lack of robust genotyping performance, we repeat genotyping by another method and data did not change. We performed only exploratory tests regarding to rs8074124 and rs12051836.

Single-locus analyses

- Association with obesity and obesity-related traits

rs8074124 was associated with obesity (2-df $p = 0.027$) and abdominal obesity (2-df $p = 0.032$) even accounting for the effects of age ($p = 0.015$ and $p = 0.008$ respectively) (Table 2). We also were able to show significant differences among genotypes for both BMI and WC ($p = 0.023$ and $p = 0.009$ respectively) even accounting for the effects of age ($p = 0.003$ and $p = 0.004$ respectively) (Table 3). rs8074124C carriers showed a lower risk for obesity ($p = 0.027$, OR = 0.66 [95%CI = 0.45–0.95]) and abdominal obesity ($p = 0.015$, OR = 0.64 [95%CI = 0.45–0.92]). Age-adjusted associations with obesity ($p = 0.005$) and abdominal obesity were significant at levels that take into account multiple testing. Moreover, rs8074124C allele carriers showed lower BMI ($p = 0.034$; 27.55 ± 3.85 vs. 28.46 ± 4.58) and WC ($p = 0.036$; 94.54 ± 10.70 vs. 96.82 ± 12.58) than genotype TT carriers. Age-adjusted associations with BMI ($p = 0.001$) and WC ($p = 0.001$) were significant at levels that take into account multiple testing.

No genotypic association was found between rs12051836 and obesity or abdominal obesity. Age-adjusted association was found between rs12051836 and BMI ($p = 0.036$) and WC ($p = 0.019$) (Table 3). Additional follow-up analysis showed that rs12051836C allele carriers had lower BMI ($p = 0.031$; age-adjusted $p = 0.032$; 27,60 ± 3,85 vs. 28,34 ± 4,50) and WC ($p = 0.024$; age-adjusted $p = 0.017$; 94,42 ± 10,96 vs. 96,61 ± 12,55) than genotype TT carriers even after adjusting for age.

- Association with lipid –related phenotypes and quantitative traits

2-df association rs8074124 and total cholesterol was found ($p = 0.039$) (Table 3). rs8074124CC carriers showed lower levels of total cholesterol ($p = 0.011$; 166.25 ± 32.29 vs. 187.95 ± 41.22) than allele T carriers (age-adjusted $p = 0.030$).

Table 2 1-df genotypic association between individual SOCS7 tagSNPs and obesity and lipid related phenotypes.

		rs8074124				rs12051836			
		Positive	Negative	p	p^a	Positive	Negative	p	p^a
MS	CC	3	20			3	19		
	CT	44	151			39	154		
	TT	128	369	0.261	0.171	141	405	0.134	0.074
Obesity	CC	3	21			4	18		
	CT	49	149			50	148		
	TT	160	345	0.027	0.015	174	381	0.125	0.079
Abdominal obesity	CC	4	19			5	17		
	CT	50	147			52	144		
	TT	170	335	0.032	0.008	185	370	0.136	0.061
HTG	CC	4	20			3	19		
	CT	58	140			52	146		
	TT	168	338	0.144	0.135	185	370	0.028	0.027
HW	CC	3	20			3	19		
	CT	51	147			45	152		
	TT	153	353	0.099	0.080	166	389	0.044	0.032
Decreased HDL-C	CC	12	12			10	12		
	CT	94	104			84	114		
	TT	231	275	0.849	0.844	259	296	0.588	0.578

MS = Metabolic Syndrome. HTG = Hypertriglyceridemia. HW = Hypertriglyceridemic waist.
[a] After accounting for age.

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Table 3 Single locus analysis of quantitative metabolic traits.

	rs8074124					rs12051836			
	Media (SD)			p	p^a	Media (SD)		p	p^a
	CC (n = 26)	CT (n = 212)	TT (n = 542)			CC (n = 22)	CT (n = 199)	TT (n = 559)		
BMI	26,17 (3,03)	27,71 (3,91)	28,46 (4,58)	0.023[b]	0.003	26,48 (3,23)	27,83 (3,84)	28,41 (4,62)	0.146[b]	0.036
WC	89,43 (10,32)	95,14 (10,61)	96,82 (12,58)	0.009[b]	0.004	90,41 (10,91)	95,13 (10,82)	96,75 (12,74)	0.045[b]	0.019
TC	166,25 (32,29)	187,97 (43,48)	187,94 (40,34)	0.039	0.334	172,36 (30,56)	187,63 (43,38)	187,92 (40,18)	0.214	0.572
TG	109,08 (72,26)	143,27 (105,04)	140,42 (95,62)	0.268	0.805	110,45 (71,57)	136,92 (103,19)	140,51 (94,32)	0.339	0.429
HDL-C	40,08 (8,07)	40,32 (10,94)	41,35 (10,31)	0.456	0.251	40,50 (7,33)	41,16 (11,12)	41,30 (10,63)	0.935	0.871
Fasting insulin	11,44 (4,98)	15,70 (9,35)	16,73 (9,44)	0.006[b]	0.063	12,26 (5,66)	15,29 (8,68)	16,65 (9,56)	0.028[b]	0.035
HOMA-IR	1,47 (0,63)	2,01 (1,19)	2,13 (1,18)	0.007[b]	0.071	1,57 (0,70)	1,96 (1,11)	2,13 (1,19)	0.034	0.038

WC = Waist circumference [cm]. TC = Total cholesterol [mg/dL]. TG = Triglycerides [mg/dL]. HDL-C [mg/dL]. Fasting insulin [μU/ml]. HOMA-IR = Homeostasis model assessment of insulin resistance. BMI [kg/m2].

[a] After accounting for age.

[b] Kruskal–Wallis p value.

rs8074124 was not associated with other categorical or continuous lipid related variable (Tables 2 and 3).

rs12051836 was associated with HTG (2-df $p = 0.028$ and $p = 0.027$ after adjusting for age) (Table 2). C allele carriers showed a lower risk for HTG than the TT genotype ($p = 0.014$; OR = 0.62 [95%CI = 0.42–0.90]). Age-adjusted analysis confirmed a significant effect ($p = 0.009$) on HTG risk. Furthermore, rs12051836 was associated with HW (2-df $p = 0.044$ and $p = 0.032$ after adjusting for age); rs12051836C carriers, compared to rs12051836 TT carriers, showed a lower risk of HW ($p = 0.008$, OR = 0.59 [95% CI = 0.40–0.88]). Age-adjusted analysis confirmed a significant effect on HW risk ($p = 0.006$). Note that allele C age-adjusted associations with HTG and HW are significant at levels that take into account multiple testing. However, there was no significant difference in TG levels between allele C and TT genotype carriers (Table 3).

- Analysis of surrogate measures of IR

rs8074124 showed differences in surrogate measures of IR between genotypes (insulin $p = 0.006$ and HOMA-IR $p = 0.007$). Differences between rs8074124 genotypes and insulin or HOMA-IR were not significant taking into account age (Table 3). rs8074124CC genotype carriers had lower fasting insulin ($p = 0.005$; 11.44 ± 4.98 vs. 16.44 ± 9.42) and HOMA-IR ($p = 0.006$; 1.47 ± 0.63 vs. 2.10 ± 1.18) than allele T carriers. Age-adjusted analysis confirmed the associations (insulin $p = 0.017$ and HOMA-IR $p = 0.018$).

rs12051836 (insulin $p = 0.028$ and HOMA-IR $p = 0.034$) showed differences in surrogate measures of IR between genotypes. Differences between rs12051836 genotypes and insulin ($p = 0.035$), and HOMA-IR ($p = 0.038$) remain significant taking into account age (Table 3). rs12051836CC carriers had a lower fasting insulin ($p = 0.032$; 12.04 ± 5.34 vs. 16.33 ± 9.18) and HOMA-IR ($p = 0.035$; 1.55 ± 0.67 vs. 2.09 ± 1.15). Age-adjusted analysis confirmed the associations (insulin $p = 0.043$ and HOMA-IR $p = 0.042$).

No association was found (2-df) between rs12051836 or rs8074124 and MS (Table 2).

Haplotype analyses

The inferred two-SNP-haplotype frequencies of rs8074124 and rs12051836 in that order were as follows (n = number of chromosomes examined): TT ($n = 1281$) 0.831, CC ($n = 226$) 0.147, CT ($n = 30$) 0.019 and TC ($n = 5$) 0.003. The most frequent haplotype TT was the one combining the most frequent allele at each site. Genotypic frequencies were as follows: TT/TT 0.69, TT/CC 0.25, TT/CT 0.03, CC/CC 0.02, TT/TC 0.006, CT/CC 0.001 and CT/CT 0.001.

We could not find any significant association between cases and controls when comparing the overall frequency differences (3-df) across four possible haplotypes.

In a separate analysis, each haplotype was compared with the most common TT. Significant differences in frequency were found only for HTG (TT vs. CC; $p = 0.035$), HW (TT vs. CC; $p = 0.044$) and decreased HDL-C (TT vs. CT; $p = 0.026$) (Table 4). These results show associations that were not identified using single markers. Furthermore, haplotype analysis of quantitative metabolic traits revealed

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Table 4 Two-SNP haplotype frequency distributions of SOCS7 tagSNPs in cases and controls and association significance. Only significant results are shown.

Haplotype	Phenotype (*n*)		*p*	OR (95% CI)
	HTG (242)	No HTG (528)		
TT	0.810	0.840	—	
CC	0.118	0.160	0.035	0.71 (0.51—0.98)
CT	0.012	0.023	NS	
	HW (216)	No HW (553)		
TT	0.856	0.822	—	
CC	0.116	0.160	0.044	0.70 (0.50—0.98)
CT	0.023	0.017	NS	
	decreased HDL-C (353)	No decreased HDL-C (417)		
TT	0.827	0.833	—	
CC	0.143	0.150	NS	
CT	0.028	0.012	0.017	0.4 (0.19—0.86)

Haplotypes are composed of variants of rs8074124 and rs12051836 in that order. Each haplotype was compared with the most common haplotype by Fisher's Exact Test. The effects of TC haplotype were not tested because of low frequency (<1%). *n* = Number of chromosomes examined. HTG = Hypertriglyceridemia. HW = Hypertriglyceridemic waist.

a significant association between CT haplotype and HDL-C ($p = 0.014$) even after adjustment for confounding variables age ($p = 0.015$) and TG ($p = 0.012$). We did not find any other significant association between two-SNP haplotypes and lipid -related variables.

No association was found between the inferred haplotypes and obesity or abdominal obesity. Haplotype load analysis revealed association of both TT and CC haplotypes with WC (haplotype TT $p = 0.004$ and haplotype CC $p = 0.010$), BMI (haplotype TT $p = 0.005$ and haplotype CC $p = 0.021$), fasting insulin (haplotype TT $p = 0.020$ and haplotype CC $p = 0.016$) and HOMA-IR (haplotype TT $p = 0.019$ and haplotype CC $p = 0.016$) even after accounting for age. No association between CT haplotype and obesity-related metabolic traits became apparent. The effects of TC haplotype were not tested because of low frequency. Note that, after the use of the stringent Bonferroni correction for multiple tests, differences were significant only for haplotype TT and WC and BMI after accounting for age.

Discussion

Few association studies have been published on SOCS common variants and obesity, DM2 or IR. Such studies have yielded different results [22—28], but there is no candidate gene association study regarding to SOCS7 variants. However, Altshuler D., Groop L. and Hughes I. performed a Genome-Wide Association Study (GWAS) in Scandinavian individuals and analyzed data to identify associations between variants and traits related to glucose, obesity, lipids and blood pressure (http://www.broadinstitute.org/diabetes/scandinavs/metatraits.html). rs4334342 (in LD with rs12051836) and rs8074124 were nominally associated with HDL-C and hypertension. To our knowledge, there is no other GWAS reporting any significant association with SOCS7 locus.

In the sample under study, we observed that rs12051836C carriers had decreased risk for HTG and HW and CC- haplotype seems to be protective against HTG and HW. rs8074124C carriers had decreased risk for obesity and abdominal obesity; and we also demonstrated that rs8074124C allele carriers and rs12051836C allele carriers had lower BMI and WC. Furthermore, SNPs in SOCS7 locus (rs8074124 and rs12051836) were associated with protection against IR estimated by insulin levels and HOMA-IR. CC-haplotype was associated with WC, BMI and surrogate measures of IR. Two-SNP haplotype (rs8074124 and rs12051836) analysis was consistent with the single locus analysis.

Although rs12051836 and rs8074124 were not associated with HDL-C in this study, an association between a haplotype containing both SNPs and HDL-C was observed. Discrepancies may be due to different structure of LD or different environmental exposure among samples or even differences in inclusion/exclusion criteria of cases and or controls or statistical analysis among studies. We should add that there is a major limitation to our study, namely that it was conducted on a sample of young male subjects (mean age 37). The present study provided only exploratory results and should be confirmed in a second and independent replication cohort.

To investigate whether tagSNPs, and even those that are in LD with them, have potential functional significance, we used the program FASTSNP [29]. At least three SNPs, rs4334342 and rs3890580 (in LD with rs12051836) and rs12051836 itself, may have a possible effect of intronic enhancer. For identification of regulatory sequences based in evolutionarily conserved noncoding regions we performed a human/mouse whole genome comparison using the BLASTz and 'subsetAxt' programs, as described in the ensembl multicontigview help page. The results indicate that rs6503550 (in LD with rs8074124), rs4334342, rs4795269, rs1045638 and rs3748725 (in LD with rs12051836), would be located in conserved regions of 837, 1462 and 2596 pb. The molecular mechanisms underlying the effects reported here are still a matter of debate. Bioinformatics analysis does not exclude the possibility of

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functional variants in SOCS7 gene that influence the expression and function.

The metabolic aterations found in this work are strongly related to Metabolic Syndrome. Metabolic Syndrome is associated with insulin and leptin resistance [30]. It may be possible that SOCS7 regulates the signal transmission of insulin and leptin receptors, and this may explain, at least in part, some of the findings reported here.

In conclusion, the present report describes for the first time associations between common variants in SOCS7 gene and obesity, central obesity, IR and disorders of lipid metabolism in nondiabetic men from Argentina. The molecular mechanisms that explain the observed associations are still unknown but rs8074124 and rs12051836 themselves, or SNPs in LD with them, may be located in regulatory regions with potential functional effects. These preliminary findings may reflect the influence of SOCS7 in insulin and leptin signaling.

Conflicts of interest/disclosure statement

The authors have nothing to disclose.

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Institutional approval

This study was carried out in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the José de San Martín Hospital of the University of Buenos Aires.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.numecd.2011.10.005.

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Further reading

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