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# Association between interleukin-1 beta polymorphism (+3953) and obesity

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ABSTRACT

It now appears that obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system as interleukin-1 beta (IL-1). Previous investigations have described a positive association between IL-1β +3953 (C > T) gene polymorphism (rs 1143634) and obesity, suggesting functional effects on fat mass, fat metabolism and body mass. However, it is necessary to determine if these results occur in other populations and if they are influenced by sex and age. Therefore, we performed a case–control study using 880 Caucasian subjects (59.7 ± 11.9 years old) from the Brazilian Aging Research Program (non-overweight = 283, overweight = 334, obese = 263) previously investigated in genetic studies, in whom we analyzed the IL-1β +3953C/T polymorphism. We observed higher T allele (CT/TT) frequency in non-overweight than overweight and obese groups. The odds ratio showed 1.340 (95% CI: 1.119–1.605) times more chance of the obese group being *CC* carriers compared to non-overweight group independent of sex and age. This study corroborates the idea that the IL-1 system is linked to the development of obesity.



## 1. Introduction

Obesity is a multifactorial disease and the genes controlling adipose tissue function may be of particular importance because increased fat mass is the most important feature of the obese phenotype (Aner, 2000). Adipose tissue has a key endocrine role as well as a metabolic function since it secretes several regulatory proteins such as cytokines. Cytokines, including interleukin 1 (IL-1) are regulatory agents in the homeostasis of energy and are produced not only in immunocompetent cells but also in adipocytes (Dianarello, 1996; Hotamisligil et al., 1995; Bruun et al., 2002).

In adipose tissue metabolism, cytokines are major regulators, particularly the IL-1 system which includes one of the most powerful inflammatory molecules. There are four main members of the IL-1 cytokine family: IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra) and IL-18. In general, the IL-1 family is considered to be pro-inflammatory and pro-atherogenic except IL-1ra which is considered an anti-inflammatory cytokine (Girn et al., 2007).

The common IL-1β polymorphism which appears to have an influence on the IL-1 system activity has been described as a C to T single nucleotide polymorphism (SNP) at nucleotide +3953, a T to C SNP at nucleotide −31 (rs 1143634) from the transcription start, and the IL-1RN gene that contains a polymorphic region in the second introns, which has an 86-bp variable number tandem repeat (VNTR). Epidemiological investigations suggest that these polymorphisms are functional and associated to chronical diseases (Zeng et al., 2003; Machado et al., 2001; El-Omar et al., 2000; Garcia-Gonzalez et al., 2003; Zienolddiny et al., 2004; Seripa et al., 2005; Licastro et al., 2004; Oda et al., 2007).

The C to T single nucleotide polymorphism (SNP) at nucleotide +3953 from the transcription start of the IL-1β gene seems to be functional because it has been associated with increased production of IL-1β *in vitro*, worsened rheumatoid arthritis, enhanced inflammatory serum parameters and decreased risk of some infections (Pociot et al., 1992; Buchs et al., 2001; Latkovski et al., 2004; Parkhill et al., 2000).

Studies have investigated the possible association between these IL-1 gene polymorphisms and obesity based on experimental evidence from IL-1 knockout mice that suggested an influence on fat mass, fat metabolism and body mass and on the development of obesity (Garcia et al., 2006).

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Two epidemiological studies performed by the same research groups investigated if common polymorphisms of the IL-1 system, which are associated with IL-1 activity such as +3953 (C > T), were associated with fat mass in 1068 young men (Strandberg et al. (2006) and elderly men (Strandberg et al., 2008). The results showed that T variants (CT and TT) of the +3953 C to T had a significantly lower total fat mass and also significantly reduced arm, leg, and trunk fat compared with CC subjects. However, the investigation performed in 3014 elderly men by Strandberg et al. (2008) did not find an association with total fat mass. Another study that investigated the association between this polymorphism and body mass index (BMI) in 181 healthy females showed a significant decrease in the incidence of the IL-1β +3953 polymorphism T allele in the overweight group compared with the lean group with a BMI < 25 kg/m$^2$ (Lee et al., 2008).

However, there have not been any previous investigations comparing the association between +3953 C to T IL-1β polymorphism and obesity. Therefore, we performed a case–control study to determine if this association occurs in other populations and if sex and age have an influence.

## 2. Subjects and methods

The present study was conducted in subjects from the Southern Brazilian Aging Research Program which investigates genetic and environmental interactions in aging and related non-transmissible diseases. Previous studies have been published and described in more detail about this research project (Tauffer et al., 2005; Prado-Lima et al., 2006). The subjects included men and women, aged 18–92 years (59.7 ± 11.9 years old).

As the study includes genetic variables, the samples were recruited by random selection of Brazilians of European ancestry from Rio Grande do Sul State. Additionally, we selected all overweight and non-overweight subjects without previous diseases such as heart disease, stroke, cancer, and other diseases or disorders that could influence the obese state, dietary pattern and genotype distribution. These exclusions are justified because these variables could interfere in the analyses. Therefore, out of 1058 subjects considered, a total of 880 subjects were selected and classified.

Obesity was determined as having a body mass index (BMI) over 30 kg/m$^2$, overweight with BMI ≥ 25 < 30 kg/m$^2$ and control group (non-overweight) with BMI < 25 kg/m$^2$. The Research Ethics Committee approved the study protocol and informed consent was obtained from all individuals whose information was collected prospectively.

Biochemical analyses were performed on blood samples collected from subjects after an overnight fasting (12 h or more); snacks and coffee were offered afterwards. The following blood tests were performed for biochemical analysis: glucose, total cholesterol, HDL-c, LDL-c and triglycerides (TG) (Um et al., 2004a,b; Lancaster et al., 2003).

Plasma glucose, serum total cholesterol and triglycerides concentrations were measured using standard enzymatic methods using Ortho-Clinical Diagnostics® reagents on the fully automated analyzer (Vitros 950® dry chemistry system; Johnson & Johnson, Rochester, NY, USA). High-density lipoprotein cholesterol was measured in the supernatant plasma after precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride as previously described (Bachorik and Albers, 1986). Low-density lipoprotein cholesterol was estimated with the Friedewald equation (1972).

Additionally, we performed an analysis if metabolic syndrome present in some subjects could be associated with IL-1B +3953 gene polymorphism. Definition of MS was that of the NCEP (Grundy et al., 2005), and a participant was deemed to have MS when three or more of the following criteria were satisfied: (1) high blood pressure: blood pressure ≥130/85 mm Hg or known treatment for hypertension; (2) hypertriglyceridemia: fasting plasma triglycerides ≥150 mg/dL; (3) low HDL: fasting HDL cholesterol < 40 mg/dL in men, <50 mg/dL in women; (4) hyperglycemia: fasting glucose level of ≥110 mg/dL or known treatment for diabetes; (5) central obesity: waist circumference >88 or >102 cm in women and men, respectively.

Genomic DNA was isolated from peripheral blood leukocytes using a GFX Genomic Blood DNA Purification (Amersham Biosciences Inc., Co.) kit. The amplification of a region from human genomic DNA in the IL-1B +3953 gene was performed using polymerase chain reaction (PCR) primers: (F) 5′-CTCAGGTGTCCTCGAAGAAATCAAA-3′ and (R) 5′-GTTTTTTGCTGTGATCC-3′. PCR was performed in a total volume of 25 μl containing 2.5 mM $MgCl_2$, 9.9 mM Tris–HCl (pH 8.8), 50 mM KCl, 0.1% Triton-X 100, 0.20 μM deoxyribonucleotide triphosphate (dNTPs), 1 U Taq DNA polymerase, and 0.2 μM of each primer. PCR conditions comprised an initial denaturation at 96 °C for 1 min followed by 30 cycles at 94 °C for 1 min, 60 °C for 1 min, and 70 °C for 1 min, with a final extension at 72 °C for 10 min. The final PCR products were digested with 10 U TaqI at 65 °C overnight. Digested products were visualized on a 6% agarose gel (Amersham Biosciences Inc., Co.) stained with ethidium bromide.

Data analysis was performed with SPSS (version 13.0.1; SPSS Inc., Chicago, IL). Existence of Hardy–Weinberg equilibrium was tested by $\chi^2$ analysis. Because the *T* variant of the IL-1β +3953 polymorphism has been reported to be dominant, we primarily pooled the CT and TT individuals for investigation of obesity, as done by several others (Buchs et al., 2001; Latkovski et al., 2004; Parkhill et al., 2000). Moreover, the TT genotype constituted only 6.5% of the population in the present study. A potential dose effect of the T allele was then investigated by comparing the CC and TT genotypes and obesity. Logistic regression (*Backward stepwise Wald* method) was used to investigate possible sex and age influence on the results. An age of 60 years was chosen as the cut-off to determine a possible age influence, because in developing countries such as Brazil persons >60 years old are considered elderly by the World Health Organization (WHO, 1995). Odds ratio values and 95% confidence intervals were also calculated. Biological and biochemical variables were also compared between obese, overweight and control subjects using one-way ANOVA analysis of variance followed by Bonferroni's *post hoc* test. The alpha value considered was set at 0.05, and all *p* values were two-tailed.

## 3. Results

The characteristics of the individuals investigated are shown in Table 1. The non-overweight group showed lower values for body weight, BMI, waist circumference and systolic blood pressure, than did overweight and obese subjects.

Multivariate analysis showed that the non-obese group showed lower weight, BMI, waist circumference and SBP than did the overweight and obese groups.

Genotype and allele frequencies of the Il-1β +3953 polymorphism gene variant in obese and non-overweight subjects are described in Table 2. Allele frequencies for C and T in the +3953 IL-1β polymorphism were 0.76 and 0.24, respectively ($p = 0.812$). Analysis showed no deviation from Hardy–Weinberg equilibrium in the sample investigated.

An analysis comparing the biomarkers between the three different genotypes did not show statistical differences. However, the genotype and allele frequencies of the IL-1β +3953 polymorphism were different between the groups compared here. Residual analysis showed that this difference is due to a reduction in the *CC* genotype and an excess of the *TC* genotype in the non-overweight group ($\chi^2 = 30.619$, $p = 0.0001$) when compared to the overweight and obese groups.

A calculated dose–allele effect confirms this association of CC versus TT plus CT genotypes: $\chi^2 = 27.976$, $p = 0.0001$). The odds ratio showed 1.623 (95% CI: 1.349–1.952) times more chance of the overweight group being *CC* carriers compared to non-overweight and 1.340 (95% CI: 1.119–1.605) times more chance of the obese group being *CC* carriers compared to non-overweight group. Regression analysis confirmed that the association observed was independent of sex (Wald 0.145, $p = 0.704$) and age (Wald 0.156, $p = 0.693$) (Fig. 1).

MS prevalence in sample subjects was 31.7% ($n = 280$). The comparison of genotype frequencies between subjects with and without MS did not show distribution differences [CC = 161 (31.6%), TT = 21 (36.8%), CT = 97 (30.6%), $\chi^2 = 2.541$, $p = 0.281$]. Regression analysis confirmed that the association between obesity and the polymorphism studied here was independent of MS (Wald 0.577, $p = 0.448$).

## 4. Discussion

We describe here an association between the CC genotype of IL-1β +3953 polymorphism and overweight and obesity in a Brazilian sample independent of sex and age. The number of studies reporting associations between DNA sequence variation in specific genes and obesity phenotypes has increased considerably, with 426 findings of positive associations with 127 candidate genes (Rankinen et al., 2006). However, the association between cytokine genes and obesity has been studied less as compared with other candi-

**Table 1**
Baseline values of characteristics of non-overweight, overweight and obese Brazilian subjects.

| Variable | | Normal weight | Overweight | Obese | *p* |
|---|---|---|---|---|---|
| Weight (kg) | Male | 61.4 ± 8.6 | 72.5 ± 8.2 | 80.7 ± 10.3 | 0.0001 |
| | Females | 57.0 ± 11.1 | 66.4 ± 9.9 | 77.1 ± 9.7 | |
| Height (cm) | Males | 160.0 ± 8.8 | 161.2 ± 8.9 | 160.0 ± 7.1 | 0.980 |
| | Females | 153.6 ± 7.3 | 152.7 ± 16.5 | 154.3 ± 6.7 | |
| BMI (kg/m$^2$) | Males | 22.8 ± 1.4 | 27.2 ± 1.3 | 32.3 ± 3.1 | 0.0001 |
| | Females | 22.7 ± 2.5 | 27.5 ± 1.4 | 33.4 ± 3.2 | |
| Waist circumference (cm) | Males | 86.2 ± 17.3 | 95.6 ± 8.2 | 103.1 ± 12.0 | 0.0001 |
| | Females | 84.3 ± 10.7 | 90.2 ± 9.5 | 99.2 ± 11.2 | |
| SBP (mmHg) | Males | 127.2 ± 53.4 | 143.9 ± 27.5 | 146.2 ± 35.9 | 0.024 |
| | Females | 126.8 ± 40.0 | 137.5 ± 35.5 | 140.8 ± 32.5 | |
| DBP (mmHg) | Males | 81.7 ± 22.2 | 80.3 ± 12.3 | 84.3 ± 17.0 | 0.249 |
| | Females | 71.3 ± 17.7 | 76.5 ± 15.1 | 77.6 ± 15.3 | |
| Glucose (mg/dL) | Males | 105.1 ± 39.6 | 103.9 ± 19.2 | 105.9 ± 30.8 | 0.926 |
| | Females | 102.7 ± 27.4 | 106.3 ± 27.4 | 105.7 ± 25.7 | |
| Cholesterol total (mg/dL) | Males | 206.0 ± 53.1 | 206.7 ± 39.2 | 205.4 ± 37.3 | 0.202 |
| | Females | 210.3 ± 38.1 | 217.0 ± 35.3 | 217.1 ± 35.3 | |
| HDL cholesterol (mg/L) | Males | 46.1 ± 8.2 | 41.4 ± 8.8 | 40.9 ± 9.4 | 0.236 |
| | Females | 45.9 ± 10.2 | 46.3 ± 7.1 | 46.3 ± 9.9 | |
| LDL cholesterol (mg/dL) | Males | 128.9 ± 54.4 | 130.6 ± 41.1 | 133.3 ± 38.2 | 0.843 |
| | Females | 136.1 ± 36.1 | 139.9 ± 36.4 | 139.1 ± 35.8 | |
| Triglycerides (mg/dL) | Males | 160.3 ± 60.7 | 168.7 ± 70.9 | 155.8 ± 67.8 | 0.149 |
| | Females | 140.0 ± 66.7 | 152.9 ± 67.5 | 160.6 ± 79.4 | |

BMI: body mass index (kg/m$^2$); normal weight: BMI < 25 kg/m$^2$; overweight: BMI > 25 < 30 kg/m$^2$; obese: BMI ≥ 30 kg/m$^2$; SD: standard deviation; *p*: significant value from one-way ANOVA followed by Bonferroni's *post hoc* test.

date genes, even though cytokines appear to be major regulators of adipose tissue metabolism, especially the IL-1 cytokine family. This family comprises four main members: IL-1α, IL-1β, IL-1 receptor antagonist (IL-1ra) and IL-18. Although IL-1 can upregulate host defenses and act as an immunoadjuvant, the family is primarily considered to be pro-inflammatory. From previous investigations conducted, some evidence suggests that interleukin system polymorphism could to be associated with obesity, such as the IL-1β polymorphism. Therefore, our results corroborated the previous investigation in Caucasian (Strandberg et al., 2006, 2008) and Korean subjects (Um et al., 2004b) as well as the suggestion that the association between IL-1 and body fat regulation in humans is robust and not substantially affected by ethnicity, gender or age (Strandberg et al., 2008). Additionally, from our results we can suggest that this association is independent of other metabolic disorders such as MS.

Considering specifically IL-1β +3953 polymorphism, previous studies indicate that this polymorphism is functional, affecting the production of inflammatory IL-1β levels. Additionally, some studies have suggested an association between IL-1β +3953 polymorphism and obesity (Um et al., 2004a,b; Strandberg et al., 2006, 2008; Lee et al., 2008). All studies described a significant decrease in the IL-1 beta T allele in the overweight and obese when compared to lean subjects. Our results corroborate the data previously published by Strandberg et al. (2006) describing that IL-1β +3953 polymorphism affects fat mass in young men. The general allele frequency was also similar between the study by Strandberg et al. (C = 0.75, T = 0.25) and the present work. This apparent discrepancy indicates a possible effect of intervening variables in the populations investigated, and thus, additional investigations need to be performed to clarify these contradicting results.

**Table 2**
Genotype and allele frequencies of IL-1β +3983 gene polymorphism in non-overweight, overweight and obese subjects.

| Genetic | Groups | | |
|---|---|---|---|
| | Non-overweight | Overweight | Obese |
| Genotype | | | |
| CC | 129 (45.6) | 222 (66.5) | 157 (59.7) |
| TT | 19 (6.7) | 22 (6.6) | 16 (6.1) |
| CT | 135 (47.7) | 90 (26.9) | 90 (34.2) |
| Allele | | | |
| C | 0.694 | 0.799 | 0.768 |
| T | 0.306 | 0.201 | 0.232 |

Non-overweight: BMI < 25 kg/m$^2$; overweight: BMI > 25 < 30 kg/m$^2$; obese: BMI > 30 kg/m$^2$.

In addition, there are studies analyzing the association between obesity and other IL-1 alpha and beta polymorphisms. Carter et al. (2008) studied single nucleotide polymorphisms in the IL-1 gene family IL-1 alpha C-889T and IL-2 beta +3954 in a Western Australian coronary heart disease (CHD) population of 556. The authors described an association between these polymorphisms and larger waist circumference. The study performed by Song et al. (2008) also described a positive association between IL-1α −880C/T polymorphism and obesity in a 182 healthy Korean females with a marked variation in BMI. Investigations analyzing the association between IL-1ra polymorphism and obesity have also been published, demonstrating that serum IL-1ra concentrations are increased in human obesity and that they are under strong genetic control. There are some studies that have described a positive association with such polymorphisms as observed in intron 2 in the IL-1ra gene (Di Renzo et al., 2007), but other investigators did not find this polymorphism to be significantly associated with obesity (Um et al., 2004a,b). Despite the relatively small number of studies, these investigations suggest a positive association between the IL-1 family and obesity, which needs to be better explored in further studies.

Strandberg et al. (2006) suggested that the possible mechanism of the effects of IL-1 could involve mediation of the effects of leptin at the hypothalamic level based on previous studies by Luheshi et al. (1999). Leptin is an adipocyte-derived hormone and cytokine that regulates energy balance through a wide range of functions. Leptin levels increase with adiposity, presumably to inform the brain regarding the quantity of stored fat (Considine et al., 1996; Seth et al., 2008). IL-1β is expressed in the hypothalamus, and its levels are increased by leptin and reduced by fasting. IL-1β has been reported to inhibit adipocyte differentiation from preadipocytes and also to decrease the lipid content of mature adipocytes (Simons et al., 2005). Moreover, IL-1 may increase leptin secretion from preadipocytes and seems essential for inflammation-induced release of leptin from adipose tissue

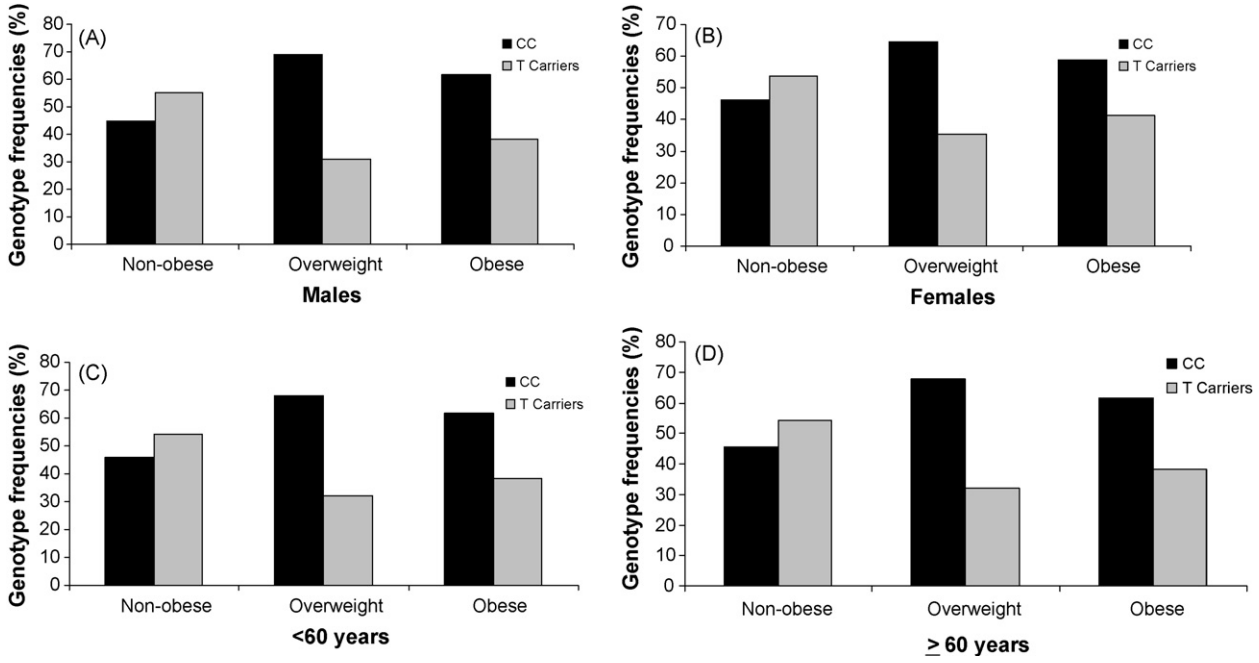


**Fig. 1.** CC and TT + CT distribution comparison of IL-1β +3983 polymorphism between males and females (A and B) and subjects <60 years and subjects ≥60 years (C and D). Sex and age did not show significant differences between genotype frequencies.

(Simons et al., 2005; Faggioni et al., 1998). Additionally, it is important to comment that IL-1 was one of the first cytokines to be implicated in vessel wall inflammation in atherosclerosis, and in facilitating early lesion formation. IL-1 molecules affect leukocyte recruitment and transmigration, allowing it to maintain a pro-inflammatory milieu once inflammation has been initiated (Dinarello, 1997; Libby et al., 1995; Girn et al., 2007).

Interleukin-1β (*IL1B*) gene, part of a cluster of genes on chromosome 2 coding for a family of IL-1 proteins, has been shown to be an important modulator of inflammatory pathways, with potential involvement in the pathogenesis of atherosclerosis and other cardiovascular diseases.

Finally, it is important to ponder some considerations associated with our methodological design. Since obesity is strongly related to other morbidities, investigators looking for an association between gene and non-morbid obesity need to consider these morbidities as intervening variables. In fact, many investigations prefer to include all subjects in the study and perform further exclusions when the statistical analyses are conducted. Of course, that approach increases the sample number and permits more consistent statistical power. However, with the great quantities of variables included in the study and the large number of positive statistical associations sometimes found in these studies, it is then very difficult to discuss the results in biological terms. For these reasons, we selected obese subjects (mainly elderly) without previous history or morbidities such as cancer or other diseases that could influence the results described here. As MS is a condition broadly related to obesity, we did not exclude the affected subjects. However, this additional analysis did not show an interference of MS with the association between CC genotype and obesity.

Additionally, another possible methodological limitation is related to fact that we used in this investigation just BMI to classify the non-overweight, overweight and obese groups. We know that BMI is a non-specific measure that is influenced by fat mass, lean tissue mass and even height (Prentice and Jebb, 2001). However, BMI is a measure largely used in population studies, and several genetic studies have used this marker to compare differences in genetic distribution between obese, overweight and non-overweight subjects (Genelhu et al., 2009; Vimaleswaran et al., 2009; Willer et al., 2009). Since the decreased BMI in IL-1β +3953 T carriers observed here was previously described by Strandberg et al. (2008) and Um et al. (2004a,b), we believe that this association is not a product of chance.

The white adipose tissue (WAT) view has changed over the last decade, from an inert triglyceride storage tissue to a highly active metabolic organ. Indeed, WAT secretes pro-inflammatory cytokines such as interleukin-1 beta, as studied here, and other important molecules, including peptides with hormone-like actions, such as adiponectin, leptin and resistin. An imbalance of these molecules with paracrine actions, caused by functional genetic variations, can contribute to creating or maintaining a systemic inflammatory state, hypertension and insulin resistance, and can also affect central control of food intake. In most obese patients, obesity is associated with a low-grade inflammation of white adipose tissue (WAT) resulting from chronic activation of the innate immune system, which can subsequently lead to insulin resistance, impaired glucose tolerance, and even diabetes (Bastard et al., 2006). These physiological alterations also create conditions that can amplify chronic vascular inflammation, which is the hallmark of atherosclerosis (Girn et al., 2007).

Additionally, it is important to comment on a critical point in genetic studies performed in populations that have different genetic origins such as the Brazilian population. In this study, we analyzed just subjects who reported European ancestry; however, we cannot consider that this population is a stratified group. Some authors have dedicated special attention to the ancestry of European descendents who live in the southern Brazilian region population, such as Alves-Silva et al. (2000), Parra et al. (2003) and Marrero et al. (2005) who observed the occurrence of massive inter-ethnic crosses and remarkable heterogeneity in the 500 years of Brazilian history and underlined the large urban areas such as the Porto Alegre metropolitan area (3.5 million inhabitants). In this population, there were no isolated ethnic groups. For this reason, we consider the sample source as a unique population, discarding possible interference associated with samples from different ethnic groups in the results described here.

In conclusion, the results described here corroborate previous investigations suggesting an associated between IL-1β +3953 polymorphism and obesity. Additional studies are needed to search for possible gene-gene and gene-environmental interactions to clarify how much this genetic variation affects body fat composition.

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