Interleukin 35 Polymorphisms Are Associated with Decreased Risk of Premature Coronary Artery Disease, Metabolic Parameters, and IL-35 Levels: The Genetics of Atherosclerotic Disease (GEA) Study

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Interleukin 35 (IL-35) is a heterodimeric cytokine involved in the development of atherosclerosis. The aim of the present study was to establish if the polymorphisms of IL-12A and EBI3 genes that encode the IL-35 subunits are associated with the development of premature coronary artery disease (CAD) in Mexican individuals. The IL-12A and EBI3 polymorphisms were determined in 1162 patients with premature CAD and 873 controls. Under different models, the EBI3 rs428253 (OR = 0.831, \( P_{\text{add}} = 0.036 \); OR = 0.614, \( P_{\text{rec}} = 0.033 \); OR = 0.591, \( P_{\text{cod}} = 0.027 \)) and IL-12A rs2243115 (OR = 0.674, \( P_{\text{add}} = 0.010 \); OR = 0.676, \( P_{\text{dom}} = 0.014 \); OR = 0.698, \( P_{\text{het}} = 0.027 \); OR = 0.694, \( P_{\text{cod1}} = 0.024 \)) polymorphisms were associated with decreased risk of developing premature CAD. Some polymorphisms were associated with clinical and metabolic parameters. Significant different levels of IL-35 were observed in EBI3 rs4740 and rs4905 genotypes only in the group of healthy controls. In summary, our study suggests that the EBI3 and IL-12A polymorphisms play an important role in decreasing the risk of developing premature CAD; it also demonstrates the relationship of the EBI3 rs4740 and rs4905 genotypes with IL-35 levels in healthy individuals.

1. Introduction

Atherosclerosis is a progressive and multifactorial disease influenced by genetic and environmental factors. A major consequence of the atherosclerosis is the coronary artery disease (CAD). It is well known that inflammation plays an important role in the pathogenesis of atherosclerosis and its complications [1]. The inflammatory phenomenon begins when circulating low density lipoprotein (LDL) particles present in the subendothelial space are oxidized, acquiring proinflammatory properties [2]. Depositions of circulating monocytes/macrophages exacerbate the inflammatory response, because the arterial proteoglycans retain and modify the lipoproteins, increasing their phagocytosis into macrophages. In addition, cell recruitment, production of adhesion molecules, chemokines, and cytokines all cause increased atheroma volume [3]. Aside from the classic cytokines known to be involved in the inflammatory process, a new cytokine, interleukin 35, has recently been described, which also plays a significant role in this phenomenon [4].
Interleukin- (IL-) 35 is a heterodimeric cytokine composed of the Epstein-Barr virus-induced 3 (EBI3) and p35 subunits; it belongs to the IL-6/IL-12 cytokine family that includes IL-12, IL-23, IL-27, and IL-35 molecules [5]. Unlike TGFβ, but similar to IL-10 and IL-27, IL-35 is minimally expressed in human tissues and is mainly induced in inflammatory conditions [4]. Unlike the other members of the IL-12 family, IL-35 is predominantly secreted by regulatory T cells (Treg). As a matter of fact, it has been shown that this cytokine represses T-cell proliferation and function in several in vitro and in vivo disease models [6–8]. Some studies have reported that IL-35 inhibits several inflammatory disorders, such as inflammatory bowel disease [9], autoimmune encephalomyelitis [10], autoimmune diabetes [11], and collagen II-induced arthritis [12]. On the other hand, decreased levels of IL-35 have been reported in patients with acute coronary syndrome (unstable angina pectoris and acute myocardial infarction) compared with a chest pain syndrome group [13]. This finding and the fact that IL-35 is strongly expressed in atherosclerotic plaque [14] suggest that this cytokine could be involved in the development of atherosclerosis. In an animal model, Wang et al. [15] have recently demonstrated the role of IL-35 in the development of atherosclerosis. Apolipoprotein E-deficient (apoE−/−) mice with an established atherosclerotic lesion displayed a lower level of IL-35 compared to the age-matched wild type C57BL/6 mice without plaque. On the other hand, the expression of the IL-35 increased significantly in apoE−/− mice with attenuated plaque.

The IL-12A gene encodes the p35 subunit of IL-35; it is located on chromosome 3q25.33 and consists of seven exons. Several polymorphisms have been described in the IL-12A gene and some of them have been associated with susceptibility to Graves’ and Alzheimer’s disease [16, 17]. The β subunit (EBI3) of IL-35 is encoded by EBI3 gene located on chromosome 19q13.3 and contains 5 exons. Zhang et al. reported that the EBI3 rs428253 polymorphism was associated with decreased risk of development of chronic rhinosinusitis and allergic rhinitis [18, 19]. Currently, no studies have examined the role of the polymorphisms present in the IL-12A and EBI3 genes regarding the susceptibility or protection to the development of CAD. Thus, the aim of the present study was to establish the effect of these polymorphisms in the genetic susceptibility to development of premature CAD in Mexican individuals. Based on the results obtained with a functional prediction analysis, we decided to study four polymorphisms from the IL-12A gene (rs2243115, rs2243123, rs583911, and rs568408) and three from the EBI3 gene (rs428253, rs4740, and rs4905) with possible functional consequences and/or with minor allele frequency > 5%. The IL-12A rs2243115 polymorphism produces binding sites for the transcription factors AP2, LRHI, and SFI, whereas the IL-12A rs568408 polymorphism produces binding sites for microRNAs. Further, the EBI3 rs428253 produces a binding site for LEFI factor and rs4740 for SR proteins. In spite of the fact that the rs4905 (EBI3 gene), rs2243123, and rs583911 (IL-12A gene) polymorphisms were not functional, they were informative (minor allele frequency > 5%) and were therefore included in the study.

2. Materials and Methods

2.1. Subjects. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Instituto Nacional de Cardiología Ignacio Chávez (INCICH). All participants provided written informed consent. The study included 1162 patients with premature CAD and 873 healthy controls belonging to the Genetics of Atherosclerotic Disease (GEA) Mexican Study. Premature CAD was defined as history of myocardial infarction, angioplasty, revascularization surgery, or coronary stenosis > 50% on angiography, diagnosed before age of 55 in men and before age of 65 in women. Controls were apparently healthy asymptomatic individuals without family history of premature CAD, recruited from blood bank donors and through brochures posted in Social Service centers. Chest and abdomen computed tomographies were performed using a 64-channel multidetector helical computed tomography system (Somatom Sensation, Siemens) and interpreted by experienced radiologists. Scans were read to assess and quantify the following: (1) coronary artery calcification (CAC) score using the Agatston method [20] and (2) total adipose tissue (TAT) and subcutaneous and visceral adipose tissue areas (SAT and VAT) as described by Kvist et al. [21]. For the present study, the control group only included individuals with CAC = 0, who were nondiabetic, and with normal glucose levels (n = 873). In the whole sample, the demographic, clinical, anthropometric, and biochemical parameters and cardiovascular risk factors were evaluated and defined as previously described [22–24]. Briefly, hypercholesterolemia was defined as total cholesterol (TC) levels ≥ 200 mg/dL. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or the use of oral antihypertensive therapy. Type 2 diabetes mellitus (T2DM) was defined with a fasting glucose ≥ 126 mg/dL and was also considered when participants reported glucose-lowering treatment or a physician diagnosis of T2DM. Obesity was defined as body mass index (BMI) ≥ 30 kg/m². Hypoalphalipoproteinemia, hypertriglyceridemia, and metabolic syndrome (MS) were defined using the criteria from the American Heart Association, National Heart, Lung, and Blood Institute Scientific Statement [25], except for central obesity that was considered when waist circumference was 90 cm in men and 80 cm in women [26]. Hyperuricemia was considered with a serum uric acid > 6.0 mg/dL and > 7.0 mg/dL for women and men, respectively [27]. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). The presence of insulin resistance was considered when the HOMA-IR values were ≥75th percentile (3.66 in women and 3.38 in men). Hyperinsulinemia was defined when insulin concentration was ≥75th percentile (16.97 μU/mL in women and 15.20 μU/mL in men). Hypoadiponectinemia was defined when adiponectin concentration was ≤25th percentile (8.67 μg/mL in women and 5.30 μg/mL in men). Increased VAT was defined as VAT ≥ 75th percentile (122.0 cm² in women and 151.5 cm² in men) and increased SAT as SAT ≥ 75th percentile (335.5 cm² in women and 221.7 cm² in men). Elevated alanine aminotransferase (ALT) was defined as ALT activity ≥ 75th percentile.
(21.0 IU/L in women and 24.5 IU/L in men). Elevated aspartate aminotransferase (AST) was defined as AST activity ≥ 75th percentile (25 IU/L in women and 28 IU/L in men) and elevated gamma glutamyltransferase (GGT) was defined as GGT ≥ 75th percentile (21.0 IU/L in women and 27.5 IU/L in men). These cutoff points were obtained from a GEA study sample of 131 men and 185 women without obesity and with normal values of blood pressure, fasting glucose, and lipids.

All GEA participants are unrelated and of self-reported Mexican-Mestizo ancestry (three generations). In order to establish the ethnic characteristics of the studied groups, we analyzed 265 ancestry informative markers (AIMs). Using the ADMIXTURE software, the Caucasian, Amerindian, and African backgrounds were determined. Similar background in premature CAD patients and healthy controls was found \( (P > 0.05) \). Patients showed 55.8% of Amerindian ancestry, 34.3% of Caucasian ancestry, and 9.8% of African ancestry, whereas controls showed 54.0% of Amerindian ancestry, 35.8% of Caucasian ancestry, and 10.1% of African ancestry.

### 2.2. IL-35 Levels Determination.
Considering that obesity is frequently associated with a chronic low grade inflammatory process, which could modify the cytokine levels, plasma concentration of IL-35 was determined in a subsample of nonobese subjects with normal values (<3 mg/L) of high sensitivity C reactive protein (hsCRP) (451 premature CAD patients and 458 healthy controls) using a Bioplex system (Bio-Rad, Contra Costa County, State of California, USA) according to manufacturer’s instructions.

### 2.3. Genetic Analysis.
The 5’ exonuclease TaqMan genotyping assays were used to determine the IL-12A (rs2243115, rs568408, rs2243123, and rs583911) and EBI3 (rs428253, rs4740, and rs4905) polymorphisms. The determinations were made on an ABI Prism 7900HT Fast Real-Time PCR system, according to manufacturer’s instructions (Applied Biosystems, Foster City, CA, USA). Samples previously sequenced of the different genotypes of the polymorphisms studied were included as positive controls.

### 2.4. Functional Prediction Analysis.
In order to predict the potential effect of the IL-12A and EBI3 polymorphisms, we used the following bioinformatics tools: FastSNP [28], SNP Function Prediction (http://snpinfo.niehs.nih.gov/snpinfo/), Human-transcriptome Database for Alternative Splicing (http://www.h-invitational.jp/h-dbas/), Splice Port: An Interactive Splice Site Analysis Tool (http://spliceport.ccbh.umd.edu/SplicingAnalyser.html), ESEfinder (http://rulai.cshl.edu/cgi-bin/tools/ESE3/esefinder.cgi), HSF (http://www.umd.be/HSF/), and SNPs3D (http://www.snp3d.org/).

### 2.5. Statistical Analysis.
The analysis was made using the SPSS version 15.0 statistical package (SPSS, Chicago, IL). Means, medians, interquartile ranges, and frequencies were calculated as the case may be. Continuous and categorical variables were analyzed by t-Student’s test, Mann–Whitney U test, Kruskal-Wallis, and Chi square or Fisher test as appropriate. The polymorphism associations with premature CAD and other variables were analyzed using logistic regression under the following inheritance models: additive (major allele homozygotes versus heterozygotes versus minor allele homozygotes), codominant 1 (major allele homozygotes versus heterozygotes), codominant 2 (major allele homozygotes versus minor allele homozygotes), dominant (major allele homozygotes versus heterozygotes + minor allele homozygotes), heterozygous (heterozygotes versus major allele homozygotes + minor allele homozygotes), and recessive (major allele homozygotes + heterozygotes versus minor allele homozygotes). For the EBI3 polymorphisms all the inheritance models were adjusted for age, gender, BMI, current smoking status, ALT, AST, and uric acid. For the IL12A polymorphisms, models were adjusted for age, gender, BMI, and current smoking status. Genotype frequencies did not deviate from Hardy-Weinberg equilibrium in any case (HWE, \( P > 0.05 \)).

### 3. Results

Tables 1 and 2 exhibit the clinical and demographic characteristics of the studied individuals. As we can see, a number of differences were observed between premature CAD patients and healthy controls. As shown in Table 1, the systolic and diastolic blood pressure are both low and within normal limits; however, some of our patients have hypertension (Table 2). The reason for this discrepancy is that some patients with hypertension are under treatment and in consequence their pressure levels were within normal range. As expected, hypercholesterolemia [TC > 200 mg/dL or low density lipoprotein cholesterol (LDL-C) ≥ 130 mg/dL], inflammation [defined as hsCRP levels ≥ 3 mg/L], and current smoking habit were significantly more frequent in controls than in premature CAD patients most likely due to the effect of statin treatment and a life style changes advice after the cardiovascular event.

#### 3.1. Association of the EBI3 and IL-12A Polymorphisms with Premature CAD.
The distribution of the EBI3 (rs4740 and rs4905) and IL-12A (rs2243123, rs568408, and rs583911) polymorphisms was similar in premature CAD and healthy controls. However, under additive, recessive, and codominant 2 models, the EBI3 rs428253 polymorphism was associated with decreased risk of developing premature CAD (\( P_{add} = 0.036, P_{rec} = 0.033, \) and \( P_{cod2} = 0.027 \)). The models were adjusted for age, gender, BMI, current smoking status, ALT, AST, and uric acid. In the same way, the IL-12A rs2243115 (\( P_{add} = 0.010, P_{dom} = 0.014, P_{het} = 0.027, \) and \( P_{cod1} = 0.024 \)) polymorphism was associated with diminished risk of developing premature CAD (Table 3) under different models adjusted for age, gender, BMI, and current smoking status.

#### 3.2. Association of the EBI3 and IL-12A Polymorphisms with Metabolic Parameters.
In premature CAD patients under different models, the EBI3 rs428253 polymorphism was associated with high levels of ALT > p75 (\( P_{add} = 0.006, P_{dom} = 0.004, P_{het} = 0.010, \) and \( P_{cod1} = 0.006 \)) and AST > p75 (\( P_{cod2} = 0.042 \)) and with decreased risk of developing T2DM (\( P_{add} = 0.033, P_{het} = 0.022, \) and \( P_{cod2} = 0.022 \)). The EBI3 rs4905 polymorphism was associated with high levels of ALT > p75...
Table 1: Clinical and metabolic characteristics of the studied groups.

|                        | Control (n = 873) | Premature CAD (n = 1162) | *P  |
|------------------------|-------------------|--------------------------|-----|
| Age (years)            | 51 ± 9            | 54 ± 8                   | <0.001 |
| Gender (% male)        | 40.7              | 81.1                     | <0.001 |
| Body mass index (kg/m²)| 27.3 [24.9–30.2]  | 28.3 [25.9–31.1]         | <0.001 |
| Waist circumference (cm)| 92 ± 11          | 98 ± 10                  | <0.001 |
| Systolic blood pressure (mmHg)| 111 [103–121] | 116 [106–127]           | <0.001 |
| Diastolic blood pressure (mmHg)| 70 [65–76] | 71 [66–78]              | 0.001 |
| Total adipose tissue (cm²)| 416 [330–514] | 425 [340–523]           | 0.147 |
| Visceral adipose tissue (cm²)| 130 [98–172] | 168 [129–215]          | <0.001 |
| Subcutaneous adipose tissue (cm²)| 280 [209–356] | 245 [193–313]         | <0.001 |
| Total cholesterol (mg/dL)| 190 [167–210] | 160 [132–193]           | <0.001 |
| High density lipoprotein cholesterol (mg/dL)| 46 [37–56] | 37 [32–44]            | <0.001 |
| Low density lipoprotein cholesterol (mg/dL)| 116 [95–133] | 91 [68–116]           | <0.001 |
| Triglycerides (mg/dL)| 138 [102–190]    | 162 [119–219]           | <0.001 |
| Non-HDL-cholesterol (mg/dL)| 141 [119–162] | 120 [93–151]           | <0.001 |
| Alanine aminotransferase (IU/L)| 23 [17–32] | 26 [19–36]             | <0.001 |
| Aspartate aminotransferase (IU/L)| 24 [20–30] | 26 [22–31]          | <0.001 |
| Glucose (mg/dL)        | 87 [82–92]       | 95 [87–117]             | <0.001 |
| Insulin (µIU/mL)       | 16 [12–21]       | 20 [15–28]              | <0.001 |
| Homeostasis model assessment of insulin resistance | 3.3 [2.4–4.7] | 5.1 [3.5–7.7]         | <0.001 |
| High sensitivity C reactive protein (mg/L) | 1.4 [0.7–2.9] | 1.2 [0.6–2.6]        | 0.005 |
| Adiponectin (µg/mL)    | 8.5 [5.3–13.6]   | 5.2 [3.2–8.1]           | <0.001 |
| Uric acid (mg/dL)      | 5.3 [4.3–6.3]    | 6.5 [5.4–7.4]           | <0.001 |

Data are shown as mean ± standard deviation, median [interquartile range], or percentage. Comparisons were made using Student’s t-test or Mann–Whitney U test, as appropriate, for continuous variables, and by Chi square analysis for categorical variables. CAD: coronary artery disease.

Table 2: Cardiovascular risk factors prevalence in the study population.

|                        | Control (n = 873) | Premature CAD (n = 1162) | *P  |
|------------------------|-------------------|--------------------------|-----|
| Total cholesterol > 200 mg/dL (%) | 36.3 | 20.3 | <0.001 |
| LDL-cholesterol ≥ 130 mg/dL (%) | 29.2 | 16.1 | <0.001 |
| Hypoalphalipoproteinemia (%) | 49.3 | 67.2 | <0.001 |
| Hypertriglyceridemia (%) | 42.8 | 56.2 | <0.001 |
| Non-HDL-cholesterol > 160 mg/dL (%) | 26.0 | 19.5 | <0.001 |
| Obesity (%) | 26.1 | 35.0 | <0.001 |
| Abdominal obesity (%) | 77.6 | 83.6 | <0.001 |
| Type 2 diabetes mellitus (%) | 0 | 35.4 | <0.001 |
| Hyperinsulinemia (%) | 45.8 | 71.4 | <0.001 |
| Insulin resistance (%) | 44.2 | 77.0 | <0.001 |
| Metabolic syndrome (%) | 29.7 | 71.9 | <0.001 |
| Hypertension (%) | 5.7 | 68.1 | <0.001 |
| High visceral adipose tissue (%) | 49.8 | 64.6 | <0.001 |
| Current smoking status (%) | 23.5 | 11.6 | <0.001 |
| Hypoadiponectinemia (%) | 40.0 | 56.5 | <0.001 |
| High sensitivity C reactive protein ≥ 3 mg/L (%) | 23.6 | 21.3 | 0.114 |
| Hyperuricemia (%) | 16.8 | 35.9 | <0.001 |

Data is shown as percentage. *Comparisons were made using Chi square analysis. CAD: coronary artery disease, LDL: low density lipoprotein, and HDL: high density lipoprotein.

(Pradd = 0.023, Pdom = 0.024, and Pcodl = 0.045). Additionally, the IL-12A rs2243123 polymorphism was associated with increased risk of T2DM (Prrec = 0.021, Pcodl = 0.028), while the rs2243115 polymorphism correlated with reduced risk of metabolic syndrome (Pradd = 0.015, Pdom = 0.017, Phet = 0.022, and Pcodl = 0.021). The rs583911 polymorphism was linked with diminished levels of inflammation (hsCRP ≥ 3 mg/L, Prrec = 0.017), high levels of AST > p75 (Pradd = 0.013, Pdom = 0.046, Prrec = 0.035, and Pcodl = 0.013), and high levels of GGT > p75 (Prec = 0.042) (Table 4).

In healthy controls, the EBI3 rs428253 polymorphism was associated with the presence of hyperuricemia (Prrec = 0.024, Pcodl = 0.032), the EBI3 rs4740 was associated with decreased risk of central obesity (Phet = 0.035, Pcodl = 0.038) and with increased risk of high levels of AST > p75 (Pradd = 0.046, Pdom = 0.014, Phet = 0.015, and Pcodl = 0.011), and the EBI3 rs4905 was linked with reduced risk of central obesity (Prrec = 0.040, Pcodl = 0.046) and increased risk of high levels of AST > p75 (Pdom = 0.020, Phet = 0.020, and Pcodl = 0.016). In addition, we found that the IL-12A rs568408 correlated with decreased risk of metabolic syndrome (Padd = 0.042) and the IL-12A rs583911 was associated with high levels of SAT (Phet = 0.004, Pcodl = 0.017) (Table 5).

3.3 Association of the EBI3 and IL-12A Genotypes with IL-35 Levels. The levels of IL-35 were determined in 451 premature CAD patients and in 458 healthy controls. Individuals with extreme outliers values were not included in the analysis (4 patients and 11 controls). Figure 1 shows that premature CAD patients have significantly higher IL-35 levels than control.
Table 3: Association between EBI3 and IL-12A gene polymorphisms and premature coronary artery disease.

| Polymorphism | Genotype frequency n (%) | MAF | Model | OR [95% CI] | P |
|--------------|--------------------------|-----|-------|-------------|---|
| (i) EBI3*    |                          |     |       |             |   |
| rs428253     | GG                       | 536 (0.614) | G>C     | 0.227       |   |
|              | GC                       | 277 (0.317) | CC       | 0.614       |   |
|              |                          |       | Additive | 0.831 [0.699–0.988] | 0.036 |
|              |                          |       | Dominant | 0.842 [0.681–1.042] | 0.115 |
|              |                          |       | Recessive | 0.614 [0.392–0.963] | 0.033 |
|              | pCAD (n = 1162)          |       | Heterozygote | 0.935 [0.750–1.167] | 0.553 |
|              |                          |       | Codominant 1 | 0.895 [0.715–1.120] | 0.334 |
|              |                          |       | Codominant 2 | 0.591 [0.375–0.933] | 0.027 |
| (ii) IL-12A**|                          |     |       |             |   |
| rs2243115    | TT                       | 746 (0.855) | T>G     | 0.077       |   |
|              | TG                       | 120 (0.137) | GG       | 0.614       |   |
|              |                          |       | Additive | 0.674 [0.499–0.909] | 0.010 |
|              |                          |       | Dominant | 0.676 [0.494–0.925] | 0.014 |
|              |                          |       | Recessive | 0.294 [0.048–1.785] | 0.183 |
|              | pCAD (n = 1162)          |       | Heterozygote | 0.698 [0.508–0.956] | 0.027 |
|              |                          |       | Codominant 1 | 0.694 [0.505–0.954] | 0.024 |
|              |                          |       | Codominant 2 | 0.282 [0.046–1.712] | 0.169 |

* Models were adjusted for age, gender, body mass index, current smoking status, alanine aminotransferase, aspartate aminotransferase, and uric acid. ** Models were adjusted for age, gender, body mass index, and current smoking status. Italic numbers indicate significant associations. The control group subjects were normoglycaemic nondiabetic. MAF: minor allele frequency; pCAD: premature coronary artery disease. Only the significant associated polymorphisms are shown.

4. Discussion

Interleukin-35 is a heterodimeric cytokine that belongs to the IL-6/IL-12 family and is composed of two chains (p35 and EBI3): one encoded by the IL-12A (p35) gene and the other by the EBI3 gene. This cytokine has been associated with the development of several inflammatory diseases. In fact, a recent study on this molecule points out its probable protective role against atherosclerosis [15]. The role of the IL-35 in the inflammatory diseases suggests that the genes that encode its different subunits could be candidates in the study of atherosclerosis and its complications (e.g., CAD). To the best of our knowledge, this is the first study that evaluates the role of IL-12A and EBI3 polymorphisms in premature CAD. In this report, we found that two polymorphisms, namely, EBI3 rs428253 and IL-12A rs2243115, were associated with reduced risk of developing premature CAD. These polymorphisms were also associated with decreased risk of T2DM (EBI3 rs428253) and metabolic syndrome (IL-12A rs2243115) in premature CAD patients. However, in healthy controls only the EBI3 rs428253 correlates with increased risk of hyperuricemia. The polymorphisms that were not linked with risk of premature CAD were associated with other clinical and metabolic parameters. In premature CAD patients, the EBI3 rs4905 was related to high levels of ALT, the IL-12A rs2243123 was associated with increased risk of T2DM, and IL-12A rs583911 correlated with inflammation, high levels of AST, and GGT. In healthy controls, the EBI3 rs4905 and EBI3 rs4740 were associated with low risk of central obesity and increased risk of high levels of AST, whereas the IL-12A rs583911 correlated with high risk of increased SAT and IL-12A rs568408 with diminished risk of metabolic syndrome.
**Table 4**: Association between EBI3 and IL-12A gene polymorphisms and metabolic abnormalities in premature coronary artery disease patients.

| Polymorphism             | Genotype frequency n (%) | MAF        | Model   | OR [95% CI]     | P       |
|--------------------------|---------------------------|------------|---------|-----------------|---------|
| (i) EBI3                 |                           |            |         |                 |         |
| rs428253                 |                           |            |         |                 |         |
| **Alanine aminotransferase > p75** |                       |            | Additive | 1.330 [1.083–1.632] | 0.006   |
| No (n = 590)             | 401 (0.679)               | 167 (0.283) | 22 (0.038) | 0.179 | Dominant | 1.429 [1.121–1.821] | 0.004   |
| Si (n = 572)             | 340 (0.594)               | 204 (0.357) | 28 (0.049) | 0.227 | Heterozygote | 1.392 [1.084–1.787] | 0.010   |
|                         |                           |            |          |                 |         |
| **Aspartate aminotransferase > p75** |                       |            |          |                 |         |
| No (n = 752)             | 489 (0.650)               | 238 (0.316) | 25 (0.034) | 0.191 | Codominant 2 | 1.823 [1.022–3.250] | 0.042   |
| Si (n = 410)             | 251 (0.613)               | 135 (0.328) | 24 (0.059) | 0.223 |          |         |         |
| **Type 2 diabetes mellitus** |                       |            |          |                 |         |
| No (n = 750)             | 458 (0.611)               | 259 (0.345) | 33 (0.044) | 0.217 | Heterozygote | 0.727 [0.554–0.954] | 0.022   |
| Si (n = 412)             | 282 (0.684)               | 112 (0.272) | 18 (0.044) | 0.180 | Codominant 1 | 0.726 [0.522–0.955] | 0.022   |
| rs4905                   |                           |            | Additive | 1.241 [1.031–1.495] | 0.023   |
| **Alanine aminotransferase > p75** |                       |            |          |                 |         |
| No (n = 750)             | 400 (0.532)               | 302 (0.402) | 50 (0.066) | 0.267 | Dominant | 1.309 [1.037–1.653] | 0.024   |
| Si (n = 410)             | 212 (0.517)               | 162 (0.394) | 36 (0.089) | 0.285 |          |         |         |
| (ii) IL-12A              |                           |            |          |                 |         |
| rs2243123                |                           |            |          |                 |         |
| **Type 2 diabetes mellitus** |                       |            |          |                 |         |
| No (n = 327)             | 286 (0.875)               | 40 (0.122)  | 1 (0.003)  | 0.064 | Dominant | 0.590 [0.381–0.912] | 0.017   |
| Si (n = 835)             | 762 (0.913)               | 72 (0.086)  | 1 (0.001)  | 0.044 | Heterozygote | 0.599 [0.386–0.929] | 0.022   |
| rs2243115                |                           |            |          |                 |         |
| **Metabolic syndrome**   |                           |            |          |                 |         |
| No (n = 930)             | 255 (0.274)               | 433 (0.466) | 242 (0.260) | 0.493 | Recessive | 0.633 [0.435–0.921] | 0.017   |
| Si (n = 232)             | 67 (0.287)                | 123 (0.532) | 42 (0.181) | 0.446 |          |         |         |
| rs58391I                 |                           |            | Additive | 0.591 [0.386–0.905] | 0.015   |
| **Inflammation**         |                           |            |          |                 |         |
| No (n = 712)             | 211 (0.296)               | 343 (0.482) | 158 (0.222) | 0.463 | Dominant | 1.318 [1.004–1.730] | 0.046   |
| Si (n = 430)             | 109 (0.242)               | 217 (0.483) | 124 (0.273) | 0.517 | Recessive | 1.344 [1.021–1.769] | 0.035   |
| GGT > p75                |                           |            |          |                 |         |
| No (n = 625)             | 178 (0.285)               | 309 (0.494) | 138 (0.221) | 0.468 | Recessive | 1.329 [1.011–1.748] | 0.042   |
| Si (n = 537)             | 141 (0.263)               | 251 (0.468) | 145 (0.269) | 0.504 |          |         |         |

Table shows the models with significant associations. Models were adjusted for age, gender, and body mass index. MAF: minor allele frequency; GGT: gamma-glutamyl transferase.
Table 5: Association between EBI3 and IL-12A gene polymorphisms and metabolic abnormalities in the control group.

| Polymorphism | Genotype frequency n (%) | MAF | Model | OR [95% CI] | P  |
|--------------|--------------------------|-----|-------|-------------|----|
| (i) EBI3     |                          |     |       |             |    |
| rs428253     |                          |     |       |             |    |
| Hyperuricemia| GG                       | 454 (0.625) | 220 (0.303) | 52 (0.072) | 0.223 Heterozygote | 1.595 [1.064–2.389] | 0.024 |
|              | GC                       | 82 (0.555)  | 57 (0.390)  | 8 (0.055)  | 0.248 Codominant 1 | 1.567 [1.038–2.365] | 0.032 |
| Central obesity| G>C                     |     |       |             |    |
| rs4740       | GG                       | 99 (0.503)  | 78 (0.400)  | 19 (0.097) | 0.296 Heterozygote | 0.391 [0.163–0.937] | 0.035 |
|              | GA                       | 377 (0.557) | 251 (0.371) | 49 (0.072) | 0.258 Codominant 2 | 0.386 [0.157–0.949] | 0.038 |
| rs4905       | AA                       | 99 (0.503)  | 78 (0.400)  | 19 (0.097) | 0.296 Recessive    | 0.404 [0.170–0.960] | 0.040 |
|              | AG                       | 374 (0.552) | 253 (0.371) | 50 (0.074) | 0.261 Codominant 2 | 0.403 [0.165–0.983] | 0.046 |
| Metabolic syndrome| A>G                    |     |       |             |    |
| rs568408     | GG                       | 542 (0.883) | 69 (0.112)  | 3 (0.005)  | 0.061 Additive     | 0.583 [0.347–0.981] | 0.042 |
|              | GA                       | 237 (0.915) | 21 (0.081)  | 1 (0.004)  | 0.044             |                     |    |
| rs583911     | AA                       | 129 (0.268) | 222 (0.459) | 132 (0.273) | 0.503 Heterozygote | 1.776 [1.203–2.622] | 0.004 |
|              | AG                       | 88 (0.226)  | 220 (0.563) | 82 (0.211) | 0.492 Codominant 1 | 1.776 [1.107–2.849] | 0.017 |
| SAT > p75    |                          |     |       |             |    |
| No (n = 196) | 99 (0.503)  | 78 (0.400)  | 19 (0.097) | 0.296       |                    |                     |    |
| Si (n = 677) | 377 (0.557) | 251 (0.371) | 49 (0.072) | 0.258       |                    |                     |    |
| No (n = 567) | 327 (0.576) | 197 (0.348) | 43 (0.076) | 0.250       |                    |                     |    |
| Si (n = 306) | 148 (0.485) | 133 (0.433) | 25 (0.082) | 0.299       |                    |                     |    |

Table shows the models with significant associations. Models were adjusted for age, gender, and body mass index. MAF: minor allele frequency, AST: aspartate aminotransferase, and SAT: subcutaneous adipose tissue.

According to the informatics tools, the two polymorphisms, which were associated with decreased risk of developing premature CAD, have a possible functional effect. Specifically, the EBI3 rs428253 modifies a binding site for the lymphoid enhancer-binding factor 1 (LEF1) that is a decisive transcription factor in the control of the granulopoiesis proliferation, proper lineage commitment, and granulocytic differentiation [29]. Furthermore, the IL-12A rs2243115 polymorphism, located in the promoter region, produces binding sites for the transcription factors AP2, LRH1, and SFI. Thus, after considering that the studied polymorphisms could have an effect in the production of IL-35, we analyzed the molecule serum levels in a group of premature CAD patients and healthy controls. Coronary patients showed significantly higher IL-35 levels than control subjects; however, the difference was small. We cannot define whether these differences could have an effect on the development of atherosclerosis. As we know, atherosclerosis is a multifactorial disease and multiple cytokines, both pro- and anti-inflammatory, play a role in the genesis and progression of the inflammatory process. In this analysis, neither EBI3 rs428253 nor IL-12A rs2243115 (the polymorphisms associated with premature CAD with
Table 6: Interleukin 35 plasma concentrations in the study groups according to the EBI3 and IL-12A polymorphisms.

| Polymorphism | Genotype | Controls (n = 447) | p* | pCAD (n = 447) | p |
|--------------|----------|-------------------|----|---------------|---|
| (i) EBI3     |          |                   |    |               |   |
| rs428253     | GG       | 275 2.72 [0.88–4.97] | 286 | 3.16 [1.03–6.51] | 0.433 |
|              | GC       | 151 3.00 [1.62–5.23] | 0.273 | 140 | 3.40 [1.19–7.76] |
|              | CC       | 21 1.98 [0.19–5.10] | 21 | 2.20 [1.63–3.78] |   |
| rs4740       | GG       | 250 2.52 [0.88–4.50] | 233 | 3.23 [1.62–7.61] | 0.311 |
|              | GA       | 160 3.00 [1.63–5.23] | 0.020 | 140 | 3.40 [1.19–7.76] |
|              | AA       | 37 3.40 [0.88–7.90] | 29 | 3.16 [1.62–5.23] |   |
| rs4905       | AA       | 248 2.52 [0.88–4.40] | 233 | 3.23 [1.62–7.61] | 0.338 |
|              | AG       | 164 3.00 [1.63–5.23] | 0.017 | 186 | 3.16 [0.95–6.15] |
|              | GG       | 35 3.78 [0.88–7.90] | 28 | 3.08 [1.62–5.23] |   |
| (ii) IL12A   |          |                   |    |               |   |
| rs2243115    | TT       | 389 2.72 [0.88–4.97] | 407 | 3.16 [1.62–6.44] |   |
|              | TG + GG  | 58 3.08 [0.83–5.23] | 0.376 | 40 | 3.56 [0.38–7.95] |
| rs568408     | GG       | 398 2.81 [0.88–4.97] | 396 | 3.23 [1.62–6.74] |
|              | GA + AA  | 49 2.32 [0.31–7.17] | 0.763 | 51 | 3.56 [0.38–7.95] |
| rs2243123    | TT       | 188 2.72 [0.59–4.97] | 183 | 3.40 [1.03–7.09] | 0.254 |
|              | TC       | 198 2.90 [0.88–5.23] | 0.702 | 190 | 3.00 [1.62–6.31] |
|              | CC       | 61 3.40 [0.88–7.90] | 74 | 3.32 [1.47–8.38] |
| rs583911     | AA       | 113 3.00 [0.88–5.23] | 130 | 3.23 [1.30–8.56] |
|              | AG       | 224 2.90 [0.88–5.23] | 0.570 | 194 | 3.00 [0.95–6.19] |
|              | GG       | 110 2.46 [0.88–4.82] | 123 | 3.40 [1.62–7.09] |

Data are shown as median [interquartile range]. Comparisons were made using Mann–Whitney U test or Kruskal-Wallis test as appropriate. italic numbers indicate significant associations.
pCAD: premature coronary artery disease.

as a possible functional effect) showed a correlation with IL-35 serum levels. The fact that the associated polymorphisms with decreased risk of developing premature CAD did not correlate with IL-35 levels could be explained considering that the production of IL-35 and other molecules is a complex mechanism that involves not only changes at DNA level but also epigenetics modifications. Moreover, it is important to consider that in our study the levels of IL-35 were measured in circulation and not at the lesion site. On the other hand, the EBI3 rs4740 and rs4905 polymorphisms were associated with different levels of IL-35. Furthermore, this association was observed only in the healthy control groups. From these two polymorphisms, only EBI3 rs4740 was functional according to the informatics tools. Interestingly, this polymorphism produces binding sites for Srp40, and SRp55, which belong to the family of SR proteins that regulate alternative splicing [30].

IL-35 is a heterodimeric cytokine that belongs to the IL-6/IL-12 cytokine family, which includes IL-12, IL-23, IL-27, and IL-35 molecules. These cytokines share subunits that are encoded by EBI3, IL-12A, IL-12B, IL-23A, and IL27p28 genes. Our research group is studying several polymorphisms located in these genes in order to establish its role in the genetic susceptibility to developing premature CAD and cardiovascular risk factors. At the moment, we have analyzed the polymorphisms of the IL27p28 gene that encode the p28 subunit of the IL-27. This analysis showed that two polymorphisms of this gene (rs26528 and rs40837) were significantly associated with a lower risk of premature CAD. Using the luciferase assay we demonstrate that the rs40837 polymorphism has a functional effect. In this study, we also determined independently the levels of IL-27. None of the studied polymorphisms were associated with IL-27 levels (personal communication).

IL-12A polymorphisms have been associated with the development of several diseases, such as rheumatoid arthritis [31], Alzheimer’s disease [17], Graves’ disease [16], and asthma [32]. In contrast, EBI3 polymorphisms have been associated with ulcerative colitis [33], pulmonary tuberculosis [34], chronic rhinosinusitis [19], and allergic rhinitis [18]. In these studies, IL-12A and EBI3 genes were analyzed independently. To the best of our knowledge, no studies so far have reported an analysis, in which both genes have been analyzed in concert for any disease.

As for the limitations, herein, we have only included the study of four polymorphisms of IL-12A and three of the EBI3 gene, which seem to be functional and/or informative based on the analysis of the prediction software results. Since this is the first work that documents the correlation of the IL-35 polymorphisms with premature CAD, and cardiovascular parameters, further studies in an independent group of patients are mandatory to validate the results. It is important
to note that one strength of our work is that the control group only included individuals without subclinical atherosclerosis (i.e., individuals without coronary artery calcification).

5. Conclusion
In summary, our results indicate that there exists a statistically significant association between the EBI3 rs428253 and IL-12A rs2243115 polymorphisms and a reduced risk of developing premature CAD. Some of the studied polymorphisms were associated with cardiovascular parameters. The EBI3 rs4740 and EBI3 rs4905 genotypes were associated with a variation in IL-35 serum levels in healthy controls. To the best of our knowledge, this is the first study that evaluates the role of IL-12A and EBI3 polymorphisms in premature CAD. For this reason, the detected associations are not yet definitive, and replicate studies in independent populations are warranted to confirm these findings.

Competing Interests
The authors declare that there are no competing interests regarding the publication of this article.

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References
[1] G. K. Hansson, “Mechanisms of disease: inflammation, atherosclerosis, and coronary artery disease,” New England Journal of Medicine, vol. 352, no. 16, pp. 1626–1695, 2005.
[2] S. Tsimikas and Y. I. Miller, “Oxidative modification of lipoproteins: mechanisms, role in inflammation and potential clinical applications in cardiovascular disease,” Current Pharmaceutical Design, vol. 17, no. 1, pp. 27–37, 2011.
[3] D. Tousoulis, E. Oikonomou, E. K. Economou, F. Crea, and J. C. Kaski, “Inflammatory cytokines in atherosclerosis: current therapeutic approaches,” European Heart Journal, vol. 37, no. 22, pp. 1723–1732, 2016.
[4] X. Li, J. Mai, A. Virtue et al., “IL-35 is a novel responsive anti-inflammatory cytokine—a new system of categorizing anti-inflammatory cytokines,” PLoS ONE, vol. 7, no. 3, Article ID e33628, 2012.
[5] D. A. A. Vignali and V. K. Kuchroo, “IL-12 family cytokines: immunological playmakers,” Nature Immunology, vol. 13, no. 8, pp. 722–728, 2012.
[6] L. W. Collison, C. J. Workman, T. T. Kuo et al., “The inhibitory cytokine IL-35 contributes to regulatory T-cell function,” Nature, vol. 450, no. 7169, pp. 566–569, 2007.
[7] L. W. Collison, M. R. Pillai, V. Chaturvedi, and D. A. A. Vignali, “Regulatory T cell suppression is potentiated by target T cells in a cell contact, IL-35- and IL-10-dependent manner,” Journal of Immunology, vol. 182, no. 10, pp. 6121–6128, 2009.
[8] M. R. Pillai, L. W. Collison, X. Wang et al., “The plasticity of regulatory T cell function,” Journal of Immunology, vol. 187, no. 10, pp. 4987–4997, 2011.
[9] S. Wirtz, U. Billmeier, T. McHeddldz, R. S. Blumberg, and M. F. Neurath, “Interleukin-35 mediates mucosal immune responses that protect against T-cell-dependent colitis,” Gastroenterology, vol. 141, no. 5, pp. 1875–1886, 2011.
[10] J.-Q. Liu, Z. Liu, X. Zhang et al., “Increased Th17 and regulatory T cell responses in EBV-induced gene 3-deficient mice lead to marginally enhanced development of autoimmune encephalomyelitis,” Journal of Immunology, vol. 188, no. 7, pp. 3099–3106, 2012.
[11] M. Bettini, A. H. Castellaw, G. P. Lennon, A. R. Burton, and D. A. A. Vignali, “Prevention of autoimmune diabetes by ectopic pancreatic β-cell expression of interleukin-35,” Diabetes, vol. 61, no. 6, pp. 1519–1526, 2012.
[12] W. Niedbala, X.-Q. Wei, B. Cai et al., “IL-35 is a novel cytokine with therapeutic effects against collagen-induced arthritis through the expansion of regulatory T cells and suppression of Th17 cells,” European Journal of Immunology, vol. 37, no. 11, pp. 3021–3029, 2007.
[13] Y. Lin, Y. Huang, Z. Lu et al., “Decreased plasma IL-35 levels are related to the left ventricular ejection fraction in coronary artery diseases,” PLoS ONE, vol. 7, no. 12, Article ID e52490, 2012.
[14] S. Kempe, P. Heinz, E. Kokai, O. Devergne, N. Marx, and T. Wirth, “Epstein-barr virus-induced gene-3 is expressed in human atheroma plaques,” American Journal of Pathology, vol. 175, no. 1, pp. 440–447, 2009.
[15] B. Wang, S. Dai, Z. Dong et al., “The modulation of endoplasmic reticulum stress by chemical chaperone upregulates immune negative cytokine IL-35 in apolipoprotein E-deficient mice,” PLoS ONE, vol. 9, no. 1, Article ID e87787, 2014.
[16] T. Guo, S. Yang, N. Liu, S. Wang, B. Cui, and G. Ning, “Association study of interleukin-12A gene polymorphisms with Graves’ disease in two Chinese populations,” Clinical Endocrinology, vol. 74, no. 1, pp. 125–129, 2011.
[17] X.-C. Zhu, L. Tan, T. Jiang, M.-S. Tan, W. Zhang, and J.-T. Yu, “Association of IL-12A and IL-12B polymorphisms with Alzheimer’s disease susceptibility in a Han Chinese population,” Journal of Neuroimmunology, vol. 274, no. 1-2, pp. 180–184, 2014.
[18] Y. Zhang, S. Duan, X. Wei, Y. Zhao, L. Zhao, and L. Zhang, “Association between polymorphisms in FOXP3 and EBI3 genes and the risk for development of allergic rhinitis in Chinese subjects,” Human Immunology, vol. 73, no. 9, pp. 939–945, 2012.
[19] Y. Zhang, C. Wang, Y. Zhao, and L. Zhang, “Some polymorphisms in Epstein-Barr virus-induced gene 3 modify the risk for chronic rhinosinusitis,” American Journal of Rhinology and Allergy, vol. 27, no. 2, pp. 91–97, 2013.
[20] G. C. Mautner, S. L. Mautner, J. Froehlich et al., “Coronary artery calcification: assessment with electron beam CT and histomorphometric correlation,” Radiology, vol. 192, no. 3, pp. 619–623, 1994.
[21] H. Kvist, B. Chowdhury, U. Grangård, U. Tylén, and L. Sjöström, “Total and visceral adipose-tissue volumes derived from measurements with computed tomography in adult men and women: predictive equations,” The American Journal of Clinical Nutrition, vol. 48, no. 6, pp. 1351–1361, 1988.
[22] R. Posadas-Sánchez, W. A. Ocampo-Arcos, A. R. López-Uribe et al., “Asociación del ácido úrico con factores de riesgo cardiovascular y aterosclerosis subclínica en adultos mexicanos,” Revista
[23] A. Medina-Urrutia, C. Posadas-Romero, R. Posadas-Sánchez et al., “Role of adiponectin and free fatty acids on the association between abdominal visceral fat and insulin resistance,” Cardiovascular Diabetology, vol. 14, no. 1, article no. 20, 2015.

[24] R. Posadas-Sánchez, A. R. López-UrIBE, C. Posadas-Romero et al., “Association of the I148M/PNPLA3 (rs738409) polymorphism with premature coronary artery disease, fatty liver, and insulin resistance in type 2 diabetic patients and healthy controls. The GEA Study,” Immunobiology, 2016.

[25] S. M. Grundy, J. I. Cleeman, S. R. Daniels et al., “Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement,” Circulation, vol. 112, no. 17, pp. 2735–2752, 2005.

[26] C. P. Sánchez-Castillo, O. Velázquez-Monroy, A. Berber, A. Lara-Esqueda, R. Tapia-CONyer, and W. P. T. James, “Anthropometric cutoff points for predicting chronic diseases in the Mexican National Health Survey 2000,” Obesity Research, vol. 11, no. 3, pp. 442–451, 2003.

[27] A. O. Ogbera and A. O. Azenabor, “Hyperuricaemia and the metabolic syndrome in type 2 DM,” Diabetology and Metabolic Syndrome, vol. 2, no. 1, article no. 24, 2010.

[28] H.-Y. Yuan, J.-J. Chiou, W.-H. Tsen et al., “FASTSNP: an always up-to-date and extendable service for SNP function analysis and prioritization,” Nucleic Acids Research, vol. 34, pp. W635–W641, 2006.

[29] J. Skokowa, M. Klimiankou, O. Klimenkova et al., “Interactions among HCLS1, HAX1 and LIF-1 proteins are essential for G-CSF-triggered granulopoiesis,” Nature Medicine, vol. 18, no. 10, pp. 1550–1559, 2012.

[30] A. Sureau, R. Gattoni, Y. Dooghe, J. Stévenin, and J. Soret, “SC35 autoregulates its expression by promoting splicing events that destabilize its mRNAs,” EMBO Journal, vol. 20, no. 7, pp. 1785–1796, 2001.

[31] L. Shen, H. Zhang, X. Zhou, and R. Liu, “Association between polymorphisms of interleukin 12 and rheumatoid arthritis associated biomarkers in a Chinese population,” Cytokine, vol. 76, no. 2, pp. 363–367, 2015.

[32] T. Chen, W. Liang, L. Gao et al., “Association of single nucleotide polymorphisms in interleukin 12 (IL-12A and -B) with asthma in a Chinese population,” Human Immunology, vol. 72, no. 7, pp. 603–606, 2011.

[33] J. K. Yamamoto-Furusuo, R. Posadas-Sánchez, E. Alvarez-León, and G. Vargas-Alarcón, “Protective role of Interleukin 27 (IL-27) gene polymorphisms in patients with ulcerative colitis,” Immunology Letters, vol. 172, pp. 79–83, 2016.

[34] R. Zheng, H. Liu, P. Song et al., “Epstein-Barr virus-induced gene 3 (EBI3) polymorphisms and expression are associated with susceptibility to pulmonary tuberculosis,” Tuberculosis, vol. 95, no. 4, pp. 497–504, 2015.