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# Polymorphisms in IL-10 and INF-γ genes are associated with early atherosclerosis in coronary but not in carotid arteries: A study of 122 autopsy cases of young adults

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ABSTRACT

Atherosclerosis is a complex disease, involving both genetic and environmental factors. However, the influence of genetic variations on its early development remains unclear. This study examined the association of 12 different polymorphisms with atherosclerosis severity in anterior descending coronary (DA, $n = 103$) and carotid arteries (CA, $n = 66$) of autopsied young adults (<30 years old). Histological sections (H-E) were classified according to the American Heart Association. Polymorphisms in ACE, TNF-α (−308G/A and −238 G/A), IFN-γ (+874 A/T), MMP-9 (−1562 C/T), IL-10 (−1082 A/G and −819 C/T), NOS3 (894 G/T), ApoA1 (rs964184), ApoE (E2E3E4 isoforms), and TGF-β (codons 25 and 10) genes were genotyped by gel electrophoresis or automatic DNA sequencing. Firearm projectile or car accident was the main cause of death, and no information about classical risk factors was available. Histological analysis showed high prevalence of type III atherosclerotic lesions in both DA (69%) and CA (39%) arteries, while severe type IV and V lesions were observed in 14% (DA) and 33% (CA). Allele frequencies and genotype distributions were determined. Among the polymorphisms studied, IFN-γ and IL-10 (−1082 A/G) were related to atherosclerosis severity in DA artery. No association between genotypes and lesion severity was found in CA. In conclusion, we observed that the high prevalence of early atherosclerosis in young adults is associated with IFN-γ ($p < 0.001$) and IL-10 ($p = 0.013$) genotypes. This association is blood vessel dependent. Our findings suggest that the vascular system presents site specialization, and specific genetic variations may provide future biomarkers for early disease identification.



## 1. Introduction

Atherosclerosis is the common underlying mechanism of ischemic heart disease and stroke, the worldwide leading mortality causes (WHO, 2011). This multifocal vascular complex disease evolves from interactions among various genetic and environmental factors [1]. Besides the classical risk factors for cardiovascular disease (*e.g.*, hypertension, cigarette smoking, diabetes, and overweight), abnormal lipid metabolism, endothelial dysfunction, and inflammation are major factors in the development of atherosclerotic lesions. Inflammation markers are also identified as "emerging risk factors" for cardiovascular disease [2].

Although atherosclerosis begins early in life, the clinical manifestation of the disease appears only later. Early clinical manifestation represents only 10% of the cases, but the prevalence of pre-clinic atherosclerotic manifestation in young adults is much higher [3,4]. Advances in molecular genetics highlight the role of genetic polymorphisms in the disease pathogenesis [1,5–11]. Previous studies report an association between atherosclerosis severity in young adults, without classical risk factor for coronary disease, and SNPs in genes related to lipid metabolism, as well as in cytokine genes [4,12]. However, the contribution of genetic variation to atherosclerosis remains unclear. We ponder that the young adult population is an interesting group to study the influence of genetic polymorphisms on atherosclerosis since they have been less exposed to classical environmental risk factors in comparison to older adults. Thus, the present study aimed to investigate the association between genetic

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variation with early atherosclerotic manifestation. To this end, polymorphisms in twelve protein-coding genes involved in different pathways related to atherosclerosis pathogenesis were analyzed and correlated with the presence and severity of atherosclerotic lesion in both coronary and carotid arteries of autopsied young adults. Polymorphisms were selected based on their involvement in injury mechanisms, such as angiotensin-converting enzyme (ACE), Th-1 and Th-2 inflammatory response (TNF-α: −308G/A and −238 G/A, IFN-γ: +874 A/T, and IL-10: −1082 A/G and −819 C/T, respectively), vascular function and remodeling (NOS3: 894 G/T, MMP-9: −1562 C/T, TGF-β codons 25 and 10), and lipid metabolism (ApoA1: rs964184, ApoE: E2E3E4 isoforms). The results showed associations between IL-10 and IFN-γ genotypes with atherosclerotic lesion severity in coronary, but not in carotid arteries.

## 2. Materials and methods

### 2.1. Population

The population studied consisted of consecutive cases of individuals, males and females, under 30 years of age who died of external causes (accidents or homicides) and were autopsied at Rio de Janeiro's Institute of Legal Medical within 18 h after death. Age, skin color (rated as light, intermediate or dark), and physical feature information were obtained by direct observation of the body. Data from 122 cases, from which the extraction of muscle DNA and histopathological evaluation of at least one artery were available, were analyzed. This study was approved by local Ethics Committee of the Instituto Médico Legal Afrânio Peixoto (Rio de Janeiro, Brazil).

### 2.2. Arterial specimens

Segments of approximately 5 cm from anterior descending coronary (DA, $n = 103$) and carotid (CA, $n = 66$) arteries were removed at autopsy and fixed with 10% buffered formaldehyde. Carotid artery samples were obtained just below common carotid artery bifurcation. Fragments of the fixed tissues were embedded in paraffin, and 4-μm-thick slices were stained with hematoxylin-eosin (H-E). Artery wall morphology was examined in each sample at ×100 and ×400 magnification on a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). Samples were classified for atherosclerotic lesion severity accordingly to the American Heart Association, where types I–VI are characterized as follows: *type I*, isolated lipid containing macrophages (*foam cells*), without extracellular lipid deposition; *type II*, multiple foam cell layers; *type III*, isolated extracellular lipid pools; *type IV*, confluent extracellular lipid (*core* formation); *type V*, *core* with fibromuscular tissue production or calcification; *type VI*, plaque with surface rupture, hematoma, or thrombosis [13,14]. Two independent observers made the histopathological analysis in a blinded manner.

### 2.3. DNA extraction and genotyping

Skeletal muscle samples were collected at autopsy and stored at −20 °C. Tissue samples were digested overnight in 0.2 μL proteinase K buffer (20 mg/mL) at 37 °C followed by DNA extraction with phenol-chloroform. After ethanol precipitation, DNA was collected, resuspended in distilled water (60 μL), and stored at −20 °C. The final DNA concentration was measured by spectrophotometry at 260 nm and aliquoted at 200 ng/μL.

ACE (ins/del intron 16), IFN-γ (+874 T/A), TNF-α (−238 G/A and −308 G/A), IL-10 (−819 C/T and −1082 A/G, rs1800871 and rs1800896, respectively), MMP-9 (−1562 C/T), NOS3 (+894G/T, rs1799983), ApoA1 (rs964184), and TGF-β (codon 25 and 10) gene polymorphisms were genotyped by sequencing PCR products with specific primers, as shown in Table 1. The amplified products were sequenced using the DYEnamicTM ET Dye Terminator Cycle Sequencing kit for MegaBACE (Amersham Pharmacia Biotech Inc). ACE genotypes were analyzed by agarose gel (1%) electrophoresis, where deleted (D) and insertion (I) alleles were identified as 190 bp and 490 bp fragment, respectively. ApoE genotyping (E2E3E4 isoforms) was determined by 5′-nuclease assay and fluorogenic allele-specific TaqMan assay (TaqMan Pre-Designed SNP Genotyping Assays, cat no. C3084793-20 and C904973-10, Applied Biosystems), following the manufacturer's instructions and using the 7500 Real-Time PCR (Applied Biosystems, USA). Sequencing analysis was performed using Geneious 4.7.5 (Biomatters, http://www.geneious.com).

### 2.4. Statistical analysis

The SPSS v15 statistical software package (Chicago, IL, USA) was used. The chi-square or Fisher's exact method was used to determine deviations from Hardy–Weinberg equilibrium and to investigate possible association between genotype or allele frequencies and histological atherosclerotic lesion severity. In all instances, the significance level was set at 5% ($p < 0.05$).

## 3. Results

The population characteristics are shown in Table 2. Population mean age was 22 ± 5.4 years old [112 males (92%) and 10 females], and the *causa mortis* mainly resulted from firearm projectile. Ninety three percent of subjects had normal or low body weight.

The severity of atherosclerotic lesions was assessed by H-E histological analysis that showed high prevalence of atherosclerotic lesions in

**Table 1**
Gene polymorphisms studied, primer sequences, and amplification conditions.

| Gene | Chr | SNPs | Pair of primers (5′–3′) | PCR conditions |
|---|---|---|---|---|
| ACE | 17 | 287 bp Ins/Del in intron 16 | F: CTGGAGACCACTCCCATCCTTTCT<br>R:GATGTGGCCATCACATTCGTCAGA | 94 °C—1 m (30 cycles), 58 °C—1 m, 72 °C—2 m |
| TNF-α | 6 | −308G/A<br>−238G/A | F: TCCTGCATCCTGTCTGGAAGT<br>R: AGGGAGCGTCTGCTGGCTGGGTG | 94 °C—30 s (35 cycles), 60 °C—45 s, 72 °C—1 m |
| IFN-γ | 12 | +874A/T | F: GGAACTTCGTTGCTCACTGGG<br>R: CTATTACATCTACTGTGC CTTCCTG | 94 °C—30 s (35 cycles), 58 °C—45 s, 72 °C—1 m |
| IL-10 | 1 | −819 T/C (rs1800871)<br>−1082A/G (rs1800896) | F: CTC GCT GCA ACC CAA CTG GC<br>R: CCT AGG TCA CAG TGA CGT GG | 94 °C—30 s (35 cycles), 55 °C—45 s, 72 °C—1 m |
| MMP-9 | 20 | −1562C/T | F: AATGCCTGGCACATAGTAGGCCCT<br>R: CTTTCTTCCTAGCCAGCCGGCATC | 95 °C—1 m (35 cycles), 62 °C—1 m, 72 °C—1 m |
| NOS3 | 7 | +894G/T (rs1799983) | F: AAGGCAGGAGACAGTGGATGGA<br>R: CCCAGTCAATCCCTTTGGTGCTCA | 94 °C—30 s (30 cycles), 60 °C—45 s, 72 °C—30 s |
| ApoA1 | 11 | G/C (rs964184) | F: AGATACCCACACAGCTCACTCCC<br>R: CAGCACTGGCCTCTGTATTGACC | 94 °C—30 s (30 cycles), 60 °C—45 s, 72 °C—30 s |
| ApoE | 19 | E2E3E4 isoforms | TaqMan assay | |
| TGF-β | | Codon 25 (G/C)<br>Codon 10 (T/C) | F: GCCCTTCTCCCTGAGGACCTC<br>R: GGCGAGCCGCAGCTTGGACAG | 94 °C—30 s (30 cycles), 60 °C—45 s, 72 °C—1 m |

**Table 2**
Population characteristics.

| Characteristic | Number of cases (%) |
|---|---|
| *Mean age* | 22 ± 5.4 years |
| *Gender* | |
| Male | 112 (92%) |
| Female | 10 (8%) |
| *Physical constitution* | |
| Slim | 40 (33%) |
| Regular | 71 (58%) |
| Obese | 11 (9%) |
| *Skin color* | |
| Light | 28 (23%) |
| Dark | 30 (25%) |
| Intermediate | 64 (52%) |

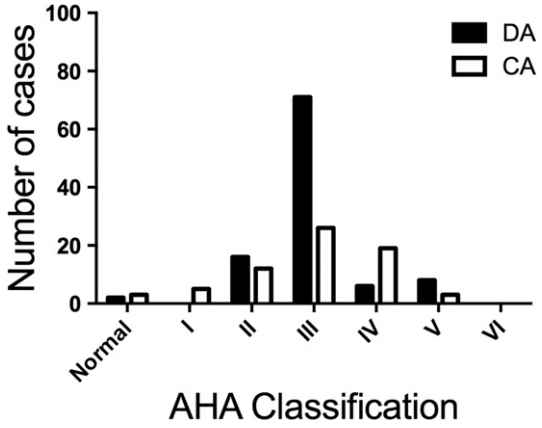


**Fig. 2.** Histological classification [according to the American Heart Association (AHA)] of atherosclerotic lesions in anterior descending coronary (DA) and carotid (CA) arteries from autopsied cases of young adults.

both DA and CA arteries, mainly type III, with only two and three cases classified as normal, respectively (Figs. 1 and 2). Despite a young age population, we observed severe atherosclerotic lesions (types IV and V) in 14% and 33% of DA and CA arteries, respectively.

Table 3 shows the allele frequencies for the genotypes studied. No deviation from Hardy–Weinberg equilibrium was observed. Tables 4 and 5 depict the distribution and association of genotypes with severity of histologic atherosclerosis in coronary and carotid arteries, respectively. Lesion severity in coronary arteries was associated with polymorphisms of IL-10 (−1082 A/G) and IFN-γ (+874 T/A) (Tables 4 and 5, respectively). On the contrary, no association between genotypes and lesion severity was observed for carotid arteries.

IL-10 levels are influenced by three single nucleotide polymorphisms (SNPs: −1082 A/G, −819 C/T and −592 C/A) of the corresponding gene, originating three haplotypes (GCC, ACC, and ATA). Based on these haplotypes, individuals are classified as follows: high IL-10 producers (GCC/GCC), intermediate (GCC/ACC, GCC/ATA), and low producers (ACC/ACC, ACC/ATA, and ATA/ATA). In our sample, IL-10 −1082 G/A and −819 C/T genotypes were found in 48 and 50 cases, respectively. We determined −592 C/A genotype from the −819 C/T, since they occur in linkage disequilibrium [12]. We determined IL-10 haplotype (−1082 A/G, −819 C/T and −592 C/A) in 42 individuals. Of these, 5 (12%) presented haplotypes associated with high IL-10 levels, 9 (21%) with intermediate and 29 (67%) with low IL-10 levels. The distribution and frequency of IL-10 genotypes and haplotypes are shown in Table 6. Although we did not find any association in carotid arteries, allele A at position −1082 of the IL-10 gene was highly prevalent in autopsy cases with atherosclerotic lesion types III and IV.

## 4. Discussion

Atherosclerosis is a complex disease, resulting from both environmental and genetic factors. Although clinical manifestations appear after the fifth decade, the atherosclerotic process begins early in life. While early onset can account for 10% of the cases, pre-clinical

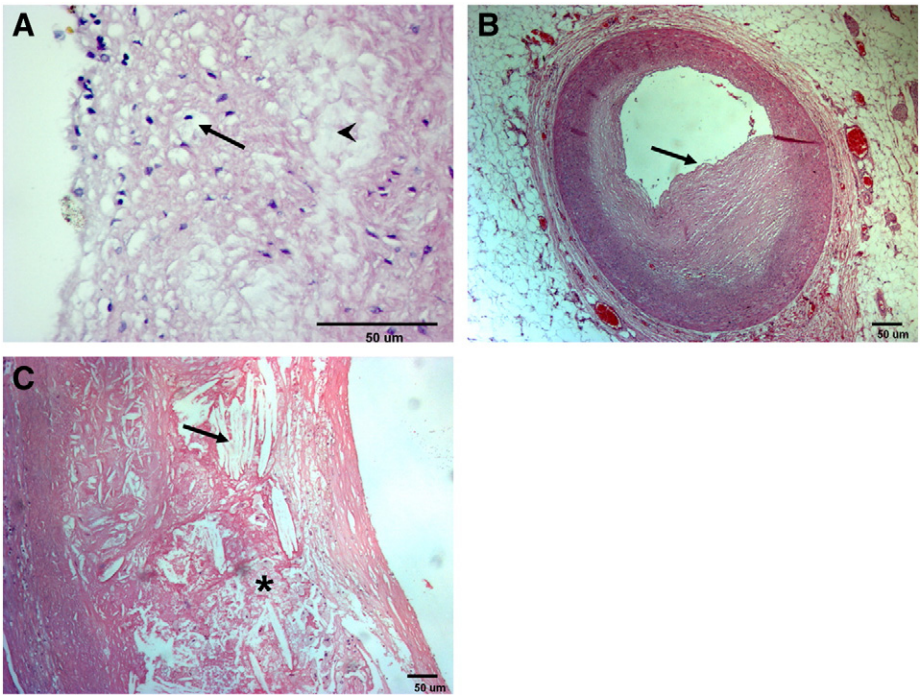


**Fig. 1.** Photomicrographs representative of atherosclerotic lesion in anterior descending coronary (A, B) and carotid (C) arteries of autopsied young adults. (A) Type III atherosclerotic lesion, with foam cell deposition (arrow) and extracellular lipid pools (arrowhead), HE ×40 magnification. (B) Type V lesion, arrow shows vessel lumen partially occluded by fibrotic atheroma plaque (HE ×10 magnification). (C) type IV lesion, note intact epithelium, and well-defined necrotic core (asterisk) with cholesterol crystals (arrow), HE ×10 magnification.

**Table 3**
Allele frequencies (*f*) for studied genotypes in 122 autopsied young adults.

| SNP | Allele | All subjects (*f*) | DA group (*f*) | CA group (*f*) |
|---|---|---|---|---|
| ACE (ins/del intron 16) ($n = 120$) | D | 0.604 | 0.636 | 0.594 |
| | I | 0.396 | 0.374 | 0.406 |
| TNF-α (−238 G/A) ($n = 94$) | G | 0.953 | 0.956 | 0.938 |
| | A | 0.047 | 0.044 | 0.063 |
| TNF-α (−308 G/A) ($n = 95$) | G | 0.787 | 0.791 | 0.787 |
| | A | 0.213 | 0.209 | 0.213 |
| IFN-γ (+874 T/A) ($n = 111$) | A | 0.757 | 0.763 | 0.712 |
| | T | 0.243 | 0.237 | 0.288 |
| MMP-9 (−1562 C/T) ($n = 68$) | C | 0.868 | 0.871 | 0.883 |
| | T | 0.132 | 0.129 | 0.117 |
| IL-10 (−1082 A/G) ($n = 48$) | A | 0.760 | 0.750 | 0.929 |
| | G | 0.240 | 0.250 | 0.071 |
| IL-10 (−819 C/T) ($n = 50$) | T | 0.380 | 0.411 | 0.375 |
| | C | 0.620 | 0.589 | 0.625 |
| NOS3 (rs1799983) ($n = 112$) | G | 0.795 | 0.814 | 0.784 |
| | T | 0.205 | 0.186 | 0.216 |
| ApoA1 (rs964184) ($n = 112$) | C | 0.843 | 0.793 | 0.817 |
| | G | 0.157 | 0.207 | 0.183 |
| TGF-β (codon 25) ($n = 111$) | G | 0.941 | 0.935 | 0.957 |
| | C | 0.059 | 0.065 | 0.043 |
| TGF-β (codon 10) ($n = 111$) | T | 0.559 | 0.543 | 0.612 |
| | C | 0.441 | 0.457 | 0.388 |

atherosclerotic manifestations in young adults is much higher [3,4]. The early appearance of arterial lesion may constitute the morphological manifestation of the genetic component of the disease. With this in mind, we reasoned that the young adult population is an interesting group to study the influence of genetic polymorphisms on atherosclerosis since they have been less exposed to classical risk factors than older adults. In this way, polymorphisms in twelve protein-coding genes involved in different pathways related to atherosclerosis pathogenesis were established. The results showed a high prevalence of atherosclerotic lesions type III in coronary and carotid arteries of autopsied young adults. Also, most severe lesions (types IV and V) were observed in 14% and 33% of DA and CA arteries, respectively. Furthermore, the degree of atherosclerotic lesion severity was associated with genetic polymorphisms in IL-10 and IFN-γ genes at coronary, but not at carotid artery.

Our data support the knowledge of atherosclerosis as an early-onset diffuse disease. The great majority of the autopsied cases resulted from deaths secondary to external causes, *e.g.*, car accidents or firearm projectiles. Only obesity could be discarded as classical risk factor in this population, since 93% of the subjects were normal or slim. Although no previous clinical information was available, epidemiological reports show low prevalence of clinical atherosclerotic manifestations under 30 years of age [3,4]. Additionally, histopathological studies suggest that lesion types I–III are usually clinically silent [15]. Clinical manifestations are associated with atherosclerotic lesions under type VI. However, lesions types IV–VI may remain silent. It is noteworthy that our study presents some limitations since individual characteristics, such as diet, body mass index, precise age and classical atherosclerotic risk factors (*e.g.*, hypertension, cigarette smoking, diabetes, and cholesterol levels), were not available. This lack of information prevented the use of multiple regression analysis with adjusted model for individual characteristics. Furthermore, we cannot rule out the use of recreational drugs and the role of stress on atherosclerotic lesion in this population.

**Table 4**
Association between genotype frequencies and atherosclerotic lesion severity in descendent coronary artery of autopsied young adults.

| SNP | Genotype | Atherosclerotic lesion in descendent anterior coronary artery (AHA classification) | | | | | | | *p* ($\chi^2$ test) |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal | I | II | III | IV | V | VI | |
| ACE (ins/del intron 16) ($n = 103$) | DD | 0.010 | 0.000 | 0.058 | 0.282 | 0.019 | 0.049 | 0.000 | |
| | DI | 0.000 | 0.000 | 0.078 | 0.291 | 0.039 | 0.010 | 0.000 | |
| | II | 0.010 | 0.000 | 0.019 | 0.117 | 0.000 | 0.019 | 0.000 | *0.48* |
| TNF-α (−238 G/A) ($n = 80$) | GG | 0.013 | 0.000 | 0.138 | 0.663 | 0.063 | 0.063 | 0.000 | |
| | GA | 0.000 | 0.000 | 0.000 | 0.038 | 0.000 | 0.000 | 0.000 | |
| | AA | 0.000 | 0.000 | 0.000 | 0.025 | 0.000 | 0.000 | 0.000 | *0.98* |
| TNF-α (−308 G/A) ($n = 79$) | GG | 0.013 | 0.000 | 0.076 | 0.494 | 0.038 | 0.051 | 0.000 | |
| | GA | 0.000 | 0.000 | 0.051 | 0.165 | 0.013 | 0.013 | 0.000 | |
| | AA | 0.000 | 0.000 | 0.013 | 0.063 | 0.013 | 0.000 | 0.000 | *0.94* |
| IFN-γ (+874 T/A) ($n = 98$) | AA | 0.020 | 0.000 | 0.041 | 0.439 | 0.041 | 0.051 | 0.000 | |
| | AT | 0.000 | 0.000 | 0.112 | 0.204 | 0.010 | 0.000 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.010 | 0.051 | 0.000 | 0.020 | 0.000 | *0.0001** |
| MMP-9 (−1562 C/T) ($n = 58$) | CC | 0.000 | 0.000 | 0.138 | 0.500 | 0.069 | 0.069 | 0.000 | |
| | CT | 0.000 | 0.000 | 0.034 | 0.121 | 0.034 | 0.000 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.000 | 0.034 | 0.000 | 0.000 | 0.000 | *0.83* |
| IL-10 (−1082 A/G) ($n = 43$) | GG | 0.023 | 0.000 | 0.047 | 0.047 | 0.023 | 0.023 | 0.000 | |
| | GA | 0.000 | 0.000 | 0.000 | 0.163 | 0.000 | 0.047 | 0.000 | |
| | AA | 0.000 | 0.000 | 0.163 | 0.442 | 0.023 | 0.000 | 0.000 | *0.013** |
| IL-10 (−819 C/T) ($n = 48$) | TT | 0.000 | 0.000 | 0.089 | 0.222 | 0.000 | 0.000 | 0.000 | |
| | TC | 0.000 | 0.000 | 0.044 | 0.133 | 0.022 | 0.000 | 0.000 | |
| | CC | 0.000 | 0.000 | 0.089 | 0.333 | 0.022 | 0.044 | 0.000 | *0.67* |
| NOS3 (rs1799983) ($n = 94$) | GG | 0.011 | 0.000 | 0.096 | 0.447 | 0.021 | 0.053 | 0.000 | |
| | GT | 0.011 | 0.000 | 0.074 | 0.223 | 0.043 | 0.021 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | *0.51* |
| ApoA1 (rs964184) ($n = 94$) | CC | 0.021 | 0.000 | 0.085 | 0.383 | 0.043 | 0.053 | 0.000 | |
| | CG | 0.000 | 0.000 | 0.064 | 0.309 | 0.021 | 0.021 | 0.000 | |
| | GG | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | *0.67* |
| ApoE (E2E3E4 isoforms) ($n = 100$) | E3E3 | 0.010 | 0.000 | 0.070 | 0.420 | 0.030 | 0.030 | 0.000 | |
| | E3E4 | 0.010 | 0.000 | 0.050 | 0.190 | 0.030 | 0.020 | 0.000 | |
| | E2E3 | 0.000 | 0.000 | 0.040 | 0.060 | 0.000 | 0.020 | 0.000 | |
| | E2E4 | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 | 0.000 | |
| | E4E4 | 0.000 | 0.000 | 0.000 | 0.010 | 0.000 | 0.000 | 0.000 | *0.93* |
| TGF-β (codon 25) ($n = 92$) | GG | 0.011 | 0.000 | 0.163 | 0.587 | 0.054 | 0.065 | 0.000 | |
| | GC | 0.000 | 0.000 | 0.000 | 0.098 | 0.011 | 0.000 | 0.000 | |
| | CC | 0.000 | 0.000 | 0.000 | 0.011 | 0.000 | 0.000 | 0.000 | *0.46* |
| TGF-β (codon 10) ($n = 92$) | TT | 0.000 | 0.000 | 0.054 | 0.196 | 0.011 | 0.032 | 0.000 | |
| | TC | 0.011 | 0.000 | 0.065 | 0.359 | 0.033 | 0.033 | 0.000 | *0.80* |
| | CC | | | | | | | | |

**Table 5**
Association between genotype frequencies and atherosclerotic lesion severity in carotid artery of autopsied young adults.

| SNP | Genotype | Atherosclerotic lesion in carotid artery (AHA classification) | | | | | | | $p$ ($\chi^2$ test) |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal | I | II | III | IV | V | VI | |
| ACE (ins/del intron 16) ($n = 64$) | DD | 0.000 | 0.016 | 0.125 | 0.141 | 0.109 | 0.000 | 0.000 | |
| | DI | 0.000 | 0.047 | 0.047 | 0.156 | 0.141 | 0.016 | 0.000 | |
| | II | 0.000 | 0.016 | 0.016 | 0.094 | 0.047 | 0.031 | 0.000 | 0.27 |
| TNF-α (−238 G/A) ($n = 48$) | GG | 0.000 | 0.063 | 0.208 | 0.292 | 0.313 | 0.042 | 0.000 | |
| | GA | 0.000 | 0.000 | 0.000 | 0.042 | 0.000 | 0.000 | 0.000 | |
| | AA | 0.000 | 0.021 | 0.000 | 0.021 | 0.000 | 0.000 | 0.000 | 0.30 |
| TNF-α (−308 G/A) ($n = 47$) | GG | 0.000 | 0.085 | 0.170 | 0.213 | 0.149 | 0.043 | 0.000 | |
| | GA | 0.000 | 0.000 | 0.021 | 0.106 | 0.128 | 0.000 | 0.000 | |
| | AA | 0.000 | 0.000 | 0.000 | 0.043 | 0.043 | 0.000 | 0.000 | 0.41 |
| IFN-γ (+874 T/A) ($n = 60$) | AA | 0.000 | 0.083 | 0.067 | 0.217 | 0.150 | 0.033 | 0.000 | |
| | AT | 0.000 | 0.000 | 0.083 | 0.150 | 0.100 | 0.000 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.017 | 0.033 | 0.050 | 0.017 | 0.000 | 0.39 |
| MMP-9 (−1562 C/T) ($n = 32$) | CC | 0.000 | 0.000 | 0.094 | 0.313 | 0.250 | 0.063 | 0.000 | |
| | CT | 0.000 | 0.063 | 0.063 | 0.063 | 0.063 | 0.031 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.06 |
| IL-10 (−1082 A/G) ($n = 21$) | GG | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| | GA | 0.000 | 0.048 | 0.000 | 0.048 | 0.000 | 0.048 | 0.000 | |
| | AA | 0.000 | 0.095 | 0.143 | 0.286 | 0.333 | 0.000 | 0.000 | 0.07 |
| IL-10 (−819 C/T) ($n = 20$) | TT | 0.000 | 0.000 | 0.050 | 0.050 | 0.150 | 0.000 | 0.000 | |
| | TC | 0.000 | 0.050 | 0.050 | 0.100 | 0.050 | 0.000 | 0.000 | |
| | CC | 0.000 | 0.100 | 0.050 | 0.200 | 0.100 | 0.050 | 0.000 | 0.78 |
| NOS3 (rs1799983) ($n = 59$) | GG | 0.000 | 0.068 | 0.102 | 0.237 | 0.119 | 0.051 | 0.000 | |
| | GT | 0.000 | 0.017 | 0.085 | 0.153 | 0.153 | 0.000 | 0.000 | |
| | TT | 0.000 | 0.000 | 0.000 | 0.017 | 0.000 | 0.000 | 0.000 | 0.62 |
| ApoA1 (rs964184) ($n = 60$) | CC | 0.017 | 0.050 | 0.117 | 0.250 | 0.167 | 0.033 | 0.000 | |
| | CG | 0.000 | 0.033 | 0.050 | 0.133 | 0.133 | 0.017 | 0.000 | |
| | GG | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.93 |
| ApoE (E2E3E4 isoforms) ($n = 100$) | E3E3 | 0.000 | 0.045 | 0.152 | 0.212 | 0.242 | 0.000 | 0.000 | |
| | E3E4 | 0.000 | 0.015 | 0.030 | 0.121 | 0.030 | 0.030 | 0.000 | |
| | E2E3 | 0.015 | 0.000 | 0.000 | 0.030 | 0.015 | 0.015 | 0.000 | |
| | E2E4 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| | E4E4 | 0.000 | 0.015 | 0.000 | 0.030 | 0.000 | 0.000 | 0.000 | 0.12 |
| TGF-β (codon 25) ($n = 58$) | GG | 0.017 | 0.069 | 0.098 | 0.228 | 0.163 | 0.033 | 0.000 | |
| | GC | 0.000 | 0.000 | 0.017 | 0.034 | 0.034 | 0.000 | 0.000 | |
| | CC | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.96 |
| TGF-β (codon 10) ($n = 58$) | TT | 0.017 | 0.034 | 0.086 | 0.138 | 0.069 | 0.034 | 0.000 | |
| | TC | 0.000 | 0.017 | 0.069 | 0.190 | 0.172 | 0.017 | 0.000 | |
| | CC | 0.000 | 0.017 | 0.017 | 0.069 | 0.051 | 0.000 | 0.000 | 0.76 |

Nevertheless, this young age group, *per se*, has suffered lower effect of environmental factors than older individuals due to the shorter exposure time to the classical risk factors. Since this is usually an asymptomatic group, investigation, direct observation, or confirmation of atherosclerotic lesion is less frequent. During the time of sample collection for this study, urban violence rate in Rio de Janeiro was high. This has allowed us to access a particular informative material, *e.g.*, arterial segments of young adults under 30 years of age, which may yield a gold standard parameter for correlation analysis studies. This group can be of particular interest for better understanding the influence of genetic variants on atherosclerosis in our population.

**Table 6**
Distributions and frequencies of IL-10 genotypes and haplotypes in all subjects and in anterior descendent coronary artery (DA) and carotid artery (CA) groups.

| Genetic markers | All subjects | DA group | CA group |
|---|---|---|---|
| *Genotypes, n (%)* | | | |
| GCC/GCC | 5 (12) | 5 (13) | 0 (0) |
| GCC/ACC | 8 (19) | 7 (18) | 3 (17) |
| GCC/ATA | 1 (2) | 1 (3) | 0 (0) |
| ACC/ACC | 10 (24) | 7 (18) | 6 (33) |
| ACC/ATA | 8 (19) | 7 (18) | 5 (28) |
| ATA/ATA | 11 (26) | 10 (26) | 4 (22) |
| *Haplotype carrier rate, n (%)* | | | |
| GCC | 14 (33) | 13 (35) | 3 (17) |
| ACC | 26 (61) | 21 (57) | 14 (78) |
| ATA | 20 (47) | 18 (49) | 9 (50) |
| *Haplotype frequency* | | | |
| GCC | 0.221 | 0.243 | 0.083 |
| ACC | 0.326 | 0.378 | 0.556 |
| ATA | 0.360 | 0.378 | 0.361 |

We observed type II atherosclerotic lesion (fatty streaks) in 22% of individuals. This prevalence is much lower than the one reported in the large PDAY study involving North American young adults, where type II lesion was observed in almost all individuals [16]. This discrepancy can be explained by the histopathological technique used, which may have underscored unanalyzed regions [17]. On the other hand, it should be pointed out that we found high prevalence of type III lesions (intimal thickening with extracellular lipid deposition), 69% for DA and 39% for CA. These findings support previous report in coronary and aortic arteries in young adults from India [18]. Such a high prevalence of type III lesion in our study is of particular relevance since this lesion is considered irreversible, as well as precursor of more severe arterial involvement [1,19,20]. Furthermore, severe type IV and even V lesions were also observed (14% in DA and 33% in CA). This prevalence is somewhat higher than previously reported in North American young adults [2,16], but close to that observed in young adults from India [3,4,18]. Since these severe atherosclerotic lesions are associated with clinical manifestations and complications [1,5–11,15], their presence in our study call our attention for the need of better understanding the influence of genetic variants in our young population and the early-onset atherosclerotic process.

In the present study, a group of SNPs was selected based on their role in different molecular pathways related to the atherosclerotic process, such as lipid metabolism (ApoA1 and ApoE), Th-1 (IFN-γ and TNF-α) and Th-2 inflammatory responses (IL-10), and endothelial function

and vascular remodeling (ACE, NOS3, MMP-9, and TGF-β). Note that the genes selected and their SNPs have been associated with either cardiovascular risk or the evolution of the disease itself [4,12]. In this study, we found correlation between IFN-γ and IL-10 gene variants and severity of histopathologic atherosclerosis lesion. We did not find any correlation for polymorphisms on ACE, ApoA1, ApoE, NOS3, MMP-9, TNF-α, or TGF-β genes. Previous study showed a role for VEGF, IL-10, and IFN-γ gene variants in the genetic background of offspring of parents with premature acute myocardial infarction [13,14,21]. Our results confirm and expand these findings since SNPs in the IL-10 and IFN-γ genes, involved in the inflammatory response, are related to the early-onset of histopathological vascular lesions in a random population, independently of parental history. The distribution of genotype frequencies observed in this study is in agreement with previous reports in the Brazilian population [22,23].

Data from human and animal studies show a predominant Th-1 pattern in atherosclerosis. The major pro-atherogenic Th-1 cytokine, IFN-γ, promotes macrophage and endothelial activation, leading to production of adhesion molecules, cytokines, chemokines, radicals, proteases, and coagulation factors. The targeted deletion of IFN-γ reduces atherosclerotic disease, whereas its administration accelerates lesion development in mice [2,12]. Furthermore, previous study reports IFN-γ variant association with high producer genotype [3–5]. Our data corroborate these findings that ascribe a role for Th-1 inflammatory response in the atherosclerosis process development.

In contrast to IFN-γ, data on the role of IL-10 in atherosclerosis are more controversial. IL-10 has both inflammatory and anti-inflammatory functions, and 75% of its variation production is genetically determined [3,4,24]. Three SNPs at positions −1082, −819, and −592 of IL-10 gene form three haplotypes (GCC, ACC, and ATA) associated with high (GCC/GCC), intermediate (GCC/ACC, GCC/ATA), and low (ATA/ATA, ACC/ATA, ACC/ACC) IL-10 production [15,25–27]. Although previous studies suggest atheroprotective effects for IL-10 [16,28–30], most of the data on humans are based on middle-aged or older subjects. In such populations, the genetic contribution may be masked by the strong covariates, such as the conventional risk factors [12,17,31]. On the other hand, one study [12,18] on young subjects reported association of IL-10 high and intermediate producers with decreased arterial elasticity, even after adjustment for conventional risk factors. Our data on autopsy cases of young adults showed a high frequency of low IL-10 producer genotype, highlighting the importance of studying young individuals in order to better observe the contribution of genetic diversity to the atherosclerotic process. Furthermore, this autopsy study allowed us to produce a morphological gold standard parameter of the initial atherosclerotic lesions in young adults, as well as its relation with the genetic diversity of the population.

Finally, it should be kept in mind that the influence of genetic polymorphisms on atherosclerosis in young adults, as observed by the associations between IL-10 and IFN-γ SNPs with lesion severity, was found only for coronary, but not for carotid artery. These findings support the hypothesis that the vascular system presents site specialization [32], which may differentially influence atherosclerosis development. The better understanding of genetic influence on the atherosclerosis process can provide future biomarkers for early-onset identification of the disease, thus improving its prevention and management, which can reduce the disease morbimortality.

## Conflict of interest

The authors declare that they have no conflict of interest.

## Transparency document

The Transparency document associated with this article can be found, in the online version.

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