HIV-Infection, Atherosclerosis and the Inflammatory Pathway: Candidate Gene Study in a Spanish HIV-Infected Population

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Abstract

Background: Higher prevalence of atherosclerosis and higher cardiovascular risk is observed in HIV-infected individuals. The biological mechanisms underlying these processes are unclear. Several studies have implicated genetic variants in the inflammatory genes in cardiovascular disease and in HIV natural course infection.

Methods & Findings: In this study we have tested the possible association between genetic variants in several inflammatory genes and asymptomatic cardiovascular disease measured by carotid intima media thickness (cIMT) and atherosclerotic plaque presence as dependent variables in 213 HIV-infected individuals. A total of 101 genetic variants in 25 candidate genes have been genotyped. Results were analyzed using Plink and SPSS statistical packages. We have found several polymorphisms in the genes ALOX (rs2115819 p = 0.009), ALOX5AP (rs4151117 p = 0.007; rs4769873 p = 0.004 and rs9315051 p = 0.0004), CX3CL1 (rs4151117 p = 0.040 and rs614230 p = 0.015) and CCL5 (rs3817655 p = 0.018 and rs2107538 p = 0.018) associated with atherosclerotic plaque. cIMT mean has been associated with CRP (1130864 p = 0.0003 and rs1800947 p = 0.008), IL1RN (rs388092 p = 0.002) and ALOX5AP (rs3885907 p = 0.02) genetic variants.

Conclusions: In this study we have found modest associations between genetic variants in several inflammatory genes and atherosclerotic plaque or cIMT. Nevertheless, our study adds evidence to the association between inflammatory pathway genetic variants and the atherosclerotic disease in HIV-infected individuals.

Background

Cardiovascular disease (CVD), has been identified as a major cause of death in HIV-infected people [1–3]. HIV-infected individuals have accelerated atherogenesis, which is associated to a high risk of suffering a cardiovascular event such as coronary artery disease (CAD), peripheral vascular disease and stroke. The prevalence of these events in HIV-infected subjects is higher than in the general population and has an earlier onset [4–6]. The biological mechanisms underlying such risk among HIV-infected people are unclear [7].

Several studies implicate inflammation processes in CVD [7–10]. Non-HIV infected cohort studies have demonstrated that markers of inflammation are strongly predictive of CVD events and mortality [8]. Inflammatory markers are elevated in HIV-infected patients in comparison with non-infected individuals [7,11]. It has been hypothesized that this increased inflammation may be the explanation for the elevated cardiovascular risk in HIV-infected individuals [9].

Mechanisms of immune activation and inflammation have been proposed as the cause of earlier CAD in HIV [7]. HIV is thought to play a crucial role in the pathogenesis of atherosclerosis in HIV-
infection [6,11]. In addition to the high prevalence of traditional CVD risk factors in HIV-infected individuals, several factors such as immunosuppression, inflammation, HIV ability to induce foam cell transformation, cumulative exposure to antiretroviral drugs, and mitochondrial and metabolic dysfunctions have been hypothesized to be involved in HIV associated atherosclerosis [11–13].

Studies on the inflammatory pathways have found genetic variants associated with atherosclerosis in the general population [10]. Inflammatory marker genes such as C-Reactive Protein (CRP) [14,15], Interleucin-6 (IL6) [10], Interleucin-1 (IL1) gene cluster [16,17], Interleucin-18 (IL18) [10], Tumor Necrosis Factor (TNF) [10], Lymphotoxin-a (LTA) [10], Fractalkine Receptor (CX3CR1) [18,19], Chemokine Receptor 5 (CCR5) [20], Chemokine Receptor 2 (CCR2) and the 5-lipoxygenase (5-LO) pathway genes [10,21] have been associated with cardiovascular events. Regarding HIV-infected patients, the most important investigation is a Genome Wide Association Study (GWAs) conducted by Shrestha et al. [22] that related two variants in the gene Ryanodine Receptor 3 (RYR3) with greater carotid Intima Media Thickness (cIMT), a surrogate marker of atherosclerosis. This finding has been replicated by the same authors in a later study [23], but there is no independent study confirming this result.

The immunological and inflammatory pathways have many shared genes that may interact in the pathogenesis of atherosclerosis in HIV-infected individuals. The aim of this study was to assess the implication of genetic variants in relevant inflammatory genes in the atherosclerotic disease of HIV infected subjects.

**Materials and Methods**

**Study population**

We performed a cross-sectional study with 213 Spanish Caucasians HIV-infected individuals attended in Hospital Universitari MutuaTerrassa (Terrassa, Catalonia, Spain). This project was approved by the local ethics committee (Comité ético de Investigación Clínica del Hospital Universitario MutuaTerrassa - Approval number: EO/0915). All Participants gave written informed consent for genetic testing. At the time of enrolment, demographic, clinical and biochemical variables were collected from each patient by interviews and from medical notes. Simultaneously ultrasonographic measures (cIMT and atherosclerotic plaque presence) were performed.

**Carotid artery Ultrasound**

Carotid Intima Media Thickness (cIMT) is the most widely used surrogate marker of atherosclerosis. It relies on the fact that the
| Variable                  | Value                          | Atherosclerotic Plaque Presence |
|--------------------------|--------------------------------|---------------------------------|
|                          |                               | Yes (n = 83)                     | No (n = 130)                     |
| Age, years ±sd           | 45.34 ± 8.20                  | 49.67 ± 8.38                    | 42.58 ± 6.79                    |
| Males, n (%)             | 166 (77.9)                    | 61 (73.5)                       | 105 (80.8)                      |
| Body Mass Index*, Kg/m² ±sd | 24.08 ± 3.76               | 24.11 ± 4.09                    | 24.06 ± 3.56                    |
| Abdominal Obesity¹, n (%) | 26 (12.2)                     | 15 (18.3)                       | 11 (8.6)                        |
| Metabolic Syndrome², n (%) | 16 (7.5)                     | 10 (12.5)                       | 6 (4.7)                         |
| Hypertension³, n (%)     | 20 (8.4)                      | 14 (17.7)                       | 6 (4.7)                         |
| Diabetes Mellitus⁴, n (%) | 8 (3.8)                      | 7 (8.4)                         | 1 (0.8)                         |
| Dyslipidemia⁵, n (%)     | 59 (27.7)                     | 28 (35.0)                       | 31 (24.0)                       |
| cIMT mean, mm ±sd        | 0.89 ± 0.21                   | 1.01 ± 0.21                     | 0.81 ± 0.17                     |
| Smoking Habits           |                               |                                 |                                 |
| Smokers, n (%)           | 150 (70.4)                    | 61 (74.4)                       | 89 (70.1)                       |
| Non Smokers, n (%)       | 59 (27.7)                     | 21 (25.6)                       | 38 (29.9)                       |
| Lipid profile            |                               |                                 |                                 |
| Total Cholesterol, mg/dl ±sd | 181.41 ± 43.13            | 193.01 ± 47.70                  | 174.01 ± 38.33                  |
| LDL Cholesterol, mg/dl ±sd | 111.70 ± 49.15              | 118.81 ± 49.23                  | 107.15 ± 48.75                  |
| HDL Cholesterol, mg/dl ±sd | 46.97 ± 16.17                | 47.98 ± 16.24                   | 46.32 ± 16.16                   |
| Non-HDL Cholesterol, mg/dl ±sd | 134.67 ± 42.28              | 145.03 ± 48.16                  | 128.05 ± 36.75                  |
| Triglycerides, mg/dl ±sd | 148.09 ± 92.78               | 160.76 ± 84.70                  | 140.00 ± 97.04                  |
| HIV Characteristics      |                               |                                 |                                 |
| Time Infected, years ±sd | 11.97 ± 7.62                  | 13.26 ± 7.50                    | 11.10 ± 7.62                    |
| Hepatitis C Coinfection, n (%) | 102 (47.9)                  | 45 (54.9)                       | 57 (44.5)                       |
| Antiretroviral Therapy**, n (%) | 175 (82.2)                  | 72 (87.8)                       | 103 (80.5)                      |
| PIs, n(%)                | 62 (29.1)                     | 24 (30.0)                       | 38 (30.4)                       |
| nRTIs, n(%)              | 166 (77.9)                    | 69 (86.2)                       | 97 (77.0)                       |
| NNRTIs, n(%)             | 101 (47.7)                    | 42 (52.5)                       | 59 (46.8)                       |
| Entry Inhibitors, n(%)   | 2 (0.9)                       | 1 (1.2)                         | 1 (0.8)                         |
| Integrase Inhibitors, n(%) | 12 (5.6)                     | 7 (8.8)                         | 5 (4.0)                         |
| Previous Antiretroviral Therapies, n (%) | 167 (78.4)                  | 68 (82.9)                       | 99 (77.3)                       |
| CD4+ cell count, cells/µl ±sd | 581.25 ± 339.53            | 610.10 ± 359.48                 | 562.83 ± 326.23                 |
| CD4+ nadir cell count, cells/µl ±sd | 301.74 ± 222.79            | 310.09 ± 242.74                 | 296.39 ± 209.88                 |
| Viral Load > 19 copies/ml, n (%) | 71 (33.3)                   | 23 (27.7)                       | 48 (36.9)                       |
| Viral Load, copies/ml (95% IQR) | 95759.65 ± 203731.45      | 14352.03 ± 61553.87             | 43494.40 ± 152702.15            |
| CDC Stage                |                               |                                 |                                 |
| A, n (%)                 | 143 (67.1)                    | 59 (72.8)                       | 84 (67.2)                       |
| B, n (%)                 | 13 (6.1)                      | 3 (3.7)                         | 10 (8.0)                        |
| C, n (%)                 | 50 (23.5)                     | 19 (23.5)                       | 31 (24.8)                       |
| Diagnosed AIDS, n (%)    | 92 (43.2)                     | 37 (45.1)                       | 55 (44.0)                       |
| Risk Group               |                               |                                 |                                 |
| Drug Users, n (%)        | 78 (36.6)                     | 35 (50.7)                       | 43 (46.0)                       |
| Sexual Transmission, n (%) | 91 (42.7)                    | 32 (46.4)                       | 59 (55.7)                       |
| Others, n (%)            | 6 (2.8)                       | 2 (2.9)                         | 4 (3.8)                         |

*Body Mass Index (BMI): the individual’s body mass divided by the square of their height.

¹Abdominal Obesity: waist circumference ≥ 102 cm in men and ≥ 88 cm in women.

²Metabolic Syndrome was defined as having two or more of the following characteristics: Diabetes Mellitus; Blood Pressure ≥ 140/90 mmHg; Dyslipidemia; Abdominal Obesity and hypertension.

³Hypertension was: systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mmHg and/or antihypertensive treatment.

⁴Diabetes Mellitus: fasting glucose levels ≥ 126 mg/dl and/or having diabetes symptoms and glucose levels ≥ 200 mg/dl in a random determination and/or diabetes treatment.

⁵Dyslipidemia: Total Cholesterol ≥ 240 mg/dl, and/or HDL Cholesterol ≤ 35 mg/dl, and/or Triglycerides ≥ 200 mg/dl and/or lipid lowering drugs.
presence of atherosclerosis in one vascular bed will correlate with the atherosclerosis in another vascular bed. High-resolution B-mode ultrasound of the carotid arteries has been well validated in the non infected population as a surrogate marker of cardiovascular risk [24].

cIMT measurements and atherosclerotic plaque presence were obtained for each patient using a B-mode ultrasound recording with a 7 to 14-MZ transducer. For the imaging studies, patients were placed in the supine position with their head in the midline position and tilted slightly upwards, and the heart in systole. For each participant, a total of eight measures were obtained: left and right carotid primitive and bulb region in both proximal and distal walls. The cIMT value was defined as the mean of all values excluding those corresponding to atherosclerotic plaque thickness. According to Manheline cIMT consensus, atherosclerotic plaque presence is defined as a focal structure encroaching into the arterial lumen by at least 0.5 mm or 50% of the surrounding IMT value [25]. It was recorded as a bimodal variable (yes/no). All measures were performed by the same operator with an experimental intra-variability of 4.7%.

Genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini kit (Qiagen, Izasa, Barcelona, Spain) following the manufacturer's instructions. Genotyping was performed using Competitive Allele PCR methodology (KBioscience, Hoddesdon, United Kingdom). Genes from the inflammatory pathway were selected. A total of 101 genetic variants or single nucleotide polymorphisms (SNPs) in 25 candidate genes were tested (Table 1). TagSNPs were selected from HapMap for maximum coverage of the genes (Selection criteria: R^2$\geq$0.8 and minor allele frequency ($maf$$\geq$0.05). We also included some relevant SNPs from other studies in HIV and non-HIV infected populations.

Statistical analysis and quality control

Statistical analyses were performed using the G*Power Calculator [26], SPSS 20 (SPSS, Chicago, IL) and Plink 1.07 [27] statistical packages. The study had an 80% power (considering $\alpha=0.05$ and $\beta=0.95$, two-sided) to capture the effect of SNPs with $maf$$\geq$0.1 and Odds ratio (OR)$\geq$2. For rarer alleles ($maf$<0.1), the study had a 75% power to capture genetic effects with OR$\geq$3. For continuous variables the study had a 95% power (considering $\alpha=0.05$ and $\beta=0.95$, two-sided) to capture the effect of SNPs with $maf$0.1.

Genotype call rate, Hardy-Weinberg equilibrium (HWE) and $maf$ was assessed for each SNP using Plink 1.07. Those SNPs with call rates<95% or $maf$<0.01 and individuals with more than 5% missing genotypes were excluded from the analyses.

Chi square analyses or Fisher exact tests (for those alleles with less than 5 counts in any group) were used to investigate the possible association of atherosclerotic plaque with genetic variants. cIMT variable was recorded as a continuous variable and it was normally distributed. Linear regression analyses were used to compare cIMT values with gene alleles.

Age, abdominal obesity, metabolic syndrome, hypertension, diabetes, total cholesterol, non-HDL cholesterol, LDL cholesterol and triglycerides were significantly associated with atherosclerotic plaque presence ($p<0.05$ in all cases). Age, hypertension, diabetes, dyslipidemia, total cholesterol, non-HDL cholesterol and LDL cholesterol were significantly associated with cIMT ($p<0.05$ in all cases).

Lipid values were highly correlated (correlation coefficient>0.75 in all cases). Only dyslipidemia was included as a clinical adjusting variable in the regression models. Metabolic syndrome was collinear with the variables diabetes, dyslipidemia, abdominal obesity and hypertension. Metabolic syndrome was not included as a clinical variable in the regression model for atherosclerotic plaque presence.

Four atherosclerotic plaque presence, age, abdominal obesity, hypertension, diabetes and dyslipidemia were included in logistic regression analyses as adjusting clinical variables for all the findings. For cIMT, age, hypertension, diabetes, and dyslipidemia were included in the regression analyses as adjusting clinical variables for all the findings. For the importance of HAART in the vascular pathogenesis, it was included in both adjustments.

**Results**

Study Population

Demographic, lipid profile and HIV baseline characteristics of the study population are shown in Table 2. Mean age was 45.34 (SD±8.20) years. Males constituted 77.9% of the samples. 82.2% of the individuals were receiving Highly Active Antiretroviral Therapy (HAART) at the time of the study. Atherosclerotic plaque presence (indicator of atherosclerotic lesion [28]) and cIMT means were collinear with the variables diabetes, dyslipidemia, abdominal obesity and hypertension. Metabolic syndrome was not included as a clinical variable in the regression model for atherosclerotic plaque presence.

| SNP | Gene | Chromosome | Position | MAF | p-value |
|-----|------|------------|----------|-----|---------|
| rs16944 | IL1B | 6 | 23832960 | 0.05 | 0.004 |
| rs4248160 | CXCL2 | 6 | 75294174 | 0.05 | 0.004 |
| rs3087263 | ALOX5 | 5 | 14770145 | 0.05 | 0.004 |

Quality Control

**Hardy-Weinberg Equilibrium.** All SNPs were in HWE except for IL1B (p = 0.044), ILIRN rs380092 (p = 0.006), CXCL12 rs2236533 and rs2236533 (p = 0.040 and p = 0.029 respectively) and ALOX5 rs3824612 (p = 0.025).

**SNP Quality control.** Three of the genotyped SNPs were excluded from the analysis, because of a call rate<95% (ILIRN rs3087263, CX3CR1 rs2669845 and IL6 rs2069833) and two because of a $maf<$0.1 (CXCL10 rs11548618 and TNF rs4248160). Two SNPs (IL1B rs55778004 and ILIRN rs4252019) were monomorphic in our population. The remaining 94 SNPs were included in the analysis.

**Single Marker Analysis**

**Atherosclerotic Plaque Presence Analysis.** Single marker analyses by Chi-square revealed 12 associations with atherosclerotic plaque presence (Table 3). ILIRN rs42452041 rare allele was associated with atherosclerotic plaque presence (p = 0.027, OR = 2.40). CXCL2 rs9131 and rs3806792 rare alleles were associated with atherosclerotic plaque absence (p = 0.035, OR = 0.65 and p = 0.026, OR = 0.63 respectively) whereas ALOX5 rs2115819 was associated with atherosclerotic plaque presence (p = 0.008, OR = 1.73). Three ALOX5AP SNPs were associated with atherosclerotic plaque absence: rs9578196
### Table 3. Summary of single marker analyses in relation to atherosclerotic plaque presence.

| Chr | Gene | SNP     | ALLELE COUNT Minor allele (MAF Aff/MAF Unaff*) | Unadjusted | Adjusted** |
|-----|------|---------|-----------------------------------------------|------------|------------|
|     |      |         |                                               | p value    | OR         | CI 95%    | p value | ji | CI 95% |
| 2   | IL1RN| rs4252041| T (0.10/0.04)                                 | 0.027      | 2.40       | 1.08–5.30 | ns      | -  | -     |
| 4   | CXCL2| rs9131   | G (0.37/0.47)                                 | 0.035      | 0.65       | 0.43–0.97 | ns      | -  | -     |
| 4   | CXCL2| rs3806792| T (0.36/0.47)                                 | 0.026      | 0.63       | 0.42–0.95 | ns      | -  | -     |
| 10  | ALOX5| rs2115819| C (0.50/0.37)                                 | 0.008      | 1.73       | 1.15–2.58 | 0.009   | 2.03| 1.19–3.47 |
| 13  | ALOX5AP| rs9578196| T (0.08/0.16)                                | 0.014      | 0.45       | 0.23–0.86 | 0.007   | 0.33| 0.14–0.73 |
| 13  | ALOX5AP| rs4769873| T (0.06/0.14)                                | 0.011      | 0.40       | 0.19–0.83 | 0.004   | 0.25| 0.10–0.65 |
| 13  | ALOX5AP| rs9315051| G (0.04/0.14)                                | 0.001      | 0.27       | 0.12–0.63 | 0.0004  | 0.15| 0.05–0.43 |
| 16  | CX3CL1| rs170361 | A (0.13/0.20)                                | 0.040      | 0.57       | 0.33–0.98 | ns      | -  | -     |
| 16  | CX3CL1| rs4151117| G (0.15/0.24)                                | 0.024      | 0.55       | 0.33–0.93 | 0.040   | 0.52| 0.28–0.97 |
| 16  | CX3CL1| rs614230 | C (0.27/0.39)                                | 0.013      | 0.59       | 0.38–0.89 | 0.015   | 0.54| 0.33–0.88 |
| 17  | CCL5  | rs3817655| A (0.23/0.15)                                | 0.035      | 1.72       | 1.04–2.85 | 0.018   | 1.96| 1.12–3.42 |
| 17  | CCL5  | rs2107538| T (0.23/0.15)                                | 0.033      | 1.71       | 1.04–2.80 | 0.018   | 1.93| 1.12–3.31 |

*maf: minor allele frequency/Aff: Affected/Unaff: Unaffected
**Adjusted by clinical variables: Age, Abdominal Obesity, Metabolic Syndrome, Hypertension, Diabetes, Dyslipidemia and Antiretroviral Therapy

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### Table 4. Summary of single marker analyses in relation to cIMT.

| Chr | Gene | SNP | Allele Count | Unadjusted | Adjusted** | p value | CI 95% |
|-----|------|-----|--------------|------------|------------|---------|--------|
| 1   | CRP  | rs1130864 | T > C        | 0.006      | 0.008      | 0.027   | -0.11 to -0.03 |
| 1   | CRP  | rs4769873  | G > A        | 0.006      | 0.006      | 0.035   | -0.13 to -0.06 |
| 1   | CRP  | rs4769873  | C > T        | 0.006      | 0.002      | 0.013   | -0.12 to -0.02 |
| 1   | CRP  | rs1800947  | C > T        | 0.006      | 0.002      | 0.013   | -0.12 to -0.02 |
| 1   | CRP  | rs380092   | T > C        | 0.006      | 0.002      | 0.013   | -0.12 to -0.02 |
| 11  | IL18  | rs2043055 | G > C        | 0.040      | 0.046      | 0.092   | -0.10 to -0.05 |
| 13  | ALOX5AP | rs3885907 | C > T        | 0.010      | 0.008      | 0.013   | -0.12 to -0.02 |
| 16  | CX3CL1 | rs170361  | A > G        | 0.008      | 0.002      | 0.013   | -0.12 to -0.02 |

*Not in HWE.

**Adjusted by clinical variables: Age, Hypertension, Diabetes, Dyslipidemia and HAART.

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### Carotid Intima Media Thickness Analysis.

Single marker analysis by logistic regression showed 6 positive associations.

- **ALOX5**: rs2115819 rare allele C was associated with atherosclerotic plaque presence ($p = 0.009$, $\beta = 2.03$). Three ALOX5AP SNPs were associated with atherosclerotic plaque absence: rs9578196 ($p = 0.007$, $\beta = 0.33$); rs4769873 ($p = 0.004$, $\beta = 0.25$); and rs9315051 ($p = 0.0004$, $\beta = 0.15$).

- **CX3CL1**: rs4151117-G and rs614230-C were associated with atherosclerotic plaque absence ($p = 0.004$, $\beta = 0.52$ and $p = 0.015$, $\beta = 0.54$ respectively). Two CCL5 SNPs were associated with atherosclerotic plaque presence: rs3817655 ($p = 0.018$, $\beta = 1.96$) and rs2107538 ($p = 0.018$, $\beta = 1.93$).

### Discussion

In this study we have tested the possible association between genetic variants in several inflammatory genes and CVD measured by cIMT and atherosclerotic plaque presence in 213 HIV-infected individuals. We have found several polymorphisms in the ALOX5, ALOX5AP, CX3CL1 and CCL5 genes significantly associated with atherosclerotic plaque, whereas cIMT mean has been associated with CRP, IL1RN and ALOX5AP genetic variants.

### Atherosclerotic Plaque

The ALOX5 rs2115819-C allele was associated with atherosclerotic plaque presence and the ALOX5AP rs9578196-T, rs4769873-G and rs9315051-G alleles with atherosclerotic plaque absence. Genetic variants in ALOX5 and ALOX5AP have been previously linked to CVD in non-HIV infected populations [21]. These two genes encode for two important proteins of the 5-LO pathway which has been previously linked to atherosclerosis [21]. Knock-out mice of ALOX5 showed less atherosclerotic plaque formation [21]. Studies in HIV-infected cultured cells have shown a diminished function of 5-LO pathway due to gp120 HIV-protein presence [30,31]. Our results, showing that ALOX5 and

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**HIV and Atherosclerosis: Candidate Gene Study**

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**CRP, IL1RN and ALOX5AP genetic variants.**

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**Atherosclerotic Plaque**

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ALOX5AP may influence CVD in HIV patients, contribute to the growing evidence on the relevance of these genes on CVD.

The CX3CL1 rs164230-C allele was found associated with absence of atherosclerotic plaque. This allele has been previously associated with smaller cIMT in a German non-HIV infected subjects (CAPS cohort) although this finding was not replicated in a French cohort (3C cohort) [18]. To our knowledge, the association found between CX3CL1 rs4151117-G allele and atherosclerotic plaque absence is a novel finding. CX3CL1 has been implicated in atherosclerotic plaque formation initial process [18,19]. CX3CR1 (CX3CL1 receptor) knockout mice and animal treated with CX3CR1 inhibitors show reduced atherosclerotic plaque formation [32,33]. The functional effects of the CX3CL1 rs164230 or rs4151117 are not known. However, these SNPs are located near a frame shift mutation that may diminish protein functionality. Our results seem to agree with the reported atheroprotective effects of CX3CL1 reduced activity.

The CCL5 rs3817655-A and rs2107538-T alleles were found associated with atherosclerotic plaque presence. The CCL5 rs2107538 polymorphism has been previously associated with higher plasma concentrations of CCL5 and increased risk of MI in Korean CAD patients [34] and Han Chinese MI patients [15]. Our results seem to agree with these findings as the T allele associated with plaque presence in our study is also associated with MI risk. The CCL5 rs3817655 finding is a novel association that has not been previously described. This SNP is near to two mutations that encode for new stop codons and truncated proteins. Our findings may reflect the functional effect of these mutations but further studies are required to confirm it.

Carotid Intima Media Thickness

The CRP rs1130864-T allele was found marginally associated with smaller cIMT and the rs1800947-C allele with greater cIMT. These results are in the opposite direction that in previous studies. The CRP rs1130864-T allele was found associated with increased CRP plasma levels [14], which are considered a marker of cardiovascular risk [35,36], and have been linked to a faster cIMT progression in HIV-infected individuals [37]. The CRP rs1800947-C allele have been previously linked to decreased CRP plasma levels [15,38], which are known to be atheroprotective. Although the functional consequences of these SNPs are not known, both of them are in regulatory regions. The presence of HIV could interact with the functionality of these genetic variants explaining the discrepancy between our findings and those published previously. These findings need to be replicated in independent cohorts to confirm the direction of the associations.

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