Effects of Fractalkine Receptor Variants on Common Carotid Artery Intima-Media Thickness

Giuseppe D. Norata, PhD; Katia Garlaschelli, BSc; Manuele Ongari, BSc; Sara Raselli, PhD; Liliana Grigore, MD, PhD; Alberico L. Catapano, PhD

Background and Purpose—Fractalkine receptor (CX3CR1) plays a key role during atherogenesis. CX3CR1 has 2 common coding polymorphisms, namely V249I and T280M, that have been associated with interindividual differences in susceptibility to atherosclerosis. In the present study, we investigated the possible association between CX3CR1 variants and intima-media thickness (IMT).

Methods—We genotyped 1256 samples from the Progression of Lesions in the Intima of the Carotid (PLIC) study (a prospective population-based study) for the presence of the V249 and the M280 variants of CX3CR1.

Results—Significantly reduced IMT was observed in subjects with the MM280 genotype (0.57±0.12 mm) compared with subjects with the TT (0.65±0.14 mm) or the TM (0.65±0.13 mm) genotype. No difference in IMT was observed within carrier of the II249, VI249, or VV249 genotype. Subjects with combined genotype VI249/MM280 and II249/MM280 showed a reduced IMT.

Conclusions—The presence of the M280 polymorphism of the fractalkine receptor is associated with a decreased common carotid artery IMT, whereas the presence of the I249 polymorphism does not play a major role on the progression of carotid atherosclerosis. (Stroke. 2006;37:1558-1561.)

Key Words: cytokines ■ genetics ■ inflammation ■ intima-media thickness

Fractalkine is the sole member of the CX3C family and has unique structural and functional attributes. Fractalkine binding to its 7-transmembrane domain G-protein–coupled receptor (CX3CR1) triggers signaling, but it also directly mediates cell adhesion. Fractalkine binds CX3CR1 rapidly and firmly, leading to tethering and arrest of leukocytes under conditions of physiological flow independent of CX3CR1 signaling. CX3CR1-expressing cells, including CD4+ T cells, CD8+ T cells, and NK cells, also express CD57 and CD11b (markers for cytotoxic lymphocytes).

CX3CR1 is believed to be a key mediator of atherogenesis and is expressed in atherosclerotic plaques and in vessels from diabetic subjects. The function of CX3CR1 in atherosclerosis was assessed by crossing CX3CR1−/− mice into the apolipoprotein E (apoE)−/− background. Lesion formation throughout the aorta, including the aortic root, and macrophage accumulation were significantly reduced in the CX3CR1−/−/apoE−/− animals, suggesting a role for fractalkine in atherogenesis.

CX3CR1 has 2 common coding polymorphisms, namely V249I and T280M; I249 and M280 have been shown to be in linkage disequilibrium and have been associated with interindividual differences in susceptibility to HIV infection, atherosclerosis, and stroke.

Despite these findings and the identification of CX3CR1 expression in atherosclerotic plaque, the majority of the studies investigated the frequency of CX3CR1 polymorphisms in case-control studies, whereas no data are available on the possible role of these polymorphisms on early markers of atherosclerosis such as intima-media thickness (IMT) of the common carotid artery. We thus investigated the effect of the M280 and I249 variants of CX3CR1 on IMT in the PLIC study (a prospective population-based study) on the Progression of Lesions in the Intima of the Carotid.

Materials and Methods

The PLIC study was designed to investigate the presence and progression of atherosclerotic lesions and IMT in the common carotid artery in a local cohort (2141 subjects). The study was approved by the ethical committee for the Center for the Study of Atherosclerosis, University of Milan, and the participating subjects signed an informed consent. A detailed description of the study has been published previously and as online supplement and includes the measurement of biochemical parameters and clinical outcome, the ultrasonography analysis, DNA extraction, and genotyping and statistical analysis.

Results

Of the 1256 DNA samples used for the genotyping test, results are available from 1168 samples; the genotype of 88 samples is missing because of technical reasons. No deviation from the Hardy–Weinberg equilibrium was observed for the
V249I and for the T280M polymorphisms, and the 2 polymorphisms resulted in partial linkage disequilibrium ($D^\prime=0.82$ and $R^2=0.36$) as already shown. The genotype frequencies are presented in Table 1. Age, gender, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, systolic and diastolic blood pressure, body mass index, and glucose levels did not differ between the subjects with the VV, the VI, and the II 249 genotype (Table 1). Similar results were obtained for the T280M polymorphism (Table 1), although triglyceride levels were lower in subjects with the MM genotype (Table 1). Although a higher number of females were observed in the MM group, the difference of male/female distribution between the genotypes was not statistically different. When data were stratified for gender, triglyceride levels resulted lower in the MM group regardless of gender, but these differences were not statistically significant because of the low number of MM subjects in each group.

### Table 1. Clinical Characteristics According to CX3CR1 Genotype (V249I or T280M)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%)</th>
<th>Age, y</th>
<th>Gender, males/females</th>
<th>Systolic blood pressure, mm Hg</th>
<th>Diastolic blood pressure, mm Hg</th>
<th>Total cholesterol, mmol L$^{-1}$</th>
<th>LDL cholesterol, mmol L$^{-1}$</th>
<th>HDL cholesterol, mmol L$^{-1}$</th>
<th>Triglycerides (mmol L$^{-1}$)</th>
<th>Glycemia, mmol L$^{-1}$</th>
<th>BMI, kg/m$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>VV</td>
<td>685 (58.6)</td>
<td>54.4 ± 10.9</td>
<td>283/402</td>
<td>132 ± 16</td>
<td>82 ± 9</td>
<td>5.76 ± 0.98</td>
<td>3.79 ± 0.91</td>
<td>1.41 ± 0.38</td>
<td>1.23 ± 0.74</td>
<td>5.08 ± 0.96</td>
<td>26.5 ± 4.2</td>
</tr>
<tr>
<td>VI</td>
<td>397 (54.0)</td>
<td>54.3 ± 11.7</td>
<td>167/229</td>
<td>132 ± 17</td>
<td>83 ± 9</td>
<td>5.67 ± 1.04</td>
<td>3.73 ± 0.96</td>
<td>1.40 ± 0.37</td>
<td>1.16 ± 0.64</td>
<td>5.03 ± 0.76</td>
<td>26.5 ± 4.3</td>
</tr>
<tr>
<td>II</td>
<td>86 (7.4)</td>
<td>54.6 ± 11.7</td>
<td>37/49</td>
<td>133 ± 16</td>
<td>84 ± 9</td>
<td>5.77 ± 1.09</td>
<td>3.81 ± 1.09</td>
<td>1.41 ± 0.43</td>
<td>1.19 ± 0.70</td>
<td>5.02 ± 0.82</td>
<td>25.9 ± 4.2</td>
</tr>
<tr>
<td>VV vs VI</td>
<td>0.89</td>
<td>0.85</td>
<td>0.90</td>
<td>0.98</td>
<td>0.98</td>
<td>0.89</td>
<td>0.93</td>
<td>0.85</td>
<td>0.85</td>
<td>0.91</td>
<td>0.19</td>
</tr>
<tr>
<td>VI vs II</td>
<td>0.82</td>
<td>0.85</td>
<td>0.90</td>
<td>0.98</td>
<td>0.98</td>
<td>0.89</td>
<td>0.93</td>
<td>0.85</td>
<td>0.85</td>
<td>0.91</td>
<td>0.27</td>
</tr>
<tr>
<td>TT vs MM</td>
<td>54.6 ± 10.8</td>
<td>54.7 ± 10.9</td>
<td>367/503</td>
<td>132 ± 17</td>
<td>82 ± 9</td>
<td>5.63 ± 1.11</td>
<td>3.70 ± 1.04</td>
<td>1.37 ± 0.37</td>
<td>1.19 ± 0.67</td>
<td>5.09 ± 0.92</td>
<td>26.5 ± 4.3</td>
</tr>
<tr>
<td>TM vs MM</td>
<td>54.3 ± 13.6</td>
<td>53.4 ± 13.6</td>
<td>121/136</td>
<td>133 ± 16</td>
<td>83 ± 9</td>
<td>5.70 ± 0.81</td>
<td>3.85 ± 0.46</td>
<td>1.40 ± 0.41</td>
<td>0.97 ± 0.43</td>
<td>5.00 ± 0.75</td>
<td>26.7 ± 4.2</td>
</tr>
<tr>
<td>P Value</td>
<td>0.28</td>
<td>0.27</td>
<td>0.98</td>
<td>0.83</td>
<td>0.94</td>
<td>0.57</td>
<td>0.91</td>
<td>0.83</td>
<td>0.83</td>
<td>0.91</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Mean ± SD. LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index.

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A

B

* $P = 0.014$

* $P = 0.007$

Association between polymorphisms of the CX3CR1 and carotid IMT.
TABLE 2. IMT According to Combined CX3CR1 Genotype

<table>
<thead>
<tr>
<th>Combined Genotype</th>
<th>V249I</th>
<th>T280M</th>
<th>n (%)</th>
<th>IMT</th>
<th>P Value vs VI/MM</th>
<th>P Value vs VI/MM + II/MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>VV</td>
<td>TT</td>
<td>671 (57.4)</td>
<td>0.64±0.14</td>
<td>0.040</td>
<td>0.017</td>
</tr>
<tr>
<td>2</td>
<td>VI</td>
<td>TT</td>
<td>166 (14.2)</td>
<td>0.66±0.15</td>
<td>0.019</td>
<td>0.007</td>
</tr>
<tr>
<td>3</td>
<td>VI</td>
<td>TM</td>
<td>219 (18.7)</td>
<td>0.66±0.13</td>
<td>0.017</td>
<td>0.006</td>
</tr>
<tr>
<td>4</td>
<td>II</td>
<td>TM</td>
<td>40 (3.4)</td>
<td>0.66±0.12</td>
<td>0.046</td>
<td>0.034</td>
</tr>
<tr>
<td>5</td>
<td>II</td>
<td>TT</td>
<td>35 (3.0)</td>
<td>0.68±0.15</td>
<td>0.014</td>
<td>0.008</td>
</tr>
<tr>
<td>6</td>
<td>II</td>
<td>MM</td>
<td>16 (1.4)</td>
<td>0.59±0.11</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>VI</td>
<td>MM</td>
<td>21 (1.8)</td>
<td>0.58±0.14</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>6 and 7</td>
<td>VI/MM</td>
<td>MM</td>
<td>37 (3.2)</td>
<td>0.58±0.12</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Mean±SD.

No association between IMT and V249I variants was observed (Figure), whereas significantly reduced IMT was observed in subjects with the MM280 genotype (0.57±0.12 mm) compared with subjects with the TT (0.65±0.14 mm) or the TM (0.65±0.13 mm) genotype (P=0.014 and P=0.007, respectively; Figure). In addition, the relationship between MM280 and lower IMT remained significant after multivariate adjustment for age, plasma lipids, glycemia, and blood pressure. When the interaction between lifestyle-related risk factors such as smoking with the T280M polymorphism was investigated, MM280 frequent smokers (>10 cigarettes for day) showed a significant lower IMT (0.63±0.13 mm) compared with TT (0.67±0.11 mm) or the TM (0.69±0.10 mm) carriers (P=0.049 and P=0.018, respectively), confirming the protective role of the MM280 genotype independent of smoking.

The analysis of the combined genotype showed that of the 9 possible genotypes, 7 genotypes were detected in our population (Table 2), with the VV249/TT280 being the most represented (56.8% of the population). Subjects with the VI249/MM280 or the II249/MM280 genotype showed significantly reduced IMT compared with subjects with the other combined genotypes (Table 2).

Discussion

The major finding of this study is that human subjects with the fractalkine receptor M280 rare allele have a reduced IMT compared with individuals homozygous or heterozygous for the T280 allele.

CX3CR1 is involved in tethering and arrest of leukocytes, thus it has been suggested as a key mediator of atherogenesis. Pioneer studies investigated the role of CX3CR1 polymorphisms in small cohorts of case-control studies showing that the presence of the I249/M280 haplotype is associated with a reduced risk of acute coronary events and coronary artery disease. In a large case-control study, only the presence of the M280 allele was associated with lower risk of cardiovascular disease, a protective effect on the occurrence of acute coronary syndrome and internal carotid artery occlusive disease, whereas the presence of the I249 in the absence of M280 was associated with a worse outcome.

Our results extend these findings as we demonstrate for the first time in a large free-living population setting a correlation between the presence of the M280 allele and carotid IMT that appears to be independent of the major risk factors for cardiovascular disease. Our data suggest a possible protective effect of the M280 allele on the early stages of atherosclerosis and the associated inflammatory response, in agreement with the observation by McDermott showing that leukocytes from homozygous M280/I249 donors have a markedly decreased adhesive function, signaling, and chemotaxis, setting the stage for further investigation in this area.

A limitation of this article is that no data on the progression of the disease are available yet. We are currently collecting prospective data at the 3-year re-examination on the whole population. A second limitation is that we have not addressed the role of diabetes on the effects of the M280 genotype of CX3CR1 on IMT. This is because of the low incidence of diabetes (3%) in the PLIC population; nevertheless, after adjustment for glucose levels, the protective effect of the M280 allele remained significant.

In conclusion, we show in a large cohort of subjects that the presence of the M280 genotype of CX3CR1 is associated with a decreased carotid IMT, suggesting that also in humans, the fractalkine receptor plays a major role on atherogenesis.

Acknowledgments

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References


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