Association study of the platelet collagen receptor glycoprotein VI gene with rheumatoid arthritis

L. Michou¹, F. Cornélis²,³
M. Baron⁴, S. Bombardieri⁵
A. Balsa⁶, R. Westhovens⁷
P. Barrera⁶, H. Alves⁸
T.R.D.J. Radstake⁶, P. Migliorini⁹
T. Bardin⁶,⁷, E. Petit-Teixeira⁴
E. Boilard⁴

¹Department of Medicine, Faculté de Médecine de l’Université Laval, CHUQ Research Centre and Division of Rheumatology, CHUQ, Quebec, Canada; ²GenHotel-Auvergne, CHU Clermont-Ferrand, France; ³GenHotel-EA3886, Évry-Val-d’Essonne University, Évry-Genopole, France; ⁴Department of Microbiology, Infectious Diseases and Immunology, Faculté de Médecine de l’Université Laval, Rheumatology and Immunology Research Center, CHUQ Research Centre, Quebec, Canada; ⁵Department of Rheumatology, Pisa University, Pisa, Italy; ⁶La Paz Hospital, Madrid, Spain; ⁷Rheumatology KU Leuven, Leuven, Belgium; ⁸Nijmegen University, Nijmegen, and Department of Rheumatology and Clinical Immunology University Medical Center Utrecht, Utrecht, The Netherlands; ⁹Porto San Joao Hospital, Porto, Portugal; ¹⁰Fédération de Rhumatologie, Pôle de l’Appareil locomoteur, Lariboisière Hospital, Paris, France.

Laëtitia Michou, François Cornélis, Morgane Baron, Stefano Bombardieri, Alejandro Balsa, René Westhovens, Pilar Barrera, Helena Alves, Timothy R.D.J. Radstake, Paola Migliorini, Thomas Bardin, Elisabeth Petit-Teixeira, Eric Boilard

Please address correspondence to: Eric Boilard, PhD, Centre de Recherche en Rhumatologie et Immunologie, Faculté de Médecine de l’Université Laval, 2705 Laurier Blvd., room T1-49, Quebec G1V 4G2, Canada.
E-mail: eric.boilard@cruqh.ulaval.ca

Received on January 9, 2013; accepted in revised form on February 13, 2013. © Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2013.

Key words: platelets, glycoprotein VI, rheumatoid arthritis, single nucleotide polymorphism, haplotype

Competing interests: none declared.

ABSTRACT

Objective. Beyond their role in haemostasis, platelets can actively contribute to immunity. The activation of the platelet collagen receptor glycoprotein VI (GPVI) promotes the release of small extracellular vesicles called microparticles. These microparticles are found in the joint bathing fluid of patients with rheumatoid arthritis (RA) and are thought to amplify inflammation. The gene coding for GPVI is localised on chromosome 19q13.4 and contains different single nucleotide polymorphisms (SNPs). Five non-synonymous SNPs define the major and minor haplotypes of GPVI. The minor haplotype is associated with higher risk of cardiovascular incidents. In this study, we examined whether this minor haplotype is also associated with RA.

Methods. Allelic discrimination of the SNPs reported to define these haplotypes encoding SKTQH and PEALN protein isoforms, ie rs1613662, rs1654416, rs2304167, rs1654413 and rs1671152, was performed in 399 RA patients and their two parents, all of Western European ethnicity. Statistical analysis relied on the transmission disequilibrium test by the use of the FBAT programme. Haplotypes were also estimated by the FBAT programme.

Results. We observed no statistically significant transmission disequilibrium for the SNPs tested. The major haplotype TAAC, which encodes the SKTQH protein isoform, was identified in 78% of our cohort individuals, and the CGGA haplotype which encodes the PEALN isoform was identified in 8% of our individuals. We observed no association of these haplotypes of the GPVI gene with RA.

Conclusions. This demonstrates that the SNPs tested within the GPVI gene are not associated with RA susceptibility and/or severity, suggesting that platelet GPVI may contribute to arthritis independently of its gene polymorphism.

Introduction

Platelets circulate in blood and play key roles in haemostasis (1). When damage to blood vessels occurs, there is exposure of the collagen-rich subendothelial matrix, thereby promoting platelet activation, platelet deposition and the formation of thrombus (1). While the formation of a platelet plug is crucial for the prevention of blood loss, platelet activation in the case of atherosclerotic plaque rupture or erosion can be particularly detrimental (1). Glycoprotein VI (GPVI) is a major receptor for collagen expressed uniquely by platelets. GPVI forms a complex with the homodimeric Fc receptor γ-chain (FcRγ) and consists of two extracellular immunoglobulin-like domains, a core region and a short cytoplasmic domain (2). Following GPVI activation, the tyrosine residues contained in the immunoreceptor tyrosine activation motif of the FcRγ-chain are phosphorylated, promoting Syk binding and the initiation of a kinase activation cascade. This process triggers the activation of phospholipase C-γ2 and calcium release and culminates in platelet aggregation (2).

Several single nucleotide polymorphisms (SNPs) of the GPVI gene have been described (3, 4). Two major haplotypes of GPVI that differs by five amino acid substitutions were identified: Ser219Pro, Lys237Glu, Thr249Ala, Gln317Leu and His322Asn. The minor haplotype is present in ~15% of the Caucasian population and is characterised by reduced surface expression and diminished functional activity (5). Intriguingly, the presence of this haplotype is associated with increased risks of coronary artery diseases, especially myocardial infarction, in various populations (6-10).

In addition to their role in haemostasis, platelets play functions in immunity (11). Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by the inflammation and the progressive destruction of the joints. Platelets are suggested to contribute to inflammatory arthritis (11); mechanistically, GPVI activation initiates platelet release of small extracellular vesicles, called microparticles, rich in inflammatory cytokine IL-1 (11). These microparticles are detected in the synovial fluid of patients with RA and are thought to amplify inflammation (11). Importantly, the mice that lack the expression of GPVI develop reduced arthritis compared to their wild...
type counterparts, pointing to a role of GPVI in the establishment of arthritis in vivo (11).

The aim of this study was to determine whether GPVI polymorphisms are associated with RA. We performed a genetic association study of non-synonymous SNPs defining a major and a minor haplotype encoding SKTQH and PEALN protein isoforms, in 399 trio families of Western European ethnicity.

**Methods**

This study was approved by the Ethics Committee of Hôpital Kremlin-Bicêtre (Paris, France) and all individuals provided an informed consent before participating in the study. Our cohort comprised 399 trio families and was previously reported in detail (12). Trio families are composed of one RA patient and both parents, all of Western European ethnicity. The families were recruited through the European Consortium on Rheumatoid Arthritis Families in France, Italy, Portugal, Spain, Belgium and the Netherlands. Index cases of trio families have a phenotype that fulfilled the ACR 1987 criteria for RA (13). 86% of our index cases were women. The mean age at RA onset was 31.1±9.5 years and the mean disease duration was 10.3±7.9 years. 72% of index cases had a positive rheumatoid factor (RF), 75% had erosions and 18% had rheumatoid nodules. Families with an additional affected sibling and RA patients under 18 years of age were excluded from the study.

We planned to genotype five non-synonymous SNPs that cover the major variability of the gene. These SNPs were previously reported to define a major and a minor haplotype encoding SKTQH and PEALN protein isoforms of the GPVI gene, i.e. rs16133662, rs1654416, rs2304167, and rs1671152 SNPs were estimated with the FBAT programme (14). For all haplotypes with an overall frequency >2%, observed haplotype frequencies were compared to expected frequencies. We also performed transmission disequilibrium tests in three clinically relevant subgroups: in a first subgroup of 298 (out of 393) trio families in whom the index cases were positive for RF, in a second subgroup of 181 (out of 287) trio families for whom the index cases were positive for anti-CCP antibodies and in a third subgroup of 307 (out of 393) trio families in whom the index cases have erosive RA. Conservative Bonferroni’s correction was applied for multiple testing and uncorrected p<0.0125 (0.05/4) was considered statistically significant in the SNP analysis, and for the haplotype analysis. The power of our sample of 399 trio families to provide an association with an OR of 1.6 is up to 80% considering the hypothesis of one gene following an additive model of inheritance, as determined by the use of the computer programme QUANTO 1.1 (http://hydra.usc.edu/gxe).

**Results**

Genotyping of our cohort of trio families revealed that the four SNPs were in Hardy-Weinberg equilibrium in the control group (data not shown). We observed no statistically significant transmission disequilibrium for the four SNPs tested (p-values=0.61, 0.60, 0.26 and 0.87, respectively) (Table I). The TDT in the three clinically relevant subgroups provided no evidence of association with RA (data not shown).

Regarding the contribution of GPVI to inflammatory arthritis, our results suggest that platelet GPVI may play a role in inflammation independently of its gene polymorphisms. Indeed, whether the different GPVI isoforms promote the release of inflammatory microparticles differently is still unknown. Further, the contribution of additional platelet receptors and factors, all capable of amplifying the GPVI response, may trigger sufficient platelet activation independently of the isoform expressed. Other genes coding for inflammatory mediators also show no association with RA: TNF-α for instance is not associated with RA in Europeans (16) although the role of TNF-α in this pathology is well documented. Only clinical studies will contribute to de-
The European Consortium on Rheumatoid Arthritis Families (ECRAF)
F. Cornélis (coordinator), T. Bardin (France); P. Migliorini, S. Bombardieri (Italy); R. Westhovens, J. Dequeker (Belgium), A. Balsa, D. Pascaule-Salcedo (Spain); P. Barrera, L. Van de Putte, P. Van Riel, T.R. Radstake (The Netherlands); H. Alves, A. Lopes-Vaz, M. Fernandes, C. Vaz (Portugal).

Funding
L. Michou is supported by a career award from the Fonds de la Recherche en Santé du Québec. E. Boilard is the recipient of a career award from the Canadian Arthritis Network and the Fonds de Recherche en Santé du Québec.

This research was financially supported by Canadian Institutes of Health Research and The Arthritis Society.

Acknowledgments
The authors are grateful to the RA patients, their family and rheumatologists for their participation in this study. The authors thank Dr Sandra Lasbleiz and Dr Pierre Fritz for reviewing clinical data, and the Association Française des Polyarthritiques, the Association Rhumatisme et Travail, the European Union for AutoCure, the Société Française de Rhumatologie, the Association Polyarthrite, the GroupeTaitbout, Genopole (France), and the AutoCure European Consortium.

References

Table I. Results of the transmission disequilibrium test according the family-based test for each SNP.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>Allele frequency</th>
<th>Number of informative families</th>
<th>S</th>
<th>E (S)</th>
<th>Var (S)</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1613662</td>
<td>C</td>
<td>0.16</td>
<td>171</td>
<td>101.0</td>
<td>97.5</td>
<td>47.25</td>
<td>0.61</td>
</tr>
<tr>
<td>rs1654416</td>
<td>G</td>
<td>0.16</td>
<td>158</td>
<td>95.0</td>
<td>91.5</td>
<td>44.75</td>
<td>0.60</td>
</tr>
<tr>
<td>rs2304167</td>
<td>G</td>
<td>0.16</td>
<td>155</td>
<td>98.0</td>
<td>90.5</td>
<td>44.25</td>
<td>0.26</td>
</tr>
<tr>
<td>rs1671152</td>
<td>A</td>
<td>0.14</td>
<td>138</td>
<td>77.0</td>
<td>78.0</td>
<td>38.5</td>
<td>0.87</td>
</tr>
</tbody>
</table>

S: observed number of transmission; E (S): expected value of S; Var (S): variance.

Table II. Results from haplotypes* using the family-based association test.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Haplotype frequency</th>
<th>Number of informative families</th>
<th>S</th>
<th>E (S)</th>
<th>Var (S)</th>
<th>Uncorrected p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAAC*</td>
<td>0.78 127</td>
<td>184.00</td>
<td>184.50</td>
<td>47.25</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>CGGA*</td>
<td>0.08 66</td>
<td>39.00</td>
<td>38.00</td>
<td>20.50</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>CAAC</td>
<td>0.06 59</td>
<td>32.00</td>
<td>30.50</td>
<td>14.75</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>TGGA</td>
<td>0.04 36</td>
<td>24.00</td>
<td>22.00</td>
<td>9.00</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

S: observed number of transmission; E (S): expected value of S; Var (S): variance.

* The major haplotype TAAC encodes the SKTQH protein isoform.
* The minor haplotype CGGA encodes the PEALN isoform of the GPVI protein.
* Haplotypes consisting of the rs1613662, rs1654416, rs2304167, and rs1671152.