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

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ABSTRACT

Objectives. *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 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 Cγ2 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.* rs1613662, rs1654416, rs2304167, rs1654413 and rs1671152 (4,5). Genotyping of SNPs relied on the TaqMan® allelic discrimination assay and was performed by KBiosciences (Hertfordshire, UK). For technical reasons, the genotyping data for rs1654413 were not obtained, but the genetic information missed by this 5th SNP is probably low since it was reported to be in strong linkage disequilibrium (D'= 0.96) with rs1671152 in Caucasians (8). Centre d'Étude du Polymorphisme Humain DNA samples were co-genotyped with all our samples, with no inconsistencies detected. The genotyping success rate was 95%. For each SNP, Hardy-Weinberg equilibrium was assessed with a conformity chi-square test in the virtual control group consisting of the two parental chromosomes, one from the mother and one from the father, which were not transmitted to the affected offspring. A transmission disequilibrium test (TDT) allowed the comparison of the transmission of a given parental allele from a heterozygous parent to the RA affected patient with an expected transmission of 50% (Mendel's law). TDT analysis SNP by SNP was performed with the FBAT programme (14). Haplotypes consisting of the rs1613662, 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 390) 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)*. The major haplotype *TAAC*, which encodes the SKTQH isoform of the GPVI protein, was identified in 78% of our cohort individuals, while the *CGGA* haplotype, which encodes the PEALN isoform, was identified in 8% of our cohort individuals. We observed no association of these haplotypes or the two other minor haplotypes with an overall frequency >2%, *i.e. CAAC* and *TGGA*, of the *GPVI* gene with RA (p-values ranging from 0.50 to 0.94) (Table II).

Discussion

Our study reveals that the minor allele frequency of each SNP and the corresponding haplotypes frequencies were similar to those previously published in Caucasian populations (8), but that there exists no association of non-synonymous SNPs and haplotypes of the *GPVI* gene with RA. Although we cannot fully rule out an insufficient power in our study and the gene was only partially covered by the selected Tag-SNPs, our results are consistent with the genome wide association studies (GWAS), which did not demonstrate any genetic association at the *19q13* locus with RA (14, 15).

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-

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

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

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

Table II. Results from haplotypes[a] using the family-based association test.

Haplotype	Haplotype frequency	Number of informative families	S	E (S)	Var (S)	Uncorrected *p*-value
TAAC*	0.78	127	184.00	184.50	47.25	0.94
CGGA[#]	0.08	66	39.00	38.00	20.50	0.83
CAAC	0.06	59	32.00	30.50	14.75	0.67
TGGA	0.04	36	24.00	22.00	9.00	0.50

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.
[a]Haplotypes consisting of the rs1613662, rs1654416, rs2304167, and rs1671152.

termine the role of platelets, and GPVI more specifically, in RA.

Thus, the platelet contribution to inflammation notwithstanding, our association study excludes GPVI as a RA gene. We suggest that the inflammatory functions mediated by platelet GPVI may take place independently of its gene polymorphism.

The European Consortium on Rheumatoid Arthritis Families (ECRAF)

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