**FCGR2B gene polymorphism rather than FCGR2A, FCGR3A and FCGR3B is associated with anti-GBM disease in Chinese**

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**Abstract**

**Background.** The Fcγ receptors play important roles in anti-glomerular basement membrane antibody disease (anti-GBM disease) in animal models, and FCGR gene polymorphisms have been reported to be associated with numerous human autoimmune diseases. We aimed to clarify the genetic association of FCGR gene polymorphisms with anti-GBM disease in Chinese patients.

**Methods.** A total of 48 patients with anti-GBM disease and 225 geographically and ethnically matched healthy controls were involved. Genotyping of the previously identified polymorphisms FCGR2A131H/R (rs1801274), FCGR2B 232I/T (rs1050501) and FCGR3A176F/V (rs396991) were detected by the TaqMan genotyping assay and FCGR3B NA1/2 by the PCR-sequence specific primer (SSP). Allele type, genotype and haplotype of identified polymorphisms were analysed between patients and controls.

**Results.** Our results revealed that FCGR2A131H/R, FCGR3A176F/V and FCGR3B NA1/2 were not associated with anti-GBM disease. The frequency of the FCGR2B 232T allele (30.2% versus 15.6%, corrected P = 0.00028, 95% CI: 1.42–3.89) and genotypes of I232T (60.4% versus 31.1%, corrected P = 0.0004, 95% CI: 1.78–6.43) was significantly increased in patients compared with controls.

**Conclusion.** The present study demonstrates the genetic association of polymorphism of FCGR2B (I232T) with susceptibility to anti-GBM disease in Chinese.

**Keywords:** anti-glomerular basement membrane antibody disease; Fcγ receptors; FCGR gene polymorphism

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**Introduction**

Anti-glomerular basement membrane antibody disease (anti-GBM disease) is an uncommon yet well-known condition because of the striking way in which it can cause rapid renal failure and lung bleeding. The aetiology of anti-GBM disease is obscure although environmental factors, including infective agents, hydrocarbon exposure, lithotripsy and urinary obstruction and cigarette smoking, as well as genetic factors were reported in the literature [1–4].

As in other autoimmune diseases, anti-GBM disease has been strongly associated with the major histocompatibility complex (MHC) [5–9]. Genotyping studies have confirmed that anti-GBM disease is associated with HLA DRB1*1501 and 1502, but the precise molecular basis of these MHC associations remains to be determined [9], and it does not by itself determine the occurrence of anti-GBM antibody-mediated disease because of the high frequency in populations [2,8]. It is therefore clear that additional genetic factors are required to explore the disease expression.

Fcγ receptors (FcγR) expressed on the surface of leukocytes bind the Fc (constant) portion of IgG. They link the humoral and cellular branches of the immune system, including phagocytosis, cytokine release, formation of reactive oxygen species and antibody-dependent cytotoxicity. Activating and inhibitory FcγRs co-exist on the surface of cells and set thresholds for immune responses [10–12]. Studies of gene-targeted mice lacking specific FcγR, either activating or inhibitory FcγR, have demonstrated that the FcγR is involved in the susceptibility of anti-GBM disease [13–16]. Growing evidence supports that FcγR dysfunction and genetic variants of FcγRIIA, IIIA, IIIB (activating FcγR) and IIB (inhibitory FcγR) are associated with human autoimmune kidney diseases, including systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA)-associated small vasculitis (AASV) [17]. However, to date there are no data examining the association of FcγR polymorphisms with anti-GBM disease.

Anti-GBM disease is a rare disease, and its genetic association study depends on the patient collection to some extent. Thus, the present study was designed to detect the identified polymorphisms of FcγRs in a comparatively large population of patients with anti-GBM disease.
Subjects and methods

Subjects

FCGR gene (encoding FcγR) polymorphisms were determined using genomic DNA from 273 Chinese, including 48 patients with anti-GBM disease and 225 healthy controls. All patients with anti-GBM disease were recruited from Peking University First Hospital, and were diagnosed by serum with a positive anti-GBM antibody, renal biopsy with crescentic glomerulonephritis and linear deposition of immunoglobulin G (IgG) along the GBM [2,3]. The control group consisted of 225 geographically and ethnically matched healthy blood donors.

The protocol for the genetic study was approved by the medical ethics committee of Peking University, and informed written consent was obtained from all participants.

Genotyping

Genomic DNA was isolated from whole blood using a modified salt extraction technique [18]. FCGR2A131H/R (rs1801274), FCGR2B 232I/T (rs1050501) and FCGR3A176V/F (rs396991) (also referred to as 158F/V by counting from the N-terminus of the mature protein after cleavage of the signal peptide) were genotyped using the Taqman assay with supplied probes and primers on an ABI PRISM Sequence Detection System 7500 (Applied Biosystems, Foster City, CA, USA). FCGR3B NA1/2 genotyping was performed by a PCR-sequence-specific primer (SSP) as previously described [19]. Genotypes were confirmed by direct sequencing of PCR products for selected cases of homozygote and heterozygote.

Statistical analysis

Statistical analyses for an association were performed by SPSS version 12.0 (SPSS, Chicago, IL, USA). Chi-square tests were used to analyse the association of the four FCGR polymorphisms with susceptibility to anti-GBM disease. When sample numbers were small, Fisher’s exact tests were used. Haplotype frequencies were estimated from typing results using the Faster Estimating Haplotypes PLUS (FALSEHPLUS) program [20,21]. The association with the disease was tested by comparing the haplotype frequencies estimated from cases and controls separately with estimates based on the combined sample, using a likelihood ratio test. The heterogeneity test within the Permutation and Model-free analysis implemented in the program was used to assess statistical significance based on 1000 permutations. Linkage disequilibria were also estimated for the four polymorphisms in the study population, using the Haploview software V3.32 (http://www.broad.mit.edu/mpg/haploview/contact.php) [22]. Two-sided P-values <0.05 were considered as significant. To account for multiple testing, the Bonferroni method was used.

Results

Clinical features of patients and controls

The average age of patients was 34.9 ± 17.0 years (range 13–82 years), including 31 males and 17 females (35.4%). All patients presented with a serum positive anti-GBM antibody and rapidly progressive glomerulonephritis. Lung haemorrhage was evident in 35.4% of (17/48) patients. All patients presented with a serum positive anti-GBM antibody, renal biopsy with crescentic glomerulonephritis and linear deposition of immunoglobulin G (IgG) along the GBM [2,3]. The control group consisted of 225 geographically and ethnically matched healthy blood donors.

The protocol for the genetic study was approved by the medical ethics committee of Peking University, and informed written consent was obtained from all participants.

The healthy controls LD analysis revealed that associated alleles were similar to previous reports [23–26]. The distributions of FCGR2A131H/R, FCGR3A176V/F, FCGR2B NA1/2 and FCGR3B NA1/2 between the patient group and control. Allele frequencies of 232I (69.8% versus 84.4%) and 232T (30.2% versus 15.6%) in FCGR2B were significantly different between patients with anti-GBM disease and healthy controls, and the presence of the 232T allele (30.2% versus 15.6%, corrected P = 0.00028, 95% CI: 1.42–3.89) and genotypes of 1232T (60.4% versus 31.1%, corrected P = 0.0004, 95% CI: 1.78–6.43) was significantly increased in patients compared with controls.

However, no significantly different distribution of FCGR2A 131H/R, FCGR3A 176V/F or FCGR3B NA1/2 between the patients and controls was observed.

Linkage disequilibrium at the FCGR genetic locus in healthy Chinese individuals and anti-GBM disease

Alignment of FCGR gene order from centromere to telomere at chromosome 1q23 is as follows: FCGR2A, FCGR3A, FCGR2C, FCGR3B and FCGR2B. LD values were generated to look for an association among the four studied polymorphisms. A pair-wise comparison of the polymorphisms, depicting the LD measures in terms of D’ by Haploview, is represented graphically in Figure 1. The combinations of associated alleles were similar to previous reports [23–26]. The healthy controls LD analysis revealed that r2 values between FCGR2A131H/R (rs1801274), FCGR3A176V/F (rs396991), FCGR3B NA1/2 and FCGR2B 232I/T (rs1050501) were 0.02, 0.03 and 0.02, respectively. On the other hand, anti-GBM analysis revealed that r2 values were 0.04, 0.05 and 0.39, respectively. Studying the linkage disequilibrium of the anti-GBM samples revealed that FCGR3B NA1/2 and FCGR2B 232I/T were in high linkage disequilibrium and transmitted as one block contrasting healthy controls.

Association of FCGR haplotypes with anti-GBM disease

The distributions of FCGR haplotypes were compared between the anti-GBM cohorts and control populations; FCGR3B–FCGR2B (χ2 = 18.65, df = 3, P = 0.0003) showed a statistically significant difference. The FCGR3B–FCGR2B NA2–232T (30.2% versus 12.4%) haplotype of patients was found at increased frequency compared with the controls.

Discussion

Anti-GBM disease, which was one of the earliest recognized human autoimmune diseases, was still a sticky challenge for nephrologists for its urgency and severity. In spite of great efforts made in the pathogenesis, the clear mechanisms were still obscure. It is a pivotal approach to seek genetic factors to elucidate its potential pathogenesis.

Receptors for the Fc region of IgG (FcγR) provide a link between the antigen-specific antibody and non-specific cellular responses of the innate immune system. They are mainly expressed on cells of haemopoietic lineage, and have
important functions in the activation and down-modulation of immune responses. Three classes of human FcyRs, RI (CD64), RII (CD32) and RIII (CD16), which generate multiple isoforms, are recognized. In humans, except for inhibitory FcγRIIb, all other FcγRs are activating receptors [10,11]. In murine experimental nephrotoxic serum nephritis (anti-GBM disease model), the disease can be prevented by lack of the activating FcγRs (FcγRI, FcγRIII or FcγRIV depending on the specific IgG subclasses) and exacerbated by lack of the inhibitory receptor FcγRIIB or transgene of human FCGR2A [15,16,27–31]. In human autoimmune diseases, previous studies have shown that genetic variants in FcγRs, especially FCGR2A131H/R, FCGR2B 232I/T, FCGR3A176V/F and FCGR3B NA1/2, were reported to be associated with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Wegener’s granulomatosis, Guillain–Barré syndrome and multiple sclerosis [10,13]. This provides important clues for anti-GBM disease and at the same time requires validation in anti-GBM disease regarding autoimmunity.

In the present study, we are the first to validate that the identified variations of FcγRs in human autoimmune diseases were involved in susceptibility to human anti-GBM disease in a comparatively large cohort. The results demonstrated an association of the FCGR2B 232I/T (rs1050501) polymorphism with susceptibility to anti-GBM disease in the Chinese population. However, a deviation from H–W equilibrium for FCGR2B 232I/T was seen (healthy controls $P = 0.006$; anti-GBM patients $P = 0.003$) because no FCGR2B 232T/T homozygotes were

### Table 1. FCGR2A, FCGR2B, FCGR3A and FCGR3B polymorphisms in patients with anti-GBM disease and healthy controls in Chinese

<table>
<thead>
<tr>
<th>Genotype frequency</th>
<th>Patients $(n = 48)$</th>
<th>Controls $(n = 225)$</th>
<th>$\chi^2$</th>
<th>$P$</th>
<th>Corrected $P$</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FCGR2A</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>131 R/R</td>
<td>9(18.8)</td>
<td>22(9.8)</td>
<td>3.164</td>
<td>0.075</td>
<td></td>
<td></td>
</tr>
<tr>
<td>131 R/H</td>
<td>17(35.4)</td>
<td>100(44.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>131 H/H</td>
<td>22(45.8)</td>
<td>103(45.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FCGR2B</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>232 T/T</td>
<td>0(0)</td>
<td>0(0)</td>
<td>14.699</td>
<td>0.0001</td>
<td>0.0004</td>
<td>3.38(1.78–6.43)</td>
</tr>
<tr>
<td>232 T/I</td>
<td>29(60.4)</td>
<td>70(31.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>232 I/I</td>
<td>19(39.6)</td>
<td>155(68.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FCGR3A</strong></td>
<td></td>
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</tr>
<tr>
<td>176 V/V</td>
<td>3(6.3)</td>
<td>16(7.1)</td>
<td>0.046</td>
<td>0.829</td>
<td></td>
<td></td>
</tr>
<tr>
<td>176 V/F</td>
<td>21(43.8)</td>
<td>94(41.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>176 F/F</td>
<td>24(50.0)</td>
<td>115(51.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FCGR3B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA 2/2</td>
<td>10(20.8)</td>
<td>48(21.3)</td>
<td>0.006</td>
<td>0.939</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA 1/2</td>
<td>30(68.8)</td>
<td>124(55.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA 1/1</td>
<td>8(16.67)</td>
<td>53(23.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Allele positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2A R present</td>
<td>26(54.2)</td>
<td>122(54.2)</td>
<td>0.139</td>
<td>0.709</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2B T present</td>
<td>29(60.4)</td>
<td>70(31.1)</td>
<td>6.07</td>
<td>0.014</td>
<td>0.055</td>
<td>1.94(1.14–3.31)</td>
</tr>
<tr>
<td>FCGR3A V present</td>
<td>24(50.0)</td>
<td>110(48.9)</td>
<td>0.002</td>
<td>0.962</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR3B NA2 present</td>
<td>40(83.3)</td>
<td>172(76.4)</td>
<td>0.102</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Allele frequency</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2A R allele</td>
<td>35(36.5)</td>
<td>144(32.0)</td>
<td>0.714</td>
<td>0.398</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR2B T allele</td>
<td>29(30.2)</td>
<td>70(15.6)</td>
<td>11.444</td>
<td>0.001</td>
<td>0.00028</td>
<td>2.35(1.42–3.89)</td>
</tr>
<tr>
<td>FCGR3A V allele</td>
<td>27(28.1)</td>
<td>126(28.0)</td>
<td>0.001</td>
<td>0.980</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FCGR3B NA2 allele</td>
<td>50(52.1)</td>
<td>228(48.9)</td>
<td>0.168</td>
<td>0.682</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| OR, odds ratio; 95% CI, 95% confidence interval. OR values are presented only for significant associations (in boldface). $P$-values were calculated by $\chi^2$-test using 2 × 2 contingency table (d.f. = 1). FCGR2A: RR versus RH + HH; R versus H. FCGR2B: TT + TI versus II; I versus T. FCGR3A: VV versus VF + FF; V versus F . FCGR3B: NA2/2 versus NA1/2 + NA1/1; NA2 versus NA1.
observed. The percentage of observed homozygotes for this locus was smaller than expected. The frequencies of the \( FCGR2B 232T/T \) genotype and the \( FCGR2B 232T \) allele vary greatly in the populations worldwide [23–26].

In the Asian and African populations, the frequency of \( FCGR2B 232T \) allele is higher than that in Europeans. The \( 232T \) allele frequency was 15.6% in our study, which was lower than that in the previous report in Asians (Japanese 22%, Chinese 22%, African American 29%) and higher than Caucasian (13%). Even assuming that the \( 232T \) allele frequency was 22% in the report from the Asian population, it was still increased in anti-GBM patients (30.2%) in our present study. For the precise genotyping (TaqMan genotyping and validation by sequencing), comparatively large sample size (48 anti-GBM disease patients) in our study, and the effect of the \( FCGR2B \) SNP being stronger in populations of Asian background than in Caucasians (strong association in Asians whereas lack of association in Caucasians) [23–26], we think the result still has indicating significance. Further validation may be required from a larger population with different ethnicity, especially Asian.

When the association of \( FCGR2B, FCGR2A, FCGR3A \) and \( FCGR3B \) polymorphisms with disease severity on diagnosis [32], such as the presence of serum creatinine >600 \( \mu \)mol/l, presence of oliguria or anuria, presence of high percentage (>85%) of glomeruli having crescents in patients with anti-GBM disease, was analysed, no notable associations were observed. On the other hand, when subdividing patients according to the presence of the \( FCGR2B 232T \) allele, patients’ clinical presentations were not associated with allele types. The possible reason may be that the sample size was limited and all the enrolled patients were in severe clinical manifestation and progressed rapidly to end-stage renal failure (ESRF).

The \( FCGR2B 232I/T \) polymorphism that induced ile232-to-thr in the transmembrane region of the \( FCGR2B \) gene was associated with SLE and malaria. Macrophages from \( FCGR2B 232T/T \) showed enhanced immune complex-triggered upregulation of surface MHC classes I and II and greater phagocytic capacity than the cells from \( 232T/T \) individuals. Peripheral B cells from \( 232T/T \) showed reduced \( FCGR2B \)-mediated inhibition of B cell receptor-triggered proliferation. Membrane separation studies in human monocytes revealed that although \( FCGR2B 232I \) readily partitioned into the raft-enriched gradient fractions, \( FCGR2B 232T \) was excluded from them. It indicated that \( FCGR2B 232T \) is unable to inhibit activating receptors because it is not present in sphingolipid rafts, resulting in unopposed proinflammatory signalling [33–35], and thus may promote anti-GBM disease. FcγR genes are in close proximity to each other on human chromosome 1; haplotypes analysis further demonstrated the role of \( FCGR2B \)-containing haplotypes in anti-GBM disease in our cohorts. Although 48 patients were not sufficient for an optimal association study, the patient cohort can hardly be enlarged for its rarity, and may deserve replication from another population. In spite of this, it was still of merit that the anti-GBM disease cohort was a ‘pure’ patient population owing to definite diagnosis criteria.

In conclusion, the present study by a comparatively large anti-GBM disease cohort has demonstrated that \( FCGR \) gene polymorphisms, specifically \( FCGR2B 232I/T \), are involved in the genetic susceptibility of anti-GBM disease in Chinese. It provides important clues for further studies on the precise mechanisms of anti-GBM disease.

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Conflict of interest statement. None declared.

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