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NA1/NA2 Heterozygote of Fcgr3b is a Risk Factor for Progression of IgA Nephropathy in Chinese

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Several studies have identified FcgRIIIb (Fcgr3b) polymorphisms that determine susceptibility to autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. The objective of the study was to clarify whether Fcgr3b allele polymorphism influence susceptibility to immunoglobulin A nephropathy (IgAN), clinical features or severity in patients with IgAN. Deoxyribonucleic acid (DNA) fragments were amplified by polymerase chain reaction (PCR) using genomic DNA from 172 unrelated, healthy blood donors and 128 IgAN patients in our Kidney Disease Centre. The present findings showed that Fcgr3b genotype influenced the disease susceptibility and severity of IgAN, although Fcgr3b polymorphism did not affect the age of the disease onset. We found that the genotype frequency of Fcgr3b heterozygote NA1/NA2 in IgAN patients in Chinese significantly higher than that of healthy donors. Furthermore, higher genotype frequency of NA1/NA2 was found also in IgAN patients with glomerulosclerosis or crescent formation than those without it. NA1/NA2 heterozygote of Fcgr3b is a risk factor for progression of IgA nephropathy in Chinese. J. Clin. Lab. Anal. 21:298–302, 2007.

Key words: Fc gamma receptor; polymorphism; IgA nephropathy

INTRODUCTION

The Fc receptors are glycoproteins found on the surface of hematopoietic cells that bind the Fc portion of immunoglobulin to provide a link between the humoral and cellular immune systems (1). By cross-linking Fc receptor-bearing cytocidal cells with the Fc portions of antibody and the Fab2 portions coating the target cells, antibodies can mediate antibody-dependent cell-mediated cytotoxicity (ADCC) (2). Cytolytic T lymphocytes (CTLs) and natural killer (NK) cells as well as macrophages and monocytes, which express Fc receptors for IgG, can mediate ADCC. The efficacy of IgG-induced Fc gamma receptor (FcγR) function displays interindividual heterogeneity due to genetic polymorphisms of three FcγR subclasses: FcγRIIa, FcγRIIIa, and FcγRIIIb (3).

FcγRIIIb (Fcgr3b, CD16) bears a neutrophil antigen polymorphism caused by four amino acid substitutions. Fcgr3b NA1 is more efficient in binding to immune complex (IC) containing IgG1 and IgG3 than Fcgr3b NA2 (2,4). The NA2 allele has been reported to be a susceptibility factor for systemic lupus erythematosus (SLE) in Japanese individuals (5).

CD16+ macrophages may be effector cells involved in the acute inflammation common to all types of proliferative glomerulonephritis including immnunoglobulin A nephropathy (IgAN) (6). To test the hypothesis that Fcgr3b polymorphism in Chinese are associated with IgAN, we measured Fcgr3b genotypes and applied this assay to 300 individuals from Zhejiang province, Eastern part of China. The aim of this retrospective study was to investigate the possibility of an association between the Fcgr3b gene polymorphism and the occurrence and severity in IgAN.

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PATIENTS AND METHODS

The study consisted of 172 healthy Chinese, and 128 biopsy-proven IgAN patients in the Kidney Disease Centre, First Affiliated Hospital, College of Medicine, Zhejiang University, China, between January 2004 and July 2006. The IgAN patients included 64 patients with grades I–II of pathological damage and 64 patients with grades IV–V of pathological damage. Healthy random blood donors were given physical examinations in the First Affiliated Hospital, College of Medicine, Zhejiang University. People who had albuminuria or hematuria were excluded from this control group. The study was approved by the local ethics committee at Zhejiang University, and informed consent was obtained from all subjects. The selection of this group was based on a diagnosis limited to primary glomerulonephritis and with a predominant deposition of IgA in the mesangium. No patients had clinical or histological evidence of systemic diseases, such as SLE, Henoch-Schönlein purpura, chronic liver disease, antineutrophil cytoplasmic antibody (ANCA)-related glomerulonephritis, or necrotizing vasculitis.

Deoxyribonucleic Acid (DNA) Samples

Genomic DNA was isolated from peripheral blood samples of 172 unrelated, healthy blood donors and 128 IgAN patients in the Kidney Disease Centre, First Affiliated Hospital, College of Medicine, Zhejiang University, after informed consent was obtained.

Fcgr3b Allotyping (NA1 and NA2)

Genotyping was performed as described previously (7). DNA fragments were amplified by polymerase chain reaction (PCR) using the following primers: Fcgr3b NA1-specific primer, 5′-CAG TGG TTT CAC AAT GTG AA-3′; Fcgr3b NA2-specific primer, 5′-CAA TGG TAC AGC GTG CTT-3′; and the reverse primer, 5′-ATG GAC TTC TAG CTG CAC-3′. The amplification procedure consisted of initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 96°C for 30 sec, annealing at 57°C for 50 sec, and extension at 72°C for 30 sec, followed by a final extension at 72°C for 10 min. The amplification products were electrophoresed in a 2% agarose gel and visualized by staining with ethidium bromide. The amplification products of NA1 and NA2 were expressed as 141 and 219 bp fragments, respectively.

Sequencing of the Fcgr3b Allele

In the above-mentioned allotyping, we performed direct sequencing using 20 PCR products of IgA nephropathy patients carrying different Fcgr3b forms according to the allele-specific PCR. PCR products were sequenced using either M13 rev or T7 primer (Invitrogen, San Diego, CA). Reactions were analyzed in a capillary sequencer (ABI Prism 310; PE Applied Biosystems, Boston, MA).

Calculation of Allele Frequencies

Calculation of allele frequencies was performed according to Steffensen et al. (8). Briefly, Fcgr3b NA1 and Fcgr3b NA2 were treated as two existing alleles of the same locus. Then the frequencies were calculated by counting the number of each allele and by calculating the percentage.

Classification of the Severity of IgAN

In this study, to avoid the possibility that the time of renal biopsy in relation to disease onset may influence the pathological severity, we also analyzed the age of onset and the duration from onset to renal biopsy in each pathological grade of IgAN patients. The onset of this disease was defined as the time of first abnormality in urinalysis. The pathological diagnoses by light microscopy of all patients with IgAN in our Kidney Centre fell within the grades I–V of pathological damage, according to the grading system of Lee et al. (9). The main levels of Lee's grading are: grade I, mostly normal glomeruli; grade II, less than half of the glomeruli show localized mesangial proliferation and sclerosis; grade III, diffuse mesangial proliferation and thickening with focal and segmental variation with focal interstitial edema and infiltrate occasionally present; grade IV, marked diffuse mesangial proliferation and sclerosis with tubular atrophy and interstitial inflammation; and grade V, similar to grade IV, but more severe.

Biopsies were performed using a 16-gauge needle guided by ultrasound localization. All patients gave their consent information. The diagnosis was based on pathological manifestation. For histological diagnosis, specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned to 2-μm-thick, and stained with hematoxylin-eosin, periodic acid-Schiff (PAS) and periodic acid silver methenamine (PASM). Two researchers examined these specimens separately in a double-blind manner.

Clinical Data

Though the study was retrospective, for each patient, clinical data including the duration from disease onset to renal biopsy and levels of serum albumin had been collected at the time of renal biopsy.

Statistical Analysis

All analyses were performed using SPSS version 11.5 (SPSS, Chicago, IL). Data were analyzed for differences

in distribution of Fcgr3b frequency among groups using the chi-squared test. A P value <0.05 was considered to be statistically significant.

RESULTS

To avoid the possibility that the time of the renal biopsy in relation to disease onset may influence the pathological severity, we analyzed the age of onset and the duration from onset to biopsy in each pathological grade of IgAN patients (Table 1). As shown in Table 1, no significant difference was observed in the age and the duration, indicating that this pathological grading can be used.

Table 2 showed an association between NA1/NA2 genotype and glomerulosclerosis or crescent formation in group of grades IV–V (Fisher's exact test).

The serum levels of IgG and IgA in Fcgr3b NA2/NA2 homozygous carriers were higher than those in NA1/NA1 homozygous carriers and NA1/NA2 heterozygous ones, although there were no significant differences (Table 3). Equally, no significant difference of serum levels of IgM was observed among the three types of carriers. However, between the group of grades I–II and the group of grades IV–V, the serum levels of IgG and IgA were significantly different (Table 3).

Fcgr3b NA1/NA2 genotype frequency showed significant difference between IgAN patients and healthy controls. In addition, the allele frequency showed no significant difference between the two groups (Table 4).

From Table 5, we found that the distributions of genotype frequency between the group of grades I–II and the group of grades IV–V were significantly different. It also suggested that homozygous carriers NA2/NA2 were observed commoner in group of grades IV–V than in grades I–II. In addition, patients with IgAN and the NA2/NA2 genotype had histologically more severe disease than IgAN patients of NA1/NA1 genotype, although there was not a significant difference in NA1 vs. NA2 allele frequency with regard to histologic severity.

DNA fragments, including the Fcgr3b polymorphic sites 141, 147, 227, and 277 from 20 IgAN patients carrying different genotypes were sequenced. No variants differing in single nucleotide position from

TABLE 1. Age of onset, and duration from onset to renal biopsy

	n	Age of onset[a]	Duration from onset to renal biopsy[b]
Grade			
Grades I–II	64	24.3±1.31	5.52±1.04
Grades IV–V	64	25.1±1.38	5.29±0.97
Genotype			
NA1/NA1	21	23.7±1.29	5.05±1.13
NA1/NA2	96	25.4±1.42	5.37±1.25
NA2/NA2	11	24.6±1.26	5.46±1.30

[a]Mean±standard error (SE) (years of age).
[b]Mean±SE (years).

TABLE 2. Association of genotypes and glomerular lesions between group of grades I–II and group of grades IV–V

	Glomerulosclerosis or crescent formation by renal biopsy		
Genotype	Yes	No	P value
Grades I–II			
NA1/NA1	6	8	>0.05
NA1/NA2	19	29	
NA2/NA2	2	1	
Grades IV–V			
NA1/NA	6	1	<0.05
NA1/NA2	43	5	
NA2/NA2	5	3	

TABLE 3. Association between Fcgr3b polymorphism and mean levels of serum Ig in IgAN patients

Genotype	IgG (mg/dL)[a]		IgA (mg/dL)[a]		IgM (mg/dL)[a]	
Grades I–II[b]						
NA1/NA1	1192±267	n.s	318±91	n.s	132±54	n.s
NA1/NA2	1216±273		325±93		129±51	
NA2/NA2	1243±279		342±99		141±60	
Grades IV–V[c]						
NA1/NA1	1337±307	n.s	384±118	n.s	142±57	n.s
NA1/NA2	1454±315		399±121		151±62	
NA2/NA2	1502±324		412±126		149±60	

[a]Values are mean±standard deviation (SD).
[b]Grades I–II vs. Grades IV–V in IgG, $P<0.05$.
[c]Grades I–II vs. Grades IV–V in IgA, $P<0.05$.

TABLE 4. Fcgr3b NA1/NA2 polymorphisms in Chinese IgAN patients and healthy controls

	IgAN (n = 128)	%	Healthy controls (n = 172)	%	χ^2	P value
Genotype frequency						
NA1/NA1	21	16.4	51	29.7	18.3	<0.005
NA1/NA2	96	75.0	87	50.6		
NA2/NA2	11	8.6	34	19.8		
Allele frequency						
NA1	138	53.9	189	54.9	0.054	>0.05
NA2	118	46.1	155	45.1		

TABLE 5. Fcgr3b NA1/NA2 polymorphisms in IgAN patients with grades I–II and grades IV–V

	Grades I–II	%	Grades IV–V	%	χ^2	*P* value
Genotype frequency						
NA1/NA1	14	21.9	7	10.9	7.73	<0.05
NA1/NA2	47	73.4	49	76.6		
NA2/NA2	3	4.7	8	12.5[a]		
Allele frequency						
NA1	75	58.6	63	49.2	2.30	>0.05
NA2	53	41.4	65	50.8		

[a]NA1/NA1 vs. NA2/NA2, Fisher's exact test, $P = 0.035$.

TABLE 6. Fcgr3b variants at nucleotide positions 141, 147, 227, and 277 with in exon 3, determined by DNA sequencing

		Nucleotide position				
Genotype pattern	IgAN (n = 20)	141	147	227	277	Fcgr3b form
NA1/NA1	3	G	C	A	G	NA1
NA1/NA2	15					
	6	G	C	A	G	NA1
	6	C	T	G	A	NA2
	2	C	T	G	G	Variant 1
	1	G	T	G	G	Variant 2
NA2/NA2	2	C	T	G	A	NA2

the known Fcgr3b NA1 form were found with in three NA1/NA1 individuals (Table 6), as well as the known Fcgr3b NA2 form with in 2 NA2/NA2 individuals. Three variants were found in 15 NA1/NA2 individuals, two were genotype variant 1, and one was variant 2.

DISCUSSION

Fc receptors, the genes of which are located in clusters across mammalian genomes, functionally link the humoral and cellular branches of the immune system and have a key role in activation and modulation of the immune response (10). FcγR polymorphisms are now considered to be heritable risk factors for autoimmune and infectious diseases, and support for a relevant role of these polymorphisms has been obtained in previous studies (11,12). Disease susceptibility linked to FcgR polymorphisms has been described for autoimmune diseases including SLE, RA, multiple sclerosis, and ANCA-positive systemic vasculitis (13).

IgAN is the most common form of primary glomerulonephritis and is characterized by depositions of IgA (mainly IgA1) or IgA-containing immune complexes (IgA-ICs) in the glomerular mesangial areas. IgA1 deposits were usually observed with complement 3 (C3) component, and often with IgG, IgM, or both in the glomerular mesangial areas (14). In addition, it has been reported that glomerular IgG deposition in the presence of normal renal function is a risk factor for renal survival in patients with IgAN (15).

In this study, we examined the association between allele polymorphism of Fcgr3b and the incidence of IgAN and histological severity in Chinese IgAN patients. The present findings showed that Fcgr3b genotype influenced the disease susceptibility and severity of IgAN, although Fcgr3b polymorphism did not affect the age of disease onset. We concluded that the genotype frequency of Fcgr3b heterozygote NA1/NA2 in IgAN patients in Chinese significantly higher than that of healthy donors. Furthermore, higher genotype frequency of NA1/NA2 was found also in IgAN patients with glomerulosclerosis or crescent formation than those without it. This study also suggested that homozygous carriers NA2/NA2 were observed commoner in group of grades IV–V than in grades I–II, but a larger number of study samples was required to draw the conclusion.

Some studies suggested that Fcgr3b deficiency does not seem to induce severe clinical complication as Fcgr3b function may be compensated by the other FcgRs (16), But women with Fcgr3b deficiency can be immunized against the antigen coded by this gene, Fcgr3b, which can induce neonatal alloimmune neutropenia in their newborns (17). In another study, some variants of the Fcgr3b gene polymorphism had been found in a Chinese population (18). Why should the allele polymorphism of the Fcgr3b predispose to IgAN? The crucial function of Fcgr3b is IC clearance. Fcgr3b can trigger the internalization of captured IC, which leads to degradation of antigen-antibody complexes, as well as directing the antigenic peptides to the major histocompatibility complex (MHC) class I or class II antigen presentation pathway (13,19). Importantly, recent findings have indicated that antigen presentation is much more efficient if the IC is internalized by FcgR rather than by nonspecific uptake mechanisms such as fluid phase pinocytosis (13,19).

Our present study showed that Fcgr3b polymorphisms had significant influence on the incidence and pathological grade in IgAN, although they did not affect the age of onset, levels of serum IgG, IgA and IgM in IgAN, suggesting that the impairment of IgG-IC clearance by this allele and subsequent glomerular deposition may also contribute to the glomerular lesions. To our knowledge, this is the first demonstration that Fcgr3b polymorphism predisposes to IgAN in Chinese. Future studies might include, for example, transcription of the gene that encodes this receptor or functional variations of this receptor involving the polymorphism of the gene in more subjects.

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