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Genetic Diversity in Human Fc Receptor II for Immunoglobulin G: Fcγ Receptor IIA Ligand-Binding Polymorphism

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Fcγ receptors, and in particular genetic variation in these receptors, are important in disorders of host defense, immunohematologic disease, and systemic autoimmune diseases. We investigated the His-Arg (CAT/CGT) polymorphism at codon 131 of the Fcγ receptor IIA gene, which influences ligand binding by the receptor. Previously, individuals had been classified phenotypically on the basis of differential binding of murine immunoglobulin G1, but the Fcγ receptor IIA genotype distribution has not been reported. We used selective PCR-based sequence analysis of genomic DNA to determine the distribution in healthy individuals. For African-Americans, the genotype distribution was determined to be A/A (14%), A/G (60%), and G/G (26%); for Caucasian Americans, the distribution was A/A (30%), A/G (51%), and G/G (19%). These data correlate well with phenotypic data. We implemented a nonradioactive single-stranded conformational polymorphism analysis to rapidly identify all three genotypes. The PCR–single-stranded conformational polymorphism analysis method will facilitate studies of the genotype distribution in individuals with disorders of immune function.

Fc receptors are found on a wide variety of hematopoietic cells and provide a bridge between the humoral and cellular branches of the immune system (19). Three distinct classes of Fcγ receptors bind the Fc portion of immunoglobulin G (IgG): Fcγ receptor I (FcγRI) (CD64), FcγRII (CDw32), and FcγRIII (CD16). The FcγR classes differ in size, cellular distribution, and function (27, 36). Interaction of FcγR with immune complexes triggers biological responses, including phagocytosis, release of inflammatory mediators, antibody-dependent cellular toxicity, enhancement of antigen presentation, and platelet activation. Fcγ receptors, and in particular genetic variation in these receptors, may be important in disorders of host defense, immunohematologic disease, and systemic autoimmune diseases (21, 37). Amino acid substitutions which influence binding of IgG ligands and alteration in gene copy number are two forms of genetic variation which have been noted in this gene family (4, 6–9, 12, 18, 22–24, 28–30, 38).

The FcγRII class is encoded by three different genes (FcγRIIA, FcγRIIB, and FcγRIIC) (3, 5, 25, 26, 31). The gene for the FcγRIIA receptor contains either G or A in codon 131, resulting in either arginine (CGT) or histidine (CAT), respectively, in the second extracellular domain (7, 14, 15, 39). This change alters the ability of the receptor to bind IgG. Cells with FcγRIIA His-131, the A/A genotype, bind human IgG2 with considerably higher affinity than those with Arg at position 131; conversely, cells with Arg-131, the G/G genotype, bind murine (m) IgG1 with considerably higher affinity than those with His at position 131 (23, 24, 29, 33). Originally, studies using monocyte interaction with an anti-CD3 antibody of the mIgG1 subclass as a trigger for T-cell proliferation classified individuals phenotypically as low responders or high responders (34). It is now known that high-responder cells in this assay have the G/G or A/G genotype while low-responder cells have the A/A genotype. Studies of platelet activation by murine antiplatelet monoclonal antibodies via the platelet FcγRIIA receptor reveal three distinct patterns of behavior most likely corresponding to the three possible genotypes (35). Phenotypic analysis has also been performed by examining the relative reactivities of two anti-FcγRII monoclonal antibodies, 41H16 and IV.3 (13, 29). 41H16 has preferential binding to FcγRIIA Arg-131 (as well as FcγRIIB and FcγRIIC) over FcγRIIA His-131, while IV.3 recognizes both forms of FcγRIIA equally well. Rapid and reliable means to determine the three possible FcγRIIA Arg-131 and His-131 genotypes are needed in order to examine whether the distribution is skewed in individuals with immune dysfunction. In addition, because the FcγRIIA phenotype varies widely among different ethnic groups, it is important to ascertain the genotype distribution in the ethnic groups of the patients of interest (1).

In this study, we investigated the frequency of the FcγRIIA A/G polymorphism in two different ethnic groups, African-Americans and Caucasian Americans, by selective PCR-based DNA sequence analysis to directly identify the FcγRIIA genotype. A single-stranded conformational polymorphism (SSCP) assay was developed for rapid determination of the FcγRIIA genotype.

MATERIALS AND METHODS

DNA isolation. Normal controls with no known immune disorder donated 4 ml of peripheral blood. Informed consent for blood drawing was obtained with the approval of the Institutional Review Board of the Children's Hospital of Philadelphia. Individuals self-identified their ethnic groups. Genomic DNA was isolated from leukocytes after selective lysis of erythrocytes with a nucleic acid extractor according to the manufacturer's instructions (Applied Biosystems, Inc., Foster City, Calif.).

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PCR amplification of the region encompassing the FcγRIIA polymorphism. Oligonucleotide primers which distinguish FcγRIIA from the highly homologous FcγRIIB and FcγRIIC genes were chosen. A sense primer from the second extracellular domain (P1 [5′-CTCTGGTCAAGGTCACATTC-3′]) was used with an antisense primer from an area of the downstream intron in which the sequences for FcγRIIA, FcγRIIB, and FcγRIIC diverge (P2 [5′-CAATTTTGCTGCTATGGGC-3′]). The resulting 278-bp PCR product contained the sequence for codons 121 through 170 of the distal second extracellular FcγRIIA domain, the splice junction, and the proximal downstream intron. In preparation for DNA sequence analysis, 300 ng of genomic DNA was used for each amplification in a 100-μl reaction mixture containing 200 pmol of each primer, 40 nmol of each deoxynucleoside triphosphate, and 1.7 U of *Taq* DNA polymerase in standard reaction buffer (50 mM KCl; 10 mM Tris-HCl, pH 8.3; 0.001% [wt/vol] gelatin; 1.5 mM $MgCl_2$). Thirty cycles (94°C for 1 min, 55°C for 2 min, and 72°C for 3 min) were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.), and samples were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels following staining with ethidium bromide. Prior to SSCP analysis, 100 ng of genomic DNA was amplified in a 100-μl reaction mixture containing 5 pmol of each primer and 25 nmol of each deoxynucleoside triphosphate in the standard reaction buffer as described above. Thirty-eight cycles of amplification (96°C for 15 s, 50°C for 30 s, 72°C for 1 min) were performed in the Perkin-Elmer 9600 thermal cycler.

Automated DNA sequence analysis. The 278-bp PCR fragment was isolated from agarose gels with Geneclean II (Bio 101, La Jolla, Calif.) and subjected to automated DNA sequence analysis with dye-labeled dideoxynucleotide chain terminators (*Taq* dyedeoxy terminator cycle sequencing; Applied Biosystems, Inc.). The DNA sequence from both strands was determined with sense or antisense primers, and reactions were analyzed on a laser-based, fluorescence emission DNA sequencer (373A; Applied Biosystems, Inc.).

SSCP assays. A 0.65-μg sample of PCR product (typically 5.4 to 6.3 μl) from genomic DNA, synthesized as described above, and 10 μl of loading buffer (95% [vol/vol] formamide, 0.05% [wt/vol] xylene cyanol, 20 mM EDTA) were heated to 100°C for 10 min before being placed immediately on wet ice (32). All subsequent steps were performed in a cold room at 4°C. Samples were loaded onto a nondenaturing 8% (wt/vol) polyacrylamide–TBE (92 mM Tris, 95 mM borate, 2.5 mM EDTA) gel (18 by 24 cm) (SE 600; Hoefer Scientific Instruments, San Francisco, Calif.) with a 37.5:1 ratio of acrylamide to bisacrylamide. The gel apparatus was further cooled by the Hoefer SE 6160 heat exchanger with a continuous flow of cold water surrounding the chamber. Electrophoresis was performed in a discontinuous buffer (25 mM Tris, 192 mM glycine [32]) at 200 V for 6 h. Gels were silver stained according to the manufacturer's instructions (silver stain kit; Bio-Rad).

Platelet aggregation. Peripheral blood samples from healthy adult volunteers of known FcγRIIA genotype were collected into polypropylene tubes (Sarstedt, Nuembrecht, Federal Republic of Germany). Platelet-rich plasma and platelet-poor plasma were obtained by differential centrifugation (800 rpm [132 × *g*] for 15 min, followed by 3,000 rpm [1,862 × *g*] for 15 min), and the platelet count of the platelet-rich plasma was adjusted to 300,000 platelets per μl with the platelet-poor plasma. Aggregation studies were performed in an aggregometer (PAP 4; Biodata, Hatboro, Pa.) with 0.5-ml samples. All samples were determined to be free of spontaneous aggregation and to aggregate normally to ADP (5 μM). Ba-6, an mIgG2 monoclonal antibody directed against CD9 (generously provided by Lawrence Brass and James Hoxie, University of Pennsylvania), and Alb-6, an mIgG1 monoclonal antibody also directed against CD9 (AMAC, Inc., Westbrook, Maine), were diluted to 200 ng/μl. Each monoclonal antibody was separately added to 0.5 ml of platelet-rich plasma at a final concentration of 1.5 μg/ml, and aggregation was monitored (17).

RESULTS

Determination of FcγRIIA genotypes. An FcγRIIA 278-bp PCR fragment containing the sequence coding for the His-Arg polymorphism at position 131 in the second extracellular domain was isolated from 97 healthy individuals, including 50 African-Americans and 47 Caucasian Americans. DNA sequence analysis revealed the following genotypes: for African-Americans, 7 A/A (14%), 30 A/G (60%), and 13 G/G (26%); for Caucasian Americans, 14 A/A (30%), 24 A/G (51%), and 9 G/G (19%). An illustration of the sequence chromatogram for a representative homozygote and heterozygote is presented in Fig. 1. There was no significant difference in genotype frequencies between Caucasians and African-Americans. Allele frequencies and heterozygote frequency were not significantly different from those calculated assuming a Hardy-Weinberg equilibrium. In addition, no mutations in the PCR products encompassing the ligand-binding domain in the FcγRIIA second extracellular domain were seen in the 194 alleles analyzed (14, 15). Platelet aggregation studies using monoclonal antibodies known to activate platelets in an Fc-dependent manner confirmed genotype assignments (35). As expected, platelets from G/G individuals aggregated after exposure to anti-CD9 monoclonal antibodies of either the mIgG2 or mIgG1 subclass, while platelets from A/A individuals aggregated in response to the mIgG2 but not to the mIgG1 subclass. Representative data are presented in Table 1. Platelets from all individuals exhibited negligible spontaneous aggregation (<10%) and aggregated normally in response to 5 μM ADP.

SSCP assay. An SSCP assay to identify the FcγRIIA genotype was developed as a rapid alternative to DNA sequence analysis. Products of PCR amplification of genomic DNA are denatured and subjected to polyacrylamide gel electrophoresis at 4°C; this step is followed by silver staining of the gel. All three genotypes are discernible by their distinct SSCP patterns. For the homozygotes, the single strands run as four major bands with differing migration distances under these conditions (Fig. 2). Note that the top pair of bands (indicated by dots between the lanes) for A/A (lanes 1 and 2) is narrower than the top pair for G/G (lanes 3 and 4), while the opposite is true for the bottom pairs of bands. The pattern for A/G heterozygotes (lanes 5 and 6) from top to bottom is a dark band, a broad smear of several bands at the positions of the corresponding homozygote bands, a lower broad band at the positions of the corresponding homozygote bands, and a final dark band. Thus, the heterozygotes have a distinct combination of the patterns for the homozygotes. A representative gel is shown in Fig. 2. In 10 separate SSCP analyses with 25 individual samples, the patterns are highly reproducible and genotype identification can be performed correctly by observers blinded to the DNA sequence results.

DISCUSSION

Two methods have been implemented in this investigation of the His-Arg polymorphism at position 131 in the FcγRIIA ligand-binding domain. We initially determined the FcγRIIA genotype by PCR-based sequence analysis of genomic DNA.

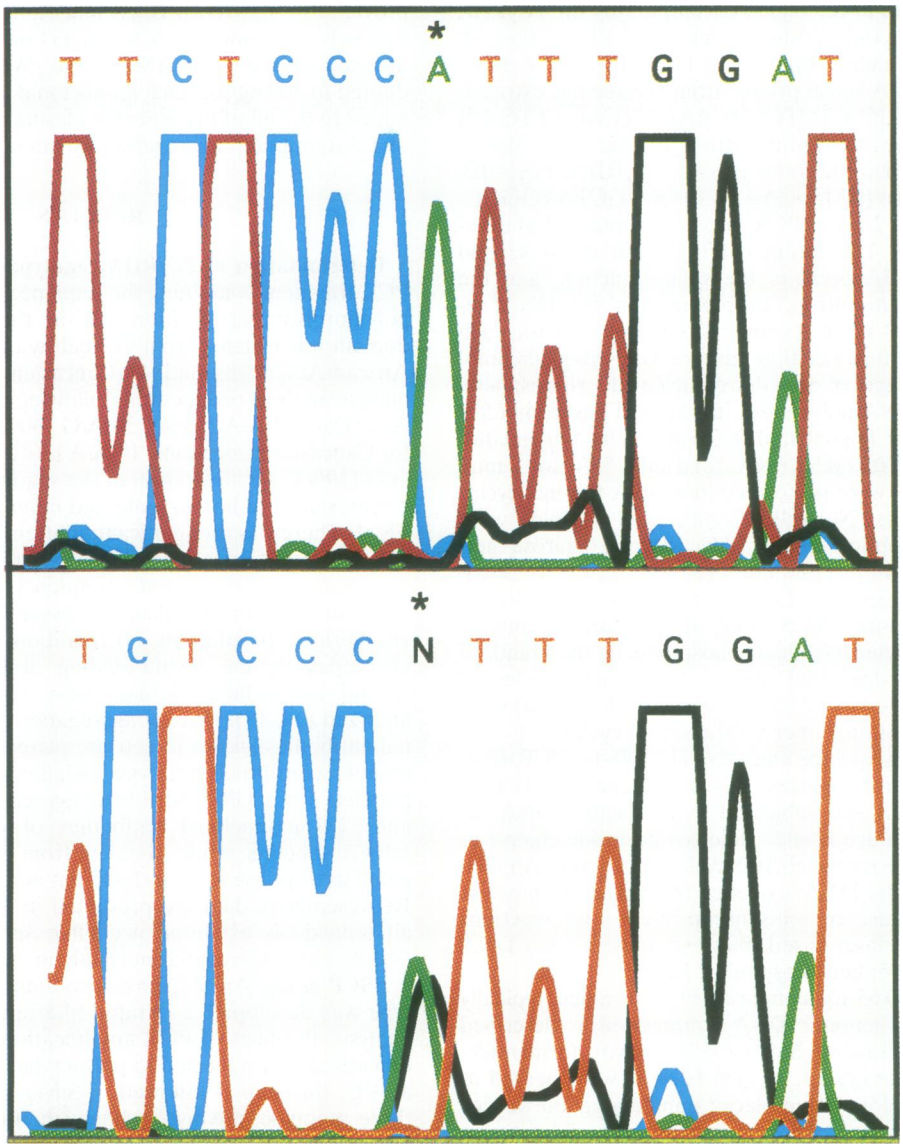

FIG. 1. FcγRIIA polymorphism genotype determination by DNA sequence analysis. DNA sequence chromatograms are shown for A/A (top) and A/G (bottom) individuals. Only an A signal is seen (top, asterisk, green peak) in A/A individuals at the position of the A/G polymorphism, while both G (black) and A (green) signals are seen in A/G heterozygotes (bottom, asterisk, denoted by "N"). Note that in the heterozygote, the two peaks at the polymorphic site are equal in height and each is half as high as the single peak in the homozygote.

The genotype distribution is the first such data reported and is in good agreement with previously described phenotype distributions of 19 to 30% low responders (A/A) among both Caucasians and African-Americans (1, 9, 34). The genotype data were corroborated by the function of the FcγRIIA protein on platelets. The dependence of platelet aggregation on the IgG subclass of murine antiplatelet antibodies has been noted, but in those studies, a direct assessment of platelet FcγRIIA genotype was not made (10, 16, 35).

TABLE 1. Platelet aggregation by the Fc end of anti-CD9 antibodies according to FcγRIIA genotype

Antibody	G/G			A/A		
	Lag time (min)	Slope (%/min)	Maximum aggregation (%)	Lag time (min)	Slope (%/min)	Maximum aggregation (%)
Alb-6 (mIgG1)	2.0	30	88	—[a]	3	2
Ba-6 (mIgG2)	2.5	37	70	1.25	35	61

[a] —, no aggregation and so lag time was infinite.

The variation in IgG ligand binding by polymorphic forms of FcγRIIA has several clinical consequences. The FcγRIIA phenotype was a critical determinant in defining whether individuals responded to an mIgG1 monoclonal antibody used to treat allograft rejection in renal transplant patients (40). FcγRIIA His-131 binds human IgG2 substantially better than FcγRIIA Arg-131 does (23, 29). In keeping with this observation, the FcγRIIA phenotype appears to affect host defense against encapsulated bacteria (2, 11, 37) and platelet activation (16, 23, 35). Preliminary data suggest a correlation between heparin-induced thrombocytopenia and the platelet FcγRIIA

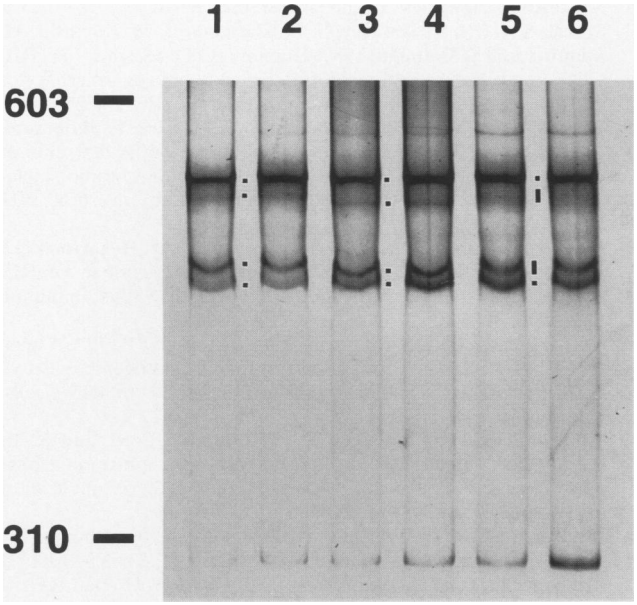

FIG. 2. FcγRIIA genotype determination by PCR-SSCP assay. PCR fragments (278 bp) from individuals whose FcγRIIA genotypes were known from DNA sequence analysis were electrophoresed in polyacrylamide gels with a discontinuous buffer as described in Materials and Methods. A/A (lanes 1 and 2), G/G (lanes 3 and 4), and A/G (lanes 5 and 6) genotypes are shown. The positions of ϕX174-*Hae*III molecular weight markers are indicated on the left.

His-Arg polymorphism at position 131, but the nature of the association has not been elucidated (20).

In future studies of the polymorphism, larger numbers of patients and a more rapid, convenient method of genotyping individuals will be needed. Phenotypic studies, involving either T-cell proliferation assays with mIgG1 anti-CD3 (24) or determination of the relative binding ratios of anti-FcγRII monoclonal antibodies 41H16 and IV.3 by FcγRII-positive cells (13), are cumbersome; the reagents may not be widely available; and the methods may be incapable of distinguishing all three groups. Since these are cellular assays, these methods are also not useful when the patient is cytopenic or cells are not available for analysis. Allele-specific reverse transcription PCR based on the A/G polymorphism has been reported, but this method requires RNA isolation and is time-consuming (9). The use of PCR for selective amplification of FcγRIIA from genomic DNA is a more direct method of genotyping and is highly reliable. However, DNA sequence analysis is not conducive to large-scale screening, and we have therefore implemented a PCR-based SSCP assay which is rapid, efficient, and cost-effective. This method distinguishes all three genotypes; this will be invaluable in further investigations of the role of this polymorphism in human disease. Continued investigation of genetic variation in the Fcγ receptors will hopefully yield insights into host defense, immunohematologic disease, and autoimmune disorders.

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