

Letter to the Editor

Functional *FCGR2B* gene variants influence intravenous immunoglobulin response in patients with Kawasaki disease

To the Editor:

Human intravenous immunoglobulin (IVIG) administered at a high dose suppresses inflammation caused by several autoimmune diseases. Although IVIG is licensed for the treatment of patients with Kawasaki disease (KD), idiopathic thrombocytopenic purpura, Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, the modes of action in these clinical syndromes require elucidation. Proposed mechanisms include F(ab')₂-dependent modulation of pathogenic autoantibody neutralization, cytokine production, and complement component neutralization.¹ However, studies with clinical and murine models show that IVIG anti-inflammatory potency depends more on the IgG Fc binding to receptors expressed on the surface of various immune cells. The Fcγ receptor (FcγR) family contains multiple activating receptors and a single inhibitory receptor, FcγRIIB. Importantly, B cells express only the inhibitory type. Presumably, IVIG modulates the balance between activating and inhibitory receptors.

FcγRIIB plays a central role in mediating Fc-dependent anti-inflammatory actions in mice.² IVIG elicits a minimal therapeutic effect in FcγRIIB-deficient mice with autoimmune disease³ but induces FcγRIIB surface expression on splenic macrophages in wild-type mice,⁴ thereby preventing antibody-induced inflammation. Also, IVIG upregulates FcγRIIB expression by monocytes and B cells among clinically responding patients with chronic inflammatory demyelinating polyneuropathy.⁵ Although these studies in mice and the limited patient data support a model for FcγRIIB involvement in IVIG actions, further clinical confirmation is required.

We thus tested our hypothesis that the gene encoding FcγRIIB participates in IVIG anti-inflammatory action in human subjects by determining whether functionally relevant polymorphisms for this gene influence IVIG response in a KD cohort. This autoimmune syndrome produces acute and diffuse vasculitis. There is predilection for the coronary arteries, in which aneurysms occur in 20% to 25% of untreated patients. IVIG (2 g/kg) with aspirin represents the only standard therapy recommended for acute KD, but the response is variable. The American Heart Association and American Academy of Pediatrics define failure to respond or IVIG refractoriness to treatment as persistent or recurrent fever (temperature >38°C) at greater than 36 hours after completing the initial IVIG infusion.⁶ The IVIG refractory rate, depending on the clinical series, is reported to be between 13% to 30%.⁶ Coronary artery vasculitis and aneurysms occur at comparatively high rates in refractory patients.⁷

With institutional review board approval and parental consent, we obtained DNA from patients with KD (n = 383) and parents (n = 578) from 3 centers in the Pacific Northwest. Qualifying patients met strict American Heart Association/American Academy of Pediatrics clinical criteria for the diagnosis of KD.⁶ The predominantly male cohort (59%) showed median KD age of onset at 34.5 months (interquartile range [15-61.5 months]). In our IVIG response analysis, we only included the patients with KD (231 responders, 76 refractory) if they received standard

treatment within 10 days, followed guidelines prescribing IVIG refractoriness,⁶ and were not participating in other treatment-related clinical trials. In all patients with KD and their parents (for frequency comparisons), we pyrosequenced (see the [Methods](#) section in this article's Online Repository at www.jacionline.org) the DNA regions containing 3 known functional polymorphisms in the *FCGR2B* gene: a single nucleotide polymorphism in the *FCGR2B* region encoding either isoleucine (I) or threonine (T) amino acid at position 232 within the transmembrane region (nucleotide position +775; IIB+775 [T/c]), which is known to alter the function of this receptor, and 2 single nucleotide polymorphisms at positions -386 (IIB-386 [G/c]) and -120 (IIB-120 [T/a]) upstream from the translation start site known to alter the promoter activity, resulting in differing levels of receptor expression.⁸ We assigned race to subjects from this heterogeneous US population using 155 ancestry informative markers (AIMs) specific for Asian, African, Native American, and European descent (see the [Methods](#) section in this article's Online Repository for more information).

However, the allele frequency for all 3 polymorphisms differed substantially among the major racial and ethnic groups. Most strikingly, the A allele at *FCGR2B*-120T/a was totally absent in Asian patients (n = 48) and their unaffected parents (n = 86) and African American patients (n = 10) and their parents (n = 16); it was rare in Hispanic patients (n = 40) and their parents (n = 67). Thus we analyzed the effect of the polymorphisms on IVIG treatment response for each race, including the heterogeneous Hispanic population, separately. In Caucasian subjects the minor allele A at *FCGR2B*-120T/a occurred more frequently among the responders (15%) than the nonresponders (5%), with an odds ratio of 3.23 (95% CI, 1.22-8.33; *P* = .01; [Table 1](#)). The overall genotypic distribution was different in responders and nonresponders (*P* = .03). Furthermore, no IVIG nonresponders were AA homozygous, whereas 5 (4%) of 128 responders were homozygotes, and a similar trend was observed with heterozygotes, occurring more frequently (23% vs 11%) among the responders ([Fig 1](#)). The Armitage trend test suggested a higher likelihood of being a responder with increased numbers of A alleles (*P* = .02). Only 2 Hispanic patients with KD were heterozygotes (AT); both were also IVIG responders. Consistent with a previous report on *FCGR2B*-386 (G/c) and *FCGR2B*-120 (T/a) haplotypes,⁹ the frequencies of G-T, C-A, and C-T haplotypes in Caucasian unaffected parents were 91%, 8%, and 1%, respectively. Among the patients, the distributions of these haplotypes differed significantly when comparing responders with nonresponders (84.5% vs 92%, 15% vs 6%, and 0.5% vs 2%, respectively; overall *P* = .02), with the increased C-A haplotype in the responders tagging *FCGR2B*-120A. In Asian subjects *FCGR2B*+775T/c, encoding the transmembrane region, showed trends related to IVIG treatment response, but the power was limited by the small sample size.

FcγRIIB inhibits immune response through coligation with either activating FcγRs or with B-cell receptors bound to immune complexes.² This coligation leads to phosphorylation of the cytoplasmic immunoreceptor tyrosine-based inhibitory motif within the transmembrane FcγRIIB portion. Immunoreceptor tyrosine-based inhibitory motif phosphorylation leads to downstream target dephosphorylation and inhibition of the activating signaling

TABLE I. Polymorphisms in the *FCGR2B* gene and response to IVIG treatment among patients with KD in all ethnic groups and only Caucasian subjects

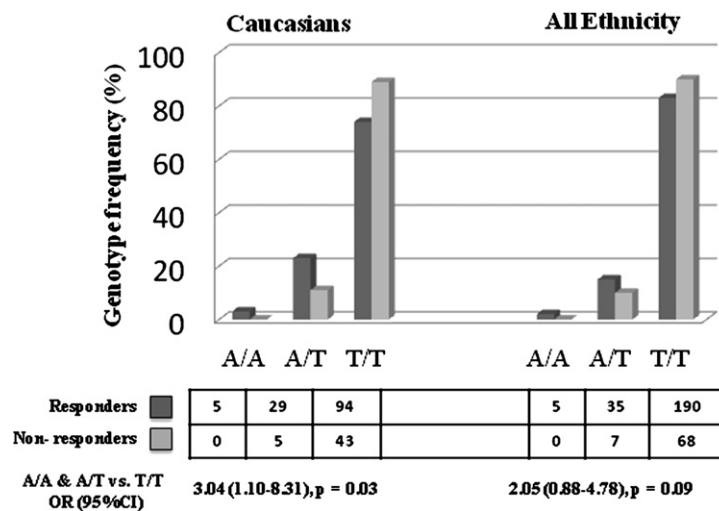
Polymorphisms	Minor allele frequency (%)			Allelic ORs (95% CI), responders vs nonresponders	P value		
	Parents	Responders	Nonresponders		Allelic*	Genotype†	Trend‡
All ethnic groups	n = 578	n = 231	n = 76				
<i>FCGR2B</i> +775T/c	15	18	14	1.35 (0.79-2.22)	.28	.42	.34
<i>FCGR2B</i> -386G/c	9	10	7	1.61 (0.79-3.23)	.18	.32	.21
<i>FCGR2B</i> -120T/a	9	10	5	2.22 (0.98-5.02)	.05	.17	.06
Caucasian subjects	n = 369	n = 128	n = 48				
<i>FCGR2B</i> +775T/c	13	12	13	0.97 (0.47-2.01)	.94	.97	.95
<i>FCGR2B</i> -386G/c	13	16	8	2.04 (0.91-4.55)	.08	.21	.10
<i>FCGR2B</i> -120T/a	12	15	5	3.23 (1.22-8.33)	.01	.03	.02
Asian subjects	n = 86	n = 40	n = 8				
<i>FCGR2B</i> +775T/c	27	40	19	1.54 (0.39-6.25)	.11	.15	.14
<i>FCGR2B</i> -386G/c	1	0	0	—	—	—	—
<i>FCGR2B</i> -120T/a	0	0	0	—	—	—	—
Hispanic subjects	n = 67	n = 29	n = 11				
<i>FCGR2B</i> +775T/c				—	.49	.65	.51
<i>FCGR2B</i> -386G/c				—	—	—	—
<i>FCGR2B</i> -120T/a				—	—	—	—

Boldfaced values are statistically significant.

OR, Odds ratio.

* χ^2 Test ($df = 1$).† χ^2 Test ($df = 2$).

‡Cochran-Armitage test.

**FIG 1.** Genotype distribution (counts) of *FCGR2B*-120T/a and association with IVIG response in Caucasian subjects and in the mixed population.

cascade. Inhibition increases with increased Fc γ RIIB surface expression, which is controlled in part at the transcriptional level. Transfection of the *FCGR2B*-386/C and *FCGR2B*-120/A promoter haplotype into BJAB or U397 cells increases constitutive and cyclic AMP-stimulated *FCGR2B* promoter activity through enhanced binding to GATA4 and Yin-Yang1 (YY1) transcription factors.⁹ Accordingly, our data, which were obtained in human subjects and linking increased *FCGR2B* promoter activity with improved clinical response, confirm the importance of Fc γ RIIB in mediating the anti-inflammatory action of high-dose IVIG.

The prevailing models derived primarily from murine experiments suggest that IVIG sensing occurs through 1 or multiple candidates, such as Fc γ RIII or DC-SIGN-related-1.² Fc γ RIIB is downstream within a signaling cascade but is required to

trigger IVIG-mediated inflammatory inhibition. Thus functional polymorphisms or other influences in 1 or multiple candidate genes along this cascade could also affect the IVIG response. The specificity of this promoter polymorphism for the Caucasian population suggests that these candidates or others, possibly involving the activating Fc γ Rs, could be responsible for the IVIG response variation in subjects of other races. This study provides the basis for defining the IVIG regulatory mechanisms in patients and for using a clinical pharmacogenomic approach to IVIG therapy.

We thank the participating patients and their parents. We also thank the investigators, pediatricians, and staffs of the participating clinics. We thank Norman Buroker for handling of the biospecimens and for DNA extraction and Aditi Shendre and Deborah S. McDuffie for genotyping.

Sadeep Shrestha, PhD^a
Howard W. Wiener, PhD^a
Aaron K. Olson, MD^b
Jeffrey C. Edberg, PhD^c
Neil E. Bowles, PhD^d
Hitendra Patel, MD^e
Michael A. Portman, MD^b

From ^athe Department of Epidemiology and ^cthe Division of Clinical Immunology and Rheumatology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Ala; ^bthe Department of Pediatrics, University of Washington, Seattle Children's Hospital, Seattle, Wash; ^dthe Department of Pediatrics, University of Utah, Salt Lake City, Utah; and ^eCardiology, Children's Hospital of Oakland, Oakland, Calif. E-mail: Michael.portman@seattlechildrens.org.

This study was supported by grant NHLBI-R21-HL90558, University of Washington Clinical and Translational Science Award Grant 1UL1 RR025014-01, and a grant from the Thrasher Foundation Research Fund.

Disclosure of potential conflict of interest: S. Shrestha receives research support from the National Heart, Lung, and Blood Institute/National Institutes of Health (NIH) and the Thrasher Foundation. A. K. Olson receives research support from the NIH and has received a Thrasher grant. M. A. Portman receives research support from the NIH and the Thrasher Foundation. The rest of the authors have declared that they have no conflict of interest.

REFERENCES

1. Negi VS, Elluru S, Siberil S, Graff-Dubois S, Mouthon L, Kazatchkine MD, et al. Intravenous immunoglobulin: an update on the clinical use and mechanisms of action. *J Clin Immunol* 2007;27:233-45.
2. Smith KG, Clatworthy MR. FcγRIIb in autoimmunity and infection: evolutionary and therapeutic implications. *Nat Rev Immunol* 2010;10:328-43.
3. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* 2001;291:484-6.
4. Kaneko Y, Nimmerjahn F, Madaio MP, Ravetch JV. Pathology and protection in nephrotoxic nephritis is determined by selective engagement of specific Fc receptors. *J Exp Med* 2006;203:789-97.
5. Tackenberg B, Jelcic I, Baerenwaldt A, Oertel WH, Sommer N, Nimmerjahn F, et al. Impaired inhibitory FcγRIIb expression on B cells in chronic inflammatory demyelinating polyneuropathy. *Proc Natl Acad Sci U S A* 2009;106:4788-92.
6. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Pediatrics* 2004;114:1708-33.
7. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, et al. Resistance to intravenous immunoglobulin in children with Kawasaki disease. *J Pediatr* 2008;153:117-21.
8. Li S, Ptacek TS, Brown EE, Edberg JC. Fcγ receptors: structure, function and role as genetic risk factors in SLE. *Genes Immun* 2009;10:380-9.
9. Su K, Li X, Edberg JC, Wu J, Ferguson P, Kimberly RP. A promoter haplotype of the immunoreceptor tyrosine-based inhibitory motif-bearing FcγRIIb alters receptor expression and associates with autoimmunity. II. Differential binding of GATA4 and Yin-Yang1 transcription factors and correlated receptor expression and function. *J Immunol* 2004;172:7192-9.

doi:10.1016/j.jaci.2011.04.027

METHODS

Genotyping

Three functional polymorphisms in the *FCGR2B* gene family were genotyped by using pyrosequencing methodology with a PCR reaction with the Failsafe PCR system (Epicenter Technologies, Madison, Wis). The PCR reactions and cycling conditions have been previously described.^{E1} Briefly, initial long-range PCR amplifications were performed with the following primers to ensure gene-specific amplification: *FCGR2B*+775 (T/c) forward CTAAGAGG AGCCCTTCCCTAT and reverse AATACGGGCCTAGATCTGAATG; *FCGR2B*−386 (G/c) forward CTCCACAGGTTACTCGTTTCTACCTTATCTTAC and reverse GCTTGCGTGGCCCTGGTTCTCA; and *FCGR2B*−120 (T/a) forward CTCCACAGGTTACTCGTTTCTACCTTATCTTAC and reverse GCTTGCGTGGCCCTGGTTCTCA. A second nested PCR reaction (using 0.25 μL of the first PCR) was performed around the single nucleotide polymorphism site by using a biotinylated primer to allow for purification of a single-stranded template for the pyrosequencing reaction as follows: *FCGR2B*+775 (T/c) forward TCCCTAACTCCCAGCTCTTCAC and reverse bio-CTACA AAACCTGAAATCCGCTTTTT; *FCGR2B*−386 (G/c) forward TCAAGA AGCATCCAGATTCCAG and reverse bio-AAACTCAGCTCAGAACCTC CTGTT; and *FCGR2B*−120 (T/a) forward AAAGAGGGTGGAAAGGGAG GAG and reverse bio-CTCTCAAAGCTTGGCGGATTCTAC. After denaturation of the PCR amplicon in 0.1 mol/L NaOH for 10 minutes, the single-strand product is immobilized to streptavidin-sepharose (Amersham Biosciences, Piscataway, NJ), washed, and annealed with 15 pmol of the following “pyrosequencing” primer: *FCGR2B*+775 (T/c), TGGCTGTGGTCAC TGGGA; *FCGR2B*−386 (G/c), TGCTGGTGACGCTGTCCT; and *FCGR2B*−120 (T/a), CCTGTGATAAAAACAGAACAT. Pyrosequencing was performed according to the manufacturer's instructions, as previously described.^{E2}

AIMs

This study is based on the potentially admixed population in the northwestern United States. One hundred fifty-five genome-wide ancestry informative markers specific for Asians, Africans, Native Americans, and Europeans were typed in the BeadExpress assay in the Illumina platform (Illumina, Inc, San Diego, Calif) to account for population stratification.

Statistical methods

Genotypes for the AIMs that successfully passed the Illumina QC test were used to calculate principal components of ancestry through use of the

principle components analysis (PCA) program from the Eigenstrat software package.^{E3} On the basis of the eigenvalues determined in this analysis, the first 3 components were chosen to be used for classification of the subjects into distinct genetic ethnicities. The values calculated by means of PCA were subjected to a discriminant analysis^{E4} by using the Discrim procedure in SAS version 9.2 (SAS Institute, Inc, Cary, NC). The subjects chosen were those who identified themselves as members of only 1 of the following ethnic groups for a formal classification analysis: Caucasian, Asian, or Hispanic. Because of small numbers, other groups were excluded. This procedure determined that a quadratic discriminant function was more appropriate than a linear function. The discriminant function calculated from subjects in these 3 self-identified groups was applied to the remainder of the subjects who had results from the PCA analysis. These included self-identified African Americans, Native Americans, those who self-identified to be in multiple groups, and those who did not provide any self-identification. The final results of this objective analysis were viewed subjectively, and subjects not clearly within a cluster were reclassified as “other mixed.”

A case-control approach was performed to examine the differential distribution of alleles and genotypes for each of the 3 variants among participants who did not respond to IVIG treatments (cases) versus those who responded (control subjects). Additionally, distributions of haplotypes based on the 2 variants in the promoter region were examined between cases and control subjects. All analyses were performed for the entire set of patients with KD and also separately for Caucasians, Asians, and Hispanics, as determined by PCA of AIMs. ORs with 95% CIs and *P* values were calculated for all analytic comparisons.

REFERENCES

- E1. Edberg JC, Langefeld CD, Wu J, Moser KL, Kaufman KM, Kelly J, et al. Genetic linkage and association of Fcγ receptor IIIA (CD16A) on chromosome 1q23 with human systemic lupus erythematosus. *Arthritis Rheum* 2002;46:2132–40.
- E2. Edberg JC, Wu J, Langefeld CD, Brown EE, Marion MC, McGwin G Jr, et al. Genetic variation in the CRP promoter: association with systemic lupus erythematosus. *Hum Mol Genet* 2008;17:1147–55.
- E3. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;38:904–9.
- E4. Rao CR. *Linear statistical inference*. New York: John Wiley & Sons; 1973.