Role of Activating FcγR Gene Polymorphisms in Kawasaki Disease Susceptibility and Intravenous Immunoglobulin Response

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Background—A functional polymorphism in the inhibitory IgG-Fc receptor gene FcγRIIB influences intravenous immunoglobulin (IVIG) response in Kawasaki disease (KD), a vasculitis preferentially affecting the coronary arteries in children. We tested the hypothesis that the polymorphisms in the activating receptors (FcγRIIA, FcγRIIIA, and FcγRIIB) also influence susceptibility, IVIG treatment response, and coronary artery disease in patients with KD.

Methods and Results—We genotyped polymorphisms in the activating FcγRIIA, FcγRIIIA, and FcγRIIB using pyrosequencing in 443 patients with KD, including 266 trios and 150 single parent-child pairs, in northwest United States and genetically determined race with 155 ancestry informative markers. We used family-based association to test for transmission disequilibrium and further generated pseudosibling controls for comparisons with the cases. The FcγRIIA-131H variant showed an association with KD (P=0.001) with an additive odds ratio (OR) of 1.51 (95% CI, 1.16–1.96; P=0.002) for the primary combined population, which persisted in both white (P=0.04) and Asian (P=0.01) subgroups and is consistent with the recent genome-wide association study. We also identified overtransmission of the FcγRIIB neutrophil antigen 1 (NA1) variant among IVIG nonresponders (P=0.0002) and specifically to white IVIG nonresponders (P=0.007). ORs for overall and white nonresponders were 3.67 (95% CI, 1.75–7.66; P=0.0006) and 3.60 (95% CI, 1.34–9.70; P=0.01), respectively. Excess NA1 transmission also occurred in patients with KD and coronary artery disease (ORadditive 2.13; 95% CI, 1.11–4.0; P=0.02).

Conclusions—A common variation in FcγRIIA is associated with increased KD susceptibility. The FcγRIIB-NA1 variant, which confers higher affinity for IgG than the NA2 variant, is a determining factor for treatment response. These activating FcγRs play an important role in KD pathogenesis and the IVIG antiinflammatory mechanism. (Circ Cardiovasc Genet. 2012;5:309-316.)

Key Words: coronary disease ■ mucocutaneous lymph node syndrome ■ pediatrics ■ Fc gamma receptors ■ immunoglobulins intravenous ■ therapy

Human pooled intravenous immunoglobulin (IVIG) is used in high doses as the primary treatment for Kawasaki disease,1,2 a prototypic vasculitis involving the coronary arteries in children.3 Prevention of coronary artery inflammation, manifested by dilation and aneurysm formation, is the primary treatment goal. Progression to a giant aneurysm requires anticoagulation to prevent thrombosis and coronary ischemia.4 Lack of appropriate therapeutic response or refractoriness is defined by a treatment goal. Progression to a giant aneurysm requires anticoagulation to prevent thrombosis and coronary ischemia.4 Lack of appropriate therapeutic response or refractoriness is defined by a joint statement from the American Heart Association and the American Academy of Pediatrics as persistent or recurrent fever extending >36 hours after completing IVIG infusion.5 Various clinical series report refractory rates between 10% and 30%.6,7 Refractory patient populations exhibit substantially higher rates of coronary inflammation and aneurysm formation than responsive individuals.7 Although various clinical risk scores have been adopted for use in Japan, where Kawasaki disease is endemic,8 their sensitivity in heterogeneous North American populations is poor.9 Kawasaki disease incidence varies considerably according to race and ethnicity. Previous investigators have suggested that the differences in the predictive value of the risk scores also reflect the genetic diversity.9

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The IVIG mechanisms of antiinflammatory action still require elucidation in humans. This knowledge deficit hinders...
identification of candidate genes involved in Kawasaki disease pathogenesis and polymorphisms that can predict treatment response. IgG-Fc region receptors (FcγRs) represent plausible Kawasaki disease mediators because of their direct interaction with IgG. FcγRs are a heterogeneous group of hematopoietic cell surface glycoproteins that are expressed primarily on human effector cells of the immune system, particularly macrophages, monocytes, myeloid cells, and dendritic cells. These molecules facilitate antibody-antigen interactions. Studies in mice lacking various forms of dendritic cells have documented their key roles in the balance between activating and inhibitory receptor signals in experimental idiopathic thrombocytopenic purpura as well as for how modulation of this balance might account for the therapeutic effects of IVIG. Although the disease processes in mice and humans are not precisely the same, the mechanisms of action of IVIG in these murine models have important connections with their human analogs. The murine models suggest an important and possibly dominant role for the inhibitory FcγRIIB in the IVIG antiinflammatory mechanism. Genetic association studies in humans support such FcγRIIB participation. However, the low frequency of the particular functional FcγRIIB polymorphisms in all populations limits its clinically relevant role.

The activating FcγRs interact with the single inhibitory receptor FcγRIIB. Thus, we hypothesized that polymorphisms in the activating receptors (FcγRIIA, FcγRIIHA, and FcγRIIIB) influence the IVIG treatment response defined by clinical parameters. We also analyzed these receptors with regard to susceptibility and persistence of coronary artery disease (CAD) in patients with Kawasaki disease. We examined the influence of functional single-nucleotide polymorphisms (SNPs) in these genes using a family-based genetic study. We performed the study in a heterogeneous US-based population of patients with Kawasaki disease and their parents comprising some ethnic and racial admixture. Substantial FcγR allelic and locus heterogeneity has been demonstrated across different ethnic and racial groups; therefore, we also performed subgroup analyses in populations of European and Asian descent.

Methods

Study Population

Patients, their parents, and available siblings were identified through clinical databases and enrolled at the following participating centers: Seattle Children’s Hospital, Oakland Children’s Hospital, and Primary Children’s Hospital of Utah. Retrospective cross-reference of the hospital database and the heart center echocardiography databases confirmed the diagnosis and treatment of all participating patients with Kawasaki disease. After approval by the Institutional Review Board at all participating institutions, parents were approached for study recruitment, and informed consent was obtained.

Clinical Diagnosis of Kawasaki Disease

The definition of complete Kawasaki disease followed the standard epidemiological criteria recommended by the American Heart Association and American Academy of Pediatrics. Patients were also included if they had at least 2 clinical criteria and coronary artery involvement as defined in the American Heart Association guidelines.

Treatment Response

Treatment response was determined in patients receiving IVIG (2 g/kg) within 11 days of initial fever. As stated in the American Heart Association/American Academy of Pediatrics-endorsed clinical report, failure to respond to IVIG treatment was defined as either persistent fever (temperature >38°C) at >36 hours or recurrent fever at >36 hours after completion of the initial IVIG infusion. Patients receiving second doses of IVIG at <36 hours were excluded from treatment response analyses unless they had persistent fever despite a second dose of IVIG.

Coronary Artery Disease

CAD was defined by echocardiography as dilation (Z score >2.5 according to Boston Z score data) or aneurysm defined by Japanese Ministry of Health criteria persisting >6 weeks after IVIG treatment (2 g/kg).

Biospecimen Collection and DNA Extraction

Most parents consented to have blood samples taken from their offspring with Kawasaki disease, and whole blood was collected in ACD (acid citrate dextrose) anticoagulant tubes. For the remainder, saliva was collected in Origene kits (DNA Genotek) by a noninvasive technique proven to preserve DNA. Briefly, participants first rinsed their mouths with water to clear food particles and then expectorated 2 mL of saliva into the Origene vial. Genomic DNA was extracted using the Versagene DNA purification kit (Gentra Systems) and quantified using the PicoGreen assay for double-stranded DNA, adjusted to a final concentration of 100 ng/μL, and stored at −80°C in Tris-EDTA.

Activating FcγR Genes and Polymorphisms

The activating FcγRIIA, FcγRIIHA, and FcγRIIB genes are all located at chromosome arm 1q23 where a span of ~200 kb, FcγRIIA has affinity for IgG and interacts with IgG or immune-complexed IgG on cell surfaces. FcγRIIB is a relatively low affinity receptor. FcγRIIIA is expressed as a membrane-spanning receptor on macrophages and natural killer cells. These 3 genes produce integral transmembrane glycoproteins that are considered functionally activating receptors.

Five well-characterized and functionally relevant SNPs were examined: (1) a common FcγRIIA SNP (rs1801274) affecting amino acid position 131 in extracellular domain 2 (FcγRIIA-131HR), with A and G alleles at position 131 coding for codominantly expressed arginine (R) and histidine (H), respectively, and known to affect affinity for IgG2 and are associated with several immune-related diseases; (2) a common FcγRIIIA SNP (rs390991) affecting amino acid position 158 in extracellular domain 2 (FcγRIIIA-158V), with G and T alleles coding either valine (V) or phenylalanine (F), respectively, and known to be associated with immune-related diseases; (3) another triallelic FcγRIIIA SNP (rs10127939) at amino acid position 48 (FcγRIIIA-48L/R/H), with T, G, and A alleles encoding for either leucine (L), arginine (R), or histidine (H), respectively, and known to influence the binding of IgG by natural killer cells; and (4) 2 FcγRIIB SNPs at positions 141 (rs403016) and 147 (rs47536) in the extracellular domain 1 that result in variable amino acid sequences resulting in 2 allotypic forms named neutrophil antigen 1 (NA1) and neutrophil antigen 2 (NA2). Although SNP 141 and SNP 147 are in a perfect linkage disequilibrium, haplotype is a conventional way to determine NA1 and NA2 allotypes, which has been well established in the literature.

Genotyping Methods

The functional polymorphisms in the FcγR family were genotyped by pyrosequencing methodology using a nested polymerase chain reaction (PCR) approach to ensure gene-specific amplification. Initial gene-specific PCR amplifications of the DNA fragments around the SNP sites were followed by second-round-nested PCRs, using 0.25 μL of the first-round PCR products as templates. All
Statistical Methods

First, genotype completeness was checked for each SNP, and Hardy-Weinberg equilibrium was examined in each ethnic group. Statistical analysis for Kawasaki disease susceptibility was performed by the transmission disequilibrium test, which tests for disequilibrium of transmission of alleles from heterozygous parents to affected child and, thus, applies to parent-child trios. The test statistic for the transmission disequilibrium test is distributed as a $\chi^2$ with 1 degree of freedom and is calculated as $(b - c)^2/(b + c)$ where $b$ and $c$ are the number of transmissions and nontransmissions, respectively, of the allele from heterozygous parents to their affected child. All analyses were performed assuming additive genetic models with a minimum informative family size set to 10. We used the family-based association test (FBAT) to allow for larger families than trios and incomplete trios with or without siblings. Single-marker FBAT analysis was used to estimate the single locus frequencies. A significant $P$ value ($<0.05$) and a positive $Z$ statistic indicated that the allele at a specific locus is more frequently transmitted to patients with Kawasaki disease than is expected under the null hypothesis of no linkage and no association, whereas a significant $P$ value and a negative $Z$ statistic indicated a protective marker allele for Kawasaki disease. For those loci that showed significant differences in FBAT analysis, case/pseudosibling control analysis was performed as previously described. The pseudosibling controls were generated from the 3 untransmitted parental genotypes, and conditional logistic regression was used to estimate odds ratios (ORs) and 95% CIs in the additive model.

As primary analysis of the study, FBAT analyses were performed separately for responders and nonresponders to determine whether alleles were differentially transmitted. Additionally, as a secondary analysis, differential transmission was examined among patients with and without CAD. All analyses were performed for the entire set of patients with Kawasaki disease and separately for whites and Asians as determined by the principal component analysis of ancestry informative markers (AIMs), as previously described. Although FBAT accounts for population admixture, the underlying assumption of the same genetic association may not hold because of allelic and genetic heterogeneity in different ethnic groups. For ethnicity-specific analyses, only families with all members clustered in the same ethnic group (all 3 for trios and 2 for the parent-child pair) were included. For quality control, Mendelian inconsistency checks were performed with the AIMs, and both parents whose data contained genotyping errors in >1 SNP were excluded from the study.

Results

The genotype frequencies for all polymorphisms met Hardy-Weinberg expectations in each population (European and Asian ancestry), and the genotype data were complete for >95% of the individuals with samples available as the genotype was redone, if they failed in the first attempt. Based on the AIMs, the first 3 principal component values were used to discriminate individuals into 4 major race/ethnic groups (Figure): (1) a homogenous European ancestry (white), (2) a more heterogeneous Asian ancestry, (3) a heterogeneous Hispanic ancestry (predominantly Mexican or Mexican American), and (4) a small group for African ancestry (black). However, there were many genetically heterogeneous individuals who could not be defined into 1 single race/ethnic group. Self-report (or as reported by parents) of all patients with Kawasaki disease and participating family members showed a slightly <90% match with the genetically determined ancestry: 570 of 599 who self-reported to be white matched, 153 of 173 who self-reported to be Asians matched, 147 of 166 who self-reported Hispanic matched, 95 self-reported multiple ethnicity, and 61 did not report any ethnicity. Based on the AIMs, the study was underpowered with a smaller sample size for blacks and Hispanics, although distinct from other race/ethnic groups as...
previously reported, was very heterogeneous. Therefore, we restricted the analysis to European and Asian ancestry. Other baseline demographic characteristics are shown in Table 1. The median age of the probands at diagnosis was 34 months (interquartile range, 15–58 months), and 62% of them were boys, with a male-to-female ratio of 1.6:1, which is similar to the ratios reported in general populations. The median age between treatment responders (35 months; interquartile range, 16–58 months) and nonresponders (31 months; interquartile range, 15–66 months) was not significantly different, and there were slightly more nonresponders (63%) than responders (60%) among boys. Of the 443 patients with Kawasaki disease recruited into the study (242 whites, 82 Asians, 88 Hispanics, and 31 blacks), 266 trios occurred for this allele among Asian responders (ORadditive, 1.40; 95% CI, 1.01–1.95; P=0.04) (Table 2). Stratification according to IVIG response (Table 2) showed that statistical significance persisted in the combined responders; however, it seemed that the effect was largely observed in Asian responders. Further, using the pseudosibling controls (Table 3), the A allele showed increased risk overall among IVIG responders (ORadditive, 1.40; 95% CI, 1.01–1.95; P=0.04). Relatively high risk occurred for this allele among Asian responders (ORadditive = 4.00; 95% CI, 1.34–11.96; P=0.01).

The FcγRIIIA-158 G allele was transmitted less in Asians (n=29, z=-2.00, P=0.05) (Table 2), including mostly IVIG responders (n=19, z=-2.04, P=0.04) (Table 3). However, FcγRIIIA-48L/R/H occurring at low frequency showed no effects. Similarly, FcγRIIB-NA1 showed no significant association with Kawasaki disease susceptibility (n=157, z=+1.88, P=0.06) (Table 2).

**IVIG Nonresponse**

We found excessive transmission of FcγRIIB-NA1 in the IVIG nonresponding subgroup (n=34, z=+3.70, P=0.0002) (Table 3). This highly significant effect was detected in whites (n=20, z=+2.71, P=0.007) (Table 3) on subgroup analysis; we lacked adequate numbers of informative families to test transmission in Asians. Pseudosibling analyses revealed an ORadditive of 3.67 (95% CI, 1.75–7.66; P=0.0006) for the combined IVIG nonresponders and an OR of 3.60 (95% CI, 1.34–9.70; P=0.01) for white IVIG nonresponders (Table 3). No such overtransmission was observed among IVIG responders, suggesting that any marginal effect detected
in the combined study population could be due to the nonresponders.

**Coronary Artery Disease**

As noted, prevention or resolution of CAD is the principal goal of Kawasaki disease therapy. Thus, we defined CAD persistence a priori as an important clinical parameter also related to IVIG response. We identified 86 patients with persistent CAD (42 whites, 18 Asians, and 26 of other ethnic groups), 331 with no CAD, and 26 with a missing diagnosis. Excess transmission of the A allele in FcγRIIA-131H/R occurred in patients with CAD (Table 4) but was not apparent in whites or Asians separately. However, this occurred in concert with excess transmission for the entire Kawasaki disease population. In contrast, FcγRIIB-NA1/NA2 excess transmission occurred in patients with CAD, consistent with findings in the IVIG nonresponders, but despite a lack of apparent effect on the entire combined patients with Kawasaki disease.

**Discussion**

The principal finding in our hypothesis-driven study is that a highly significant association between IVIG response and FcγRIIB genotype in patients with Kawasaki disease has important pharmacogenomic and clinical implications. Reinforcing the demonstration that parental transmission of NA1 genotype substantially decreases the odds of appropriate clinical IVIG response, the study also showed that this genotype confers substantially greater risk of persistent CAD. To our knowledge, no prior investigation has specifically evaluated the impact of polymorphisms for genes transcribing FcγRs on clinically defined IVIG response. However, few studies analyzed associations between FcγRs, including NA1/NA2 and coronary artery lesions. Although they did not define coronary artery phenotype, only Bezieveld et al. reported slightly decreased risk (OR, 0.42; 95% CI, 0.16–0.69) of persistent CAD in white patients with Kawasaki disease with the genotype NA1/NA2 compared with NA1/NA1. Likely, the present study’s greater number of informative patients combined with parent data, with genetically determined homogenous populations, within the transmission disequilibrium test and FBAT framework provided adequate power to detect these NA1 associations with clearly defined phenotypes.

The potential biological mechanisms responsible for different IVIG responses between the NA1 and NA2 isoforms require elucidation. FcγRIIB is expressed almost exclusively on neutrophils, although recent data show low-level expression in human basophils. NA1 confers greater neutrophil IgG-dependent phagocytic capacity than NA2. This may

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**Table 2. TDT and Pseudosibling-Based Case-Control Analysis of Polymorphisms in Activating FcγRs With Susceptibility to Kawasaki Disease in Three Racial/Ethnic Groups**

<table>
<thead>
<tr>
<th>Polymorphisms/Genes Ethnicity</th>
<th>Associated Allele Frequency</th>
<th>Informative Families*</th>
<th>Z Statistic (P Value)</th>
<th>OR (95% CI)†</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIIA-131H/R</td>
<td>(A)</td>
<td>All ethnic groups</td>
<td>0.57</td>
<td>182</td>
<td>+3.12 (0.001)</td>
</tr>
<tr>
<td>FcγRIIA-48L/R</td>
<td>(T/g/a)</td>
<td>All ethnic groups</td>
<td>G=0.04</td>
<td>21/35/48</td>
<td>-0.85 (0.40)</td>
</tr>
<tr>
<td>FcγRIIA-158V/F</td>
<td>(G/T)</td>
<td>All ethnic groups</td>
<td>A=0.07</td>
<td>1.07 (0.83–1.39)</td>
<td>0.59</td>
</tr>
<tr>
<td>FcγRIIA-131H/R</td>
<td>(A/G)</td>
<td>All ethnic groups</td>
<td>0.35</td>
<td>179</td>
<td>+0.46 (0.64)</td>
</tr>
<tr>
<td>FcγRIIB-NA1/NA2 (NA1)</td>
<td>0.32</td>
<td>29</td>
<td>-2.00 (0.05)</td>
<td>0.55 (0.27–1.10)</td>
<td>0.09</td>
</tr>
<tr>
<td>FcγRIIB-NA1/NA2 (NA2)</td>
<td>0.60</td>
<td>157</td>
<td>+1.88 (0.06)</td>
<td>1.28 (0.97–1.69)</td>
<td>0.07</td>
</tr>
<tr>
<td>FcγRIIB-NA1/NA2 (NA2)</td>
<td>0.66</td>
<td>85</td>
<td>+1.60 (0.11)</td>
<td>1.32 (0.90–1.93)</td>
<td>0.15</td>
</tr>
<tr>
<td>FcγRIIB-NA1/NA2 (NA2)</td>
<td>0.47</td>
<td>30</td>
<td>+1.37 (0.17)</td>
<td>1.62 (0.81–3.23)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

TDT indicates transmission disequilibrium test; FcγR, IgG-Fc region receptor; OR, odds ratio; H, histidine; R, arginine; L, leucine; V, valine; F, phenylalanine; NA, neutrophil antigen.

*TDT statistical analyses were only performed where there were \( \geq 10 \) informative families.

†OR (additive) based on the genotype of the patients with Kawasaki disease and pseudosibling controls derived from the 3 alternate genotypes based on the untransmitted alleles.
Table 3. TDT and Pseudosibling-Based Case-Control Analysis of Activating FcγR Gene Variants Among IVIG Responding and IVIG Nonresponding Patients With Kawasaki Disease in Three Racial/Ethnic Groups

<table>
<thead>
<tr>
<th>Genes and Polymorphisms</th>
<th>TDT Statistics</th>
<th>Pseudosibling Case-Control*</th>
<th>TDT Statistics</th>
<th>Pseudosibling Case-Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Informative</td>
<td>Z Statistic (P Value)</td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td></td>
<td>Families†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcγRIIA-131H/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>115</td>
<td>+2.1 (0.04)</td>
<td>1.40 (1.01–1.95)</td>
<td>0.04</td>
</tr>
<tr>
<td>White</td>
<td>67</td>
<td>+0.97 (0.33)</td>
<td>1.24 (0.81–1.90)</td>
<td>0.33</td>
</tr>
<tr>
<td>Asian</td>
<td>17</td>
<td>+2.40 (0.02)</td>
<td>4.00 (1.34–11.96)</td>
<td>0.01</td>
</tr>
<tr>
<td>FcγRIIA-158V/F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>108</td>
<td>−0.25 (0.80)</td>
<td>1.00 (0.72–1.39)</td>
<td>1</td>
</tr>
<tr>
<td>White</td>
<td>67</td>
<td>+0.43 (0.67)</td>
<td>1.10 (0.72–1.68)</td>
<td>0.67</td>
</tr>
<tr>
<td>Asian</td>
<td>19</td>
<td>−2.04 (0.04)</td>
<td>4.00 (1.34–11.96)</td>
<td>0.01</td>
</tr>
<tr>
<td>FcγRIIB-NA1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>95</td>
<td>+1.51 (0.13)</td>
<td>1.28 (0.89–1.82)</td>
<td>0.18</td>
</tr>
<tr>
<td>White</td>
<td>48</td>
<td>+1.21 (0.23)</td>
<td>1.29 (0.79–2.10)</td>
<td>0.32</td>
</tr>
<tr>
<td>Asian</td>
<td>22</td>
<td>+0.82 (0.41)</td>
<td>2.14 (0.87–5.26)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

TDT indicates transmission disequilibrium test; FcγR, IgG-Fc region receptor; IVIG, intravenous immunoglobulin; OR, odds ratio; H, histidine; R, arginine; V, valine; F, phenylalanine; NA, neutrophil antigen.

*OR (additive) is based on the genotype of the patients with Kawasaki disease and pseudosibling controls derived from the 3 alternate genotypes based on the untransmitted alleles.

†TDT statistical analyses were only performed where there were ≥10 informative families.

Table 4. Results of TDT and Pseudosibling-Based Case-Control Analysis of Polymorphisms in Activating FcγRs Among Patients With KD With and Without CAD in Three Racial/Ethnic Groups

<table>
<thead>
<tr>
<th>Genes &amp; Polymorphisms</th>
<th>TDT Statistics</th>
<th>Pseudosibling Case-Control*</th>
<th>TDT Statistics</th>
<th>Pseudosibling Case-Control*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Informative</td>
<td>Z Statistic (P Value)</td>
<td>OR (95% CI)</td>
<td>P Value</td>
</tr>
<tr>
<td></td>
<td>Families†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FcγRIIA-131H/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>127</td>
<td>+1.79 (0.07)</td>
<td>1.33 (1.02–1.82)</td>
<td>0.07</td>
</tr>
<tr>
<td>White</td>
<td>79</td>
<td>+2.05 (0.04)</td>
<td>1.52 (1.01–2.22)</td>
<td>0.04</td>
</tr>
<tr>
<td>Asian</td>
<td>17</td>
<td>+1.15 (0.25)</td>
<td>0.58 (0.23–1.48)</td>
<td>0.26</td>
</tr>
<tr>
<td>FcγRIIA-158V/F</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>134</td>
<td>−0.23 (0.82)</td>
<td>0.96 (0.71–1.31)</td>
<td>0.81</td>
</tr>
<tr>
<td>White</td>
<td>91</td>
<td>0 (1)</td>
<td>1.00 (0.69–1.55)</td>
<td>1</td>
</tr>
<tr>
<td>Asian</td>
<td>21</td>
<td>−1.63 (0.10)</td>
<td>0.50 (0.21–1.17)</td>
<td>0.11</td>
</tr>
<tr>
<td>FcγRIIB-NA1</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>All ethnic groups</td>
<td>113</td>
<td>+0.65 (0.52)</td>
<td>1.09 (0.80–1.49)</td>
<td>0.58</td>
</tr>
<tr>
<td>White</td>
<td>63</td>
<td>+1.07 (0.29)</td>
<td>1.24 (0.81–1.85)</td>
<td>0.28</td>
</tr>
<tr>
<td>Asian</td>
<td>21</td>
<td>+0.82 (0.41)</td>
<td>1.41 (0.62–3.15)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

TDT indicates transmission disequilibrium test; FcγR, IgG-Fc region receptor; KD, Kawasaki disease; CAD, coronary artery disease; OR, odds ratio; H, histidine; R, arginine; V, valine; F, phenylalanine; NA, neutrophil antigen; L, leucine.

*OR (additive) is based on the genotype of the patients with KD and pseudosibling controls derived from the 3 alternate genotypes based on the untransmitted alleles.

†TDT statistical analyses were only performed where there were ≥10 informative families (several single-nucleotide polymorphisms, specifically FcγRIIA-48R/L, did not have enough informative families, especially in Asians and Hispanics).
persistent CAD.29 The present data offer an intriguing possibility that IVIG manipulates NA1/NA2-dependent activity in Kawasaki disease.

We also found excess transmission of the more potent FcγRIIA-131H variant among patients with Kawasaki disease. This finding validates a recent report from a genome-wide association study by an international Kawasaki disease consortium.30 The polymorphism (A/g) in FcγRIIA-131H/R alters recognition of the ligand in that the receptor encoded by FcγRIIA-131H (A allele) shows greater binding affinity for IgG231 and, thus, more effective phagocytosis of IgG2-opsosized particles. This receptor variant also shows decreased binding affinity for C-reactive protein, which shares several functional activities with IgG2 and is markedly elevated during acute Kawasaki disease. Tanuichi et al26 previously reported an FcγRIIA-131H association with CAD in a small set of Japanese patients with Kawasaki disease. However, the present data suggest that this allele relates to overall Kawasaki disease susceptibility rather than to specific CAD risk.

The multiple FcγRs interact with FcγRIIB (inhibitory), with one another, or both through their coligation at the immune effector cell membrane. Thus, functional polymorphisms within the genes regulating these receptors can alter the balance between activation and inhibition and thereby influence their interaction. Strengths of associations between individual FcγR SNPs and inflammatory disease susceptibility clearly vary by race and may be explained by the racial variation in the gene sequences of the other receptors. As previously noted, FcγRIIB polymorphisms influence IVIG response in patients with Kawasaki disease in a racially dependent manner. The presence of FcγRIIB (-120 A and -386 C) minor alleles in white patients improved their chance for positive IVIG therapeutic response.12 Functionality for these SNPs within the human FcγRIIB promoter region has been confirmed in that they enhance transcription factor binding. Yet, these SNPs were absent in the Asians studied, further emphasizing that balance between the activating and inhibitory receptors varies by ethnicity. Although these FcγRs are located in the same region, with high linkage disequilibrium, the SNPs were not highly correlated. The correlations, however, also varied between the 2 ethnic groups.

In following, we logically performed ethnicity-specific analyses for the FcγRs in the current study. There are limitations related to the ethnic stratification. The low number of informative families for the SNPs within these subgroups limited the power of the observations. Correction for multiple testing is conventionally performed for noncandidate-based studies, such as genome-wide association studies, which evaluate numerous variants. Some might suggest that our stratification introduces the requirement for such correction. However, the need for correction in a hypothesis-driven study evaluating functional SNPs remains controversial. With regard to the observed associations related to the ethnic stratification as well as to CAD, significance is somewhat less. We cautiously report these latter findings, which will require validation with larger subject numbers in these ethnic groups.

Acknowledgments

The data were presented in part at the Scientific Sessions of the American Heart Association, Orlando, Florida, November 14, 2011. We are grateful to the participating patients and their parents as well as to the investigators, pediatricians, and staff members of the participating clinics. We thank Dolenia Ledee for handling the biospecimens and DNA extraction and Deborah S. McDuffie for genotyping.

Sources of Funding

This study was supported by National Heart, Lung, and Blood Institute grant R21-HL90558 and a grant from the Thrasher Foundation Research Fund.

Disclosures

None.

References


Disclosures

None.

References


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**CLINICAL PERSPECTIVE**

Kawasaki disease is a prototypic vasculitis in children and is diagnosed based on the presence of fever, clinical features, and supportive laboratory criteria. The vasculitis shows a predilection for the coronary arteries. Coronary artery inflammation can result in dilation and aneurysm formation. Intravenous immunoglobulin (IVIG) infusion with aspirin successfully treats fever and prevents coronary artery inflammation. The mechanism of IVIG action has not been substantiated. Many patients demonstrate refractoriness to IVIG treatment with persistent or recurrent fever and remain at high risk for development of coronary artery disease. Prior studies suggested that IgG-Fc region receptors (FCRs) represent plausible Kawasaki disease mediators because of their direct interaction with IgG. In the current study, we demonstrate that polymorphisms for genes regulating the activating FCRs are associated with both IVIG treatment response and susceptibility to Kawasaki disease. In particular, family-based testing shows that the FCγRIIB neutrophil antigen I haplotype is excessively transmitted to non-IVIG-responding patients in the Kawasaki disease study population. Confirmation of this association in an independent Kawasaki disease population would further substantiate genotyping for this polymorphism as a mode of predicting IVIG response. Thus, the genotype could be used early in the disease to determine whether alternative forms of treatment would be beneficial. Additionally, we offer confirmation of other studies that the FCγRIIA-131H polymorphism relates to disease susceptibility. This genotype could be pursued as a tool for diagnosis and evaluation of Kawasaki disease pathogenesis.
Role of Activating $Fc\gamma R$ Gene Polymorphisms in Kawasaki Disease Susceptibility and Intravenous Immunoglobulin Response

_Circ Cardiovasc Genet_. 2012;5:309-316; originally published online May 7, 2012; doi: 10.1161/CIRCGENETICS.111.962464
_Circulation: Cardiovascular Genetics_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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**Supplementary Table 1. PCR and pyrosequencing primers used in the analysis of functional variants in FcγRIIA, FcγRIIIA, and FcγRIIIB**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism*</th>
<th>First Round Nested PCR (5'-3')</th>
<th>Second Round PCR (5'-3')</th>
<th>Pyrosequencing Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>FcγRIIA</td>
<td>FcγRIIA-131H/R (A/g)</td>
<td>F: TGCCATAAGAGAATGCTCACA R: TCAAGTGAACAACAGCCTGACT</td>
<td>F: CATGCTGAGGTGCCACAG R: bio-ACAGCGTGGATGCTATGGTTC</td>
<td>CCCAGAAATTCTCCC</td>
</tr>
<tr>
<td>FcγRIIA</td>
<td>FcγRIIA-158V/F (T/g)</td>
<td>F: CTGGTGTTTACATTGAGTTCT R: CTGATTCGGAGGCTGTTCTACA</td>
<td>F: bio-AGGCAGGAAGTATTTTCT</td>
<td>GACACATTTTTACTCCCAA</td>
</tr>
<tr>
<td>FcγRIIIA</td>
<td>FcγRIIIA-48L/R/H (T/g/a)</td>
<td>F: TTCAAGAAAAAGAAATGGTG R: CCCAACACCTACTAGAGCTA</td>
<td>F: TACAGGCTCCAGAACGA R: CGAGGCCTGGCTTGA</td>
<td>AGTGGTTCAACAATGAG</td>
</tr>
<tr>
<td>FcγRIIIB</td>
<td>NA1/NA2</td>
<td>F: TTCAAGAAAAAGAAACTGGCA R: CCCAACCTACTAGAGCTA</td>
<td>F: AAGATCTCCCCCCAGGTGTG</td>
<td>GCCTCAATGGTACAG</td>
</tr>
</tbody>
</table>

*The first allele is the most frequent allele in our population in all ethnic group.*