Role of Activating $Fc\gamma R$ Gene Polymorphisms in Kawasaki Disease
Susceptibility and Intravenous Immunoglobulin Response

Running title: Shrestha et al.; $Fc\gamma R$ genes and Kawasaki Disease

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Abstract:

**Background** - A functional polymorphism in the inhibitory IgG-Fc receptor 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 (CAD) in KD patients.

**Methods and Results** - We genotyped polymorphisms in the activating FcγRIIA, FcγRIIIA and FcγRIIB genes using pyrosequencing in 443 KD patients, including 266 trios and 150 single parent-child pairs, in northwest US and genetically determined race with 155 ancestry information markers. We used the FBAT program to test for transmission disequilibrium and further generated pseudo-sibling controls for comparisons to the cases. The FcγRIIA +311H variant showed an association with KD (p = 0.001) with ORadditive = 1.51 [1.16-1.96], p = 0.002) for the primary combined population, which persisted in both Caucasian (p = .04) and Asian (p = .01) subgroups and is consistent with the recent genome-wide association study. We also identified over-transmission of FcγRIIB-NA1 among IVIG non-responders (p = 0.0002), and specifically to Caucasian IVIG non-responders (p = 0.007). Odds ratios for overall and Caucasian non-responders were respectively 3.67 [1.75-7.66], p = 0.0006 and 3.60 [1.34-9.70], p = 0.01. Excess NA1 transmission also occurred to KD with CAD (ORadditive = 2.13 [1.11-4.0], p = 0.02).

**Conclusions** - A common variation in FcγRIIA is associated with increased KD susceptibility. The FcγRIIB-NA1, which confers higher affinity for IgG compared to NA2, is a determining factor for treatment response. These activating FcγRs play an important role in KD pathogenesis and mechanism of IVIG anti-inflammatory.

**Key words:** coronary disease; Kawasaki disease; pediatrics; FCgammaReceptors; IVIG treatment response
Introduction

Human pooled intravenous immunoglobin (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 or aneurysm formation, is the primary treatment goal. Progression to a giant aneurysm requires anti-coagulation 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 more than 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 KD is endemic,\(^8\) their sensitivity in heterogeneous North American populations is poor.\(^7\) KD incidence varies considerably according race to and ethnicity. Previous investigators have suggested that the differences in the predictive value of the risk scores also reflect the genetic diversity.\(^9\)

The IVIG mechanisms of anti-inflammatory action still require elucidation in humans. This knowledge deficit hinders identification of candidate genes involved in KD pathogenesis, and polymorphisms which can predict treatment response. IgG-Fc region receptors (FC\(\gamma\)Rs) represent plausible KD mediators due to their direct interaction with immunoglobulin G. FC\(\gamma\)Rs 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.\(^10\) These molecules facilitate antibody-antigen interactions. Studies in mice lacking various forms of FC\(\gamma\)R have documented their key roles in the balance
between activating and inhibitory receptor signals in experimental idiopathic thrombocytopenic purpura (ITP), as well as for how modulation of this balance might account for the therapeutic effects of IVIG.\textsuperscript{11} 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 analogues. The murine models suggest an important and possibly dominant role for the inhibitory FcγRIIB in the IVIG anti-inflammatory mechanism. Genetic association studies in humans support such FcγRIIB participation. However, the low frequency of the particular functional FcγRIIB polymorphisms in all the populations limits its clinically relevant role\textsuperscript{12}.

The activating Fcγ receptors interact with the single inhibitory receptor FcγRIIB. Thus, we hypothesized that polymorphisms in the activating receptors (FcγRIIA, FcγRIIIA and FcγRIIB) influence the IVIG treatment response defined by clinical parameters. We also analyzed these receptors with regards to susceptibility, and persistence of coronary artery disease in KD patients. 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 U.S based population of KD patients and their parents containing 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 participating centers - Seattle Children’s Hospital, Oakland Children’s Hospital, and Primary Children’s Hospital of Utah. Retrospective cross-referencing of the
hospital database and the Heart Center echocardiography databases confirmed the diagnosis and treatment of all participating KD patients. After approval by the IRB at all participating institutions, parents were approached for study recruitment and informed consent was obtained.

**Clinical Diagnosis of KD:** The definition of complete KD 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 two clinical criteria and coronary artery involvement as defined in the AHA guidelines.5

**Treatment Response:** Treatment response was determined in patients receiving IVIG (2 gm/kg) within 11 days of initial fever.5 As stated in the AHA/AAP Endorsed Clinical Report,13 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):** CAD was defined by echocardiography as dilation (Z-score>2.5, according to Boston Z-score data14 or aneurysm defined by Japanese Ministry of Health Criteria persisting>6 weeks after IVIG treatment (2 gm/kg).

**Bio-specimen Collection and DNA Extraction:** Most parents consented to have blood samples taken from their KD offspring, and whole blood was collected in ACD (citrated) anticoagulant tubes. For the remainder saliva was collected in Oragene™ kits (DNA Genotek, Ottawa) by a noninvasive technique proven to preserve DNA. Briefly, participants first rinsed their mouth with water to clear food particles and then expectorated 2 mL of saliva into the Oragene™ vial. Genomic DNA was extracted using the Versagene™ DNA purification kit (Gentra Systems, Minneapolis, MN) and quantified using the PicoGreen assay for double-stranded DNA, adjusted
to a final concentration of 100ng/μL, and stored at -80°C in TE.

Activating FcγR genes and polymorphisms

The activating FcγRIIA, FcγRIIA and FcγRIIB genes are all located at chromosome position 1q2311,15 within a span of about 200kb. FcγRIIA has affinity for IgG and interacts with IgG or immune complexed IgG on cell surfaces. FcγRIIB is a relatively low affinity receptor. FcγRIIA is expressed as a membrane-spanning receptor on macrophages and natural killer cells. These three genes produce integral transmembrane glycoproteins that are considered functionally activating receptors.

Five well-characterized and functionally relevant SNPs were examined: i) a common FcγRIIA SNP (rs1801274) affecting amino acid position 131 in EC2 region (FcγRIIA-131H/R), A and G alleles at position 131 coding for codominantly expressed arginine (R) and histidine (H), respectively and are known to affect affinity for IgG2 and associated with several immune related diseases;10,16,17 ii) a common FcγRIIA SNP (rs396991), G and T alleles coding either valine (V) or phenylalanine (F), respectively at position 158 in EC2 region (FcγRIIA-158V/F) known to be associated with immune related diseases,16-18 where valine (V) has higher affinity for IgG1 and IgG3 than the phenylalanine (F) isoform; iii) another triallelic FcγRIIA SNP (rs10127939) at amino acid position 48 (FcγRIIA-48L/R/H), T, G and A alleles encoding for either leucine (L), arginine (R) or histidine (H), respectively and are known to influence the binding of IgG by NK cells19; and iv) two FcγRIIB SNPs at positions 141 (rs403016) and 147 (rs447536) in the extracellular domain 1 (EC1) that result in variable amino acid sequences resulting in two allotypic forms named neutrophil antigen NA1 and NA2. While 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.20
Genotyping methods

The functional polymorphisms in the FcγR gene 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 a second round nested PCR reactions using 0.25 ul of the first round PCR products as templates. All PCR reactions were carried out with Taq polymerase (Invitrogen, Carlsbad, CA). Biotinylated second round PCR products amplified with primer pairs (one of primer pairs is labeled with biotin) were used for subsequent pyrosequencing reactions. The PCR reactions and cycling conditions have been previously described. Briefly, amplification is performed with one biotinylated primer to allow for purification of a single stranded template for the pyrosequencing reaction. Following denaturation of the PCR amplicon in 0.1 M NaOH for 10 minutes, the single stranded product is immobilized to streptavidin-sepharose (Amersham Biosciences), washed and annealed with 15 pmoles of a “Pyrosequencing” primer. The primers and probes for the two rounds of PCR and pyrosequencing primers are listed in supplementary Table 1. Pyrosequencing was performed according to the manufacturer’s instructions as previously described.

Statistical methods

First, genotype completeness was checked for each SNP and Hardy-Weinberg equilibrium (HWE) was examined in each ethnic group. Statistical analysis for KD susceptibility was performed by the transmission disequilibrium test (TDT), 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 TDT is distributed as a chi-square with 1 df and is calculated as \((b-c)^2/(b+c)\) where \(b\) and \(c\) are, respectively, the number of transmissions and non-
transmissions of the allele from heterozygous parents to their affected children. All analyses
were performed assuming additive genetic models with a minimum informative family size set to
ten. We used the Family Based Association Test 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 KD than 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 KD. 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 (OR) and
95% confidence intervals (CI) in the additive model.

As primary analysis of the study, FBAT analyses were performed separately for
responders and non-responders to determine if alleles were differentially transmitted.

Additionally, as a secondary analysis, differential transmission was examined among CAD and
non-CAD patients. All analyses were performed for the entire set of KD patients and also
separately for Caucasians and Asians, as determined by the principal component analysis (PCA)
of ancestry informative markers (AIMs), as previously described. While FBAT accounts for
population admixture, the underlying assumption of the same genetic association may not hold
due to allelic and genetic heterogeneity in different ethnic groups. For ethnicity-specific
analyses, only families with all members clustered in the same ethnic group (all three for trios
and two 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 more than 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 was complete for over 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 three principal component values were used to discriminate individuals into four major race/ethnic groups (Figure 1); one for a homogenous European ancestry, one for a more heterogeneous Asian ancestry, another for a heterogeneous Hispanic ancestry (predominantly Mexican or Mexican-American) and the fourth small one for an African ancestry. However, there were many genetically heterogeneous individuals that could not be defined to one single race/ethnic group. Self-report (or as reported by parents) of all KD patients and participating family members showed slightly less than 90% match with the genetically determined ancestry: 570 out of 599 who self-reported to be Caucasians matched; 153 out of 173 who self-reported to be Asian/Asian American matched; 147 out of 166 who self-reported Hispanic matched; 95 self-reported multiple ethnicity and 61 did not report any ethnicity. Based on the AIMS, we were underpowered with smaller African American sample size and the Hispanic ancestry though distinct from other race/ethnic groups as previously reported was very heterogeneous. Therefore, we restricted our analysis in this study 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 (IQR: 15-58 months) and 62% of them were males, with a male-to-female ratio of 1.6:1, similar to the ratios reported in general populations. The median
age between the treatment responders and the non-responders was not significantly different (35 [16-58] vs. 31 [15-66] months), and there were slightly more non-responders (63%) than responders (60%) among males. Of the 443 KD patients recruited into the study (242 Caucasians, 82 Asians, 88 Hispanics and 31 African Americans), 266 trios (156, 44, 52 and 14 from the four ethnic groups, respectively) and 150 single parent-child pair (75, 29, 30 and 16, respectively) were included in the TDT analyses. Seven families were excluded due to Mendelian errors with AIMs. From the review of medical records, 267 patients (141 Caucasians, 57 Asians, 51 Hispanics and 18 of other ethnic groups) could be classified as IVIG treatment responsive and 86 (51 Caucasians, 9 Asians, and 20 Hispanics and 6 of other ethnic groups) patients as non-responsive, with genetic data available for at least one biological parent participating in the study. Interestingly, \( Fc\gamma RI A-48 \) was tri-allelic mostly in Caucasians, but below 5% frequency in other ethnic groups.

**Susceptibility**

We tested the primary hypothesis relating to IVIG treatment response in patients of European (Caucasian) and Asian ancestry (Asian). However, these polymorphisms influence susceptibility for other inflammatory diseases. Accordingly, we first analyzed their association with disease within the entire study population. Most importantly, we showed excess A allele (histidine) transmission in \( Fc\gamma RI A-131H/R \) from heterozygous parents to affected offspring \( (n = 182, z = +3.12, p = 0.001) \) in the additive model (Table 2). Ethnic stratification revealed differences in the A allele frequency between Asian and other racial groups. Excess A allele transmission occurred in Caucasians \( (n = 105, z = +2.04, p = 0.04) \) and Asian families \( (n = 26, z = +2.34, p=0.02) \).

Analyses using pseudosibling controls for comparisons with KD patients demonstrated A allele association with KD \( (OR_{\text{additive}} = 1.51 [1.16-1.96], p = 0.002) \) for the primary combined study.
population, for Asians (OR_additive = 2.75 [1.22-6.25], p = 0.01) and for Caucasians (OR_additive = 1.43 [1.02-2.00], 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 (OR_additive = 1.40 [1.01-1.95], p = 0.04) overall among IVIG responders. Relatively high risk occurred for this allele among Asian responders (OR_additive = 4.00 [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 KD susceptibility (n = 157, z = +1.88, p = 0.06, table 2).

**IVIG Non-response**

We found FcγRIIB-NA1 excessive transmission in the IVIG non-responding subgroup (n = 34, z = +3.70, p = 0.0002, table 3). This highly significant effect was detected in Caucasians (n = 20, z = +2.71, p = 0.007,Table 3) on subgroup analysis; we lacked adequate numbers of informative families to test transmission in the Asians. Pseudosibling analyses revealed the corresponding odds ratios for the combined and Caucasian IVIG non-responders (OR_additive = 3.67 [1.75-7.66], p = 0.0006 and 3.60 [1.34-9.70], p = 0.01), respectively (Table 3). No such over-transmission was observed among IVIG responding patients, suggesting that any marginal effect detected in the combined study population, could be due to the non-responders.

**Coronary Artery Disease (CAD):** As noted, prevention or resolution of CAD is the principal goal of KD 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
Caucasians and 18 Asians and 26 of other ethnic groups), 331 with no CAD and 26 with a missing diagnosis. Excess transmission of the A in FcγRIIA-131H/R occurred in CAD patients (Tables 4), but was not apparent in Caucasians or Asians separately. However, this occurred in concert with excess transmission for the entire KD population. In contrast, FcγRIIIB- NA1 excess transmission occurred in CAD patients, consistent with findings in the IVIG non-responders, but despite lack of apparent effect on the entire combined KD patients.

**Discussion**

The principal finding in our hypothesis driven study, a highly significant association between IVIG response and FcγRIIIB genotype in KD patients, has important pharmacogenomic and clinical implications. Reinforcing demonstration that parental transmission of NA1 genotype substantially decreases the odds of appropriate clinical IVIG response, our study also showed that this genotype confers substantially greater risk of persistent coronary artery disease. No prior investigation has specifically evaluated the impact of polymorphisms for genes transcribing FCγ activating receptors on clinically defined IVIG response. However, few studies analyzed associations between FCγRs including NA1/NA2 and coronary artery lesions.25,26 Though they did not define coronary artery phenotype, only Biezeveld et al25 reported slightly decreased risk (OR = 0.42 [0.16-1.06]; p = 0.06) of coronary artery lesions in Caucasian KD patients with the genotype NA1/NA2 compared to NA1/NA1. Likely, our greater number of informative patients combined with parent data, with genetically determined homogenous populations, within the TDT 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.27 NA1 confers greater neutrophil IgG dependent phagocytic capacity than NA2.10 This may relate to differences in the number of functionally relevant N-linked glycosylation sites that affect their interaction with IgG as NA1 has a higher binding affinity for IgG1 and IgG3. Alternatively, NA1 and NA2 may interact differently with other cell surface receptors such as β2 integrin, CD11b/CD18.

Some clinical and pathological evidence suggests an important role for neutrophils in KD and coronary artery pathogenesis.28 In particular, CD11b expression on neutrophils increases during the acute disease phase, declines after treatment, and remains elevated in patients with persistent coronary artery disease.29 Our data offer an intriguing possibility that IVIG manipulates NA1/NA2 dependent activity in KD.

We also found excess transmission of the more potent FcγRIIA-131H variant among KD patients. This finding validates a recent report from a genome-wide association study by an international KD consortium.30 The polymorphism (A/g) in FcγRIIA-131H/R alters recognition of ligand in that the receptor encoded by FcγRIIA-131H shows greater binding affinity for IgG2,31 and thus more effective phagocytosis of IgG2 opsonized 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 KD. Tanuichi et al previously reported an FcγRIIA-131H association with CAD in a small set of Japanese KD patients.26 However, our data suggest that this allele relates to overall KD susceptibility rather than specific coronary artery disease risk.

The multiple FcγR activating receptors interact with FcγRIIB (inhibitory) and/or each other through their co-ligation 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 FcγRs 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 KD patients in a racially dependent manner. The presence of FcγRIIB (-120 A and -386 C) minor alleles in Caucasian patients improved their chance for positive IVIG therapeutic response. 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. While these FcγR genes are located in the same region, with high linkage disequilibrium (D’), the SNPs were not highly correlated. The correlations (r²), however also varied between the two 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 our observations. Correction for multiple testing is conventionally performed for non-candidate based studies such as GWAS, 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 our observed associations related to the ethnic stratification as well as coronary artery disease, significance is somewhat less. We cautiously report these latter findings, which will require validation with larger subject numbers in these ethnic groups.
Acknowledgments: This data was presented in part at the Scientific Sessions of the American Heart Association, Orlando, November 14, 2011. We are grateful to participating patients and their parents as well as the investigators, pediatricians and staffs of the participating clinics. We thank Dolena Ledee for handling of the biospecimens and DNA extraction and Deborah S McDuffie for genotyping.

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Conflict of Interest Disclosures: None

References:


Table 1. Demographic characteristics of participating KD patients

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>ALL KD patients</th>
<th>IVIG Responders</th>
<th>IVIG Non-responders</th>
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<th>CAD-</th>
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<tr>
<td>Mean (sd)</td>
<td>41.9 (33.5)</td>
<td>41.8 (32.4)</td>
<td>41.7 (33.2)</td>
<td>33.5 (37.5)</td>
<td>44.5 (32.1)</td>
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<td>Median (IQR)</td>
<td>34 (15-58)</td>
<td>35 (16-58)</td>
<td>31 (15-66)</td>
<td>21 (8-50)</td>
<td>40 (19-60)</td>
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<td>161</td>
<td>102</td>
<td>29</td>
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<td>133</td>
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</table>

*89 KD patients either did not have IVIG response data or were in different treatment, 12 did not have CAD data, 30 did not have the age listed and 19 did not have the gender; †race/ethnicity based on Principal component analysis (PCA) and discrimination procedures using 155 Ancestry Informative Markers (AIMS)

Table 2. TDT and pseudosibling based case-control analysis of polymorphisms in activating FcγR genes with susceptibility to Kawasaki disease in three racial/ethnic groups

<table>
<thead>
<tr>
<th>Polymorphisms/Genes</th>
<th>Associated allele</th>
<th>Informative families</th>
<th>z-statistics (p-value)</th>
<th>OR (95% CI)*</th>
<th>p</th>
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<tr>
<td>FcγRIIa-131H/R(a/g)</td>
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<tr>
<td>All Ethnic groups</td>
<td>0.57</td>
<td>182</td>
<td>+3.12 (0.001)</td>
<td>1.51 (1.16-1.96)</td>
<td>0.002</td>
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<td>Caucasians</td>
<td>0.54</td>
<td>105</td>
<td>+2.04 (0.04)</td>
<td>1.43 (1.02-2.01)</td>
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<td>2.75 (1.22-6.18)</td>
<td>0.01</td>
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<td>-0.60 (0.55)</td>
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<td>FcγRIIa-158V/F(t/g)</td>
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<tr>
<td>All Ethnic groups</td>
<td>0.35</td>
<td>179</td>
<td>+0.46 (0.64)</td>
<td>1.07 (0.83-1.39)</td>
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<tr>
<td>Asians</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-NA/NA2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Ethnic groups</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>Caucasians</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>Asians</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 statistics was only performed where there were 10 or more informative families; †OR (additive) based on the genotype of the KD patients 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 non-responding Kawasaki patients in three racial/ethnic groups†

<table>
<thead>
<tr>
<th>Genes &amp; Polymorphisms</th>
<th>IVIG Responders</th>
<th>TDT statistics</th>
<th>Pseudosibling case-control†</th>
<th>IVIG non-responders</th>
<th>TDT statistics</th>
<th>Pseudosibling case-control†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Informative</td>
<td>z-statistics</td>
<td>OR (95% CI) p</td>
<td>Informative</td>
<td>z-statistics</td>
<td>OR (95% CI) p</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>+2.1 (0.04)</td>
<td>1.40 (1.01-1.95) 0.04</td>
<td>+2.71 (0.007)</td>
<td>3.6 (1.34-9.70) 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>+0.97 (0.33)</td>
<td>1.24 (0.81-1.90) 0.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>+2.40 (0.02)</td>
<td>4.00 (1.34-11.96) 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRIIA-158V/F</td>
<td>All</td>
<td>-0.25 (0.80)</td>
<td>1.00 (0.72-1.39)</td>
<td>40</td>
<td>+1.48 (0.14)</td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>+0.43 (0.67)</td>
<td>1.10 (0.72-1.68) 0.67</td>
<td></td>
<td>42</td>
<td>+0.82 (0.41)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>-2.04 (0.04)</td>
<td>4.00 (1.34-11.96) 0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRIIB-NA1</td>
<td>All</td>
<td>+1.51 (0.13)</td>
<td>1.28 (0.89-1.82)</td>
<td>34</td>
<td>+2.71 (0.002)</td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>+1.21 (0.23)</td>
<td>1.89 (0.79-2.10) 0.32</td>
<td></td>
<td>20</td>
<td>+2.71 (0.007)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>+0.82 (0.41)</td>
<td>2.14 (0.87-5.26) 0.10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TDT statistics was only performed where there were 10 or more informative families; †OR (additive) based on the genotype of the KD patients and pseudosibling controls derived from the 3 alternate genotypes based on the untransmitted alleles.

Table 4. Results of TDT and pseudosibling based case-control analysis of polymorphisms in activating FcγR genes among Kawasaki disease patients with and without coronary artery disease (CAD) in three racial/ethnic groups†

<table>
<thead>
<tr>
<th>Genes &amp; Polymorphisms</th>
<th>KD patients without CAD (n = 331)</th>
<th>TDT statistics</th>
<th>Pseudosibling case-control†</th>
<th>KD patients with CAD (n = 86)</th>
<th>TDT statistics</th>
<th>Pseudosibling case-control†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Informative</td>
<td>z-statistics</td>
<td>OR (95% CI) p</td>
<td>Informative</td>
<td>z-statistics</td>
<td>OR (95% CI) p</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>+1.79 (0.07)</td>
<td>1.33 (1.02-1.82) 0.07</td>
<td>+2.45 (0.01)</td>
<td>1.89 (1.10-3.33) 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>+2.05 (0.04)</td>
<td>1.52 (1.01-2.22) 0.04</td>
<td></td>
<td>20</td>
<td>0 (1)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>+1.15 (0.25)</td>
<td>0.58 (0.23-1.48) 0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRIIA-158V/F</td>
<td>All</td>
<td>-0.23 (0.82)</td>
<td>0.96 (0.71-1.31) 0.81</td>
<td>40</td>
<td>+1.66 (0.10)</td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>0 (1)</td>
<td>1.00 (0.69-1.55) 1</td>
<td></td>
<td>18</td>
<td>+0.85 (0.39)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>-1.63 (0.10)</td>
<td>0.50 (0.21-1.71) 0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FcγRIIB-NA1</td>
<td>All</td>
<td>+0.65 (0.52)</td>
<td>1.09 (0.80-1.49) 0.58</td>
<td>35</td>
<td>+2.41 (0.02)</td>
</tr>
<tr>
<td></td>
<td>Caucasians</td>
<td>+1.07 (0.29)</td>
<td>1.24 (0.81-1.85) 0.28</td>
<td></td>
<td>17</td>
<td>+1.34 (0.18)</td>
</tr>
<tr>
<td></td>
<td>Asians</td>
<td>+0.82 (0.41)</td>
<td>1.41 (0.62-3.15) 0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TDT statistics was only performed where there were 10 or more informative families (several SNPs, specifically FcγRIIA-48R/L did not have enough informative families especially in Asians and Hispanics); †OR (additive) based on the genotype of the KD patients and pseudosibling controls derived from the 3 alternate genotypes based on the untransmitted alleles.
Figure Legend:

**Figure.** The first, second and third principal components (PC1, PC2 and PC3) based on 155 ancestry informative markers (AIMs) for the entire study population of Kawasaki disease patients and their parents. The clustering of different ethnic groups is shown by different colors; Caucasian (blue), African/African American (pink), Asian/Asian American (green), Hispanic (red).
Role of Activating FcγR Gene Polymorphisms in Kawasaki Disease Susceptibility and Intravenous Immunoglobulin Response

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