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

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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 *FcγRIIIB*) 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 *FcγRIIIB* 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-131H* variant showed an association with KD ($p = 0.001$) with $OR_{additive} = 1.51$ [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 *FcγRIIIB-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 ($OR_{additive} = 2.13$ [1.11–4.0], $p = 0.02$).

Conclusion—A common variation in *FcγRIIA* is associated with increased KD susceptibility. The *FcγRIIIB-NA1*, which confers higher affinity for IgG compared to NA2, is a determining

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Disclosures

None

factor for treatment response. These activating Fc γ R_s play an important role in KD pathogenesis and mechanism of IVIG anti-inflammatory.

Keywords

coronary disease; pediatrics; Kawasaki disease; IVIG treatment response; Fc γ R

Introduction

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 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.⁹ 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 γ R_s) represent plausible KD mediators due to their direct interaction with immunoglobulin G. Fc γ R_s 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 γ 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.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 Fc γ RIIB in the IVIG anti-inflammatory mechanism. Genetic association studies in humans support such Fc γ RIIB participation. However, the low frequency of the particular functional Fc γ RIIB polymorphisms in all the populations limits its clinically relevant role.¹²

The activating Fc γ receptors interact with the single inhibitory receptor Fc γ RIIB. Thus, we hypothesized that polymorphisms in the activating receptors (Fc γ RIIA, Fc γ RIIA and Fc γ RIIB) 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 Fc γ R 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 1q23^{11, 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 *FcγRIIIA* SNP (rs10127939) at amino acid position 48 (*FcγRIIIA*-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 *FcγRIIIB* 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.²⁰ 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.^{20, 21} 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.²²

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.¹³ 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.²³ 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.¹² 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 reported²⁴ 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, *FcγRIIIA*-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 *FcγRIIA-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 ($OR_{\text{additive}} = 2.75$ [1.22–6.25], $p = 0.01$) and for Caucasians ($OR_{\text{additive}} = 1.43$ [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 ($OR_{\text{additive}} = 1.40$ [1.01–1.95], $p = 0.04$) overall among IVIG responders. Relatively high risk occurred for this allele among Asian responders ($OR_{\text{additive}} = 4.00$ [1.34–11.96], $p = 0.01$).

The *FcγRIIIA-158G* 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, *FCGR3A-48L/R/H* occurring at low frequency showed no effects. Similarly, *FcγRIIIB-NA1* showed no significant association with KD susceptibility ($n = 157$, $z = +1.88$, $p = 0.06$, table 2).

IVIG Non-response

We found *FcγRIIIB-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 ($OR_{\text{additive}} = 3.67$ [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 FC γ R_s 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. *Fc γ RIIB* 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 β 2 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 rises during the acute disease phase, declines after treatment, and remains elevated in 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 *Fc γ RIIA-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 *Fc γ RIIA-131H/R* alters recognition of ligand in that the receptor encoded by *Fc γ RIIA-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 *Fc γ RIIA-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 *Fc γ R* activating receptors interact with *Fc γ RIIB* (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 *Fc γ R* 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, *Fc γ RIIB* polymorphisms influence IVIG response in KD patients in a racially dependent manner. The presence of *Fc γ RIIB* (–120 A and –386 C) minor alleles in Caucasian patients improved their chance for positive IVIG therapeutic response.¹² Functionality for these SNPs within the human *Fc γ RIIB* 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 *Fc γ R* genes are located in the same region, with high linkage disequilibrium (D'), the SNPs were not highly correlated. The correlations (r^2), however also varied between the two ethnic groups.

In following, we logically performed ethnicity specific analyses for the *Fc γ R*s 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Kawasaki Disease is a prototypic vasculitis in children and is diagnosed by 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 or aneurysm formation. Intravenous gamma globulin (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 have suggested that IgG-Fc region receptors (FC γ Rs) represent plausible KD mediators due to their direct interaction with immunoglobulin G. In the current study we demonstrate that polymorphisms for genes regulating the activating FC γ Rs are associated both with IVIG treatment response and susceptibility to KD. In particular, family based testing shows that the FC γ RIIB-NA1 haplotype is excessively transmitted to non-IVIG responding patients in our KD population. Confirmation of this association in an independent KD 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 for other studies that FC γ RIIA-131H polymorphism relates to disease susceptibility. This genotype could be pursued as a tool for diagnosis and evaluation of KD pathogenesis.

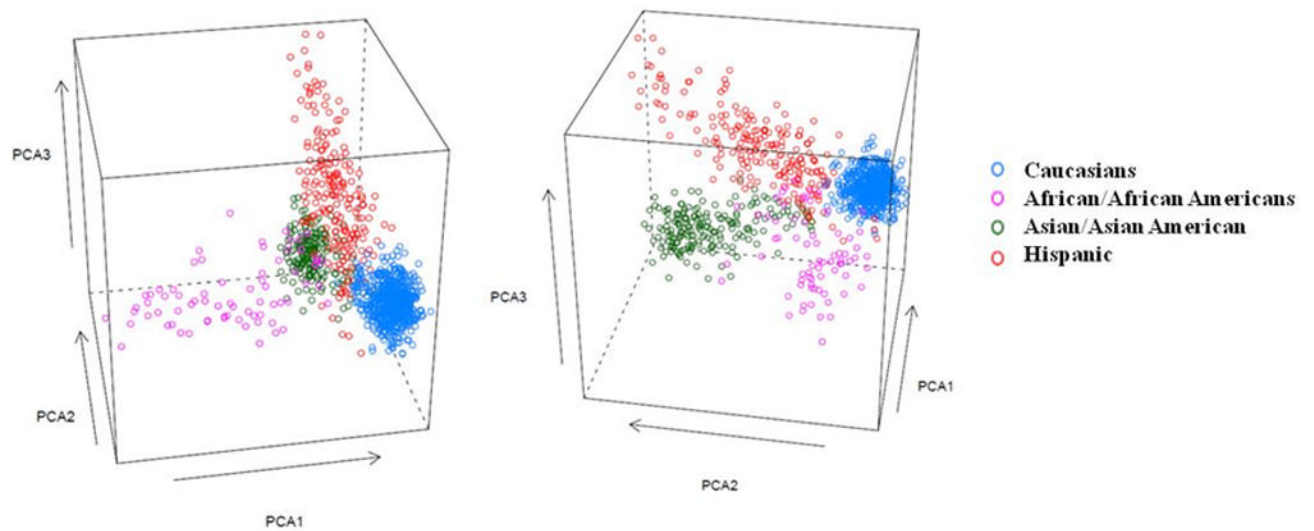


Figure 1.

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).

Table 1

Demographic characteristics of participating KD patients

	ALL KD patients*				
Age (months)	IVIG Responders	IVIG Non-responders	CAD+	CAD-	
Mean (sd)	41.8(32.4)	41.7(33.2)	33.5(37.5)	44.5(32.1)	
Median (IQR)	34 (15–58)	31(15–66)	21(8–50)	40(19–60)	
Race/ethnicity[‡]					
Caucasian	242	141	51	42	190
Asian	82	57	9	18	59
Hispanic	88	51	20	21	58
African American	31	15	5	5	24
Gender					
Male	263	151	50	59	194
Female	161	102	29	23	133

* 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 pseudosibbling based case-control analysis of polymorphisms in activating *FcγR* genes with susceptibility to Kawasaki disease in three racial/ethnic groups

Polymorphisms/Genes Ethnicity	Associated allele frequency	Informative families*	z-statistics (p-value)	OR (95% CI) [†]	P
<i>FcγRIIA-131H/R</i> (a/g)					
(A)					
All Ethnic groups	0.57	182	+3.12 (0.001)	1.51 (1.16–1.96)	0.002
Caucasians	0.54	105	+2.04 (0.04)	1.43 (1.02–2.01)	0.04
Asians	0.73	26	+2.34(0.02)	2.75 (1.22–6.18)	0.01
<i>FcγRIIA-48L/R/H</i> (t/g/a)					
(G/T)					
All Ethnic groups	G = 0.04 T = 0.07	21/35/48	-0.85 (0.40)/+1.00 (0.32)/-0.27 (0.79)	-	-
Caucasians	G = 0.06 T = 0.09	17/29/38	-0.47 (0.64)/+1.10 (0.27)/-0.60 (0.55)	-	-
Asians	G = .007 T = 0.02	-	-	-	-
<i>FcγRIIA-158V/F</i> (t/g)					
(G)					
All Ethnic groups	0.35	179	+0.46 (0.64)	1.07 (0.83–1.39)	0.59
Caucasians	0.37	111	+0.25 (0.80)	1.04 (0.75–1.46)	0.80
Asians	0.32	29	-2.00 (0.05)	0.55 (0.27–1.10)	0.09
<i>FcγRIIB-NA/NA2</i>					
(NA1)					
All Ethnic groups	0.60	157	+1.88 (0.06)	1.28 (0.97–1.69)	0.07
Caucasians	0.66	85	+1.60 (0.11)	1.32 (0.90–1.93)	0.15
Asians	0.47	30	+1.37 (0.17)	1.62 (0.81–3.23)	0.17

* TDT statistics was only performed where there were 10 or more informative families;

[†]OR (additive) based on the genotype of the KD patients and pseudosibbling controls derived from the 3 alternate genotypes based on the untransmitted alleles

Table 3

TDT and pseudosibbling based case-control analysis of activating *FcγR* gene variants among IVIG responding and IVIG non-responding Kawasaki patients in three racial/ethnic groups[†]

Genes & Polymorphisms	IVIG Responders						IVIG non-responders					
	TDT statistics			Pseudosibbling case-control [‡]			TDT statistics			Pseudosibbling case-control [‡]		
	Informative families*	z-statistics (p-value)	OR (95% CI)	P	Informative families*	z-statistics (p-value)	OR (95% CI)	P	Informative families*	z-statistics (p-value)	OR (95% CI)	P
<i>FcγRIIA-131H/R</i>												
All	115	+2.1 (0.04)	1.40 (1.01–1.95)	0.04	41	+1.81 (0.07)	1.72 (0.96–3.08)	0.07				
Caucasians	67	+0.97 (0.33)	1.24 (0.81–1.90)	0.33	21	+0.76 (0.45)	1.33 (0.63–2.82)	0.45				
Asians	17	+2.40 (0.02)	4.00 (1.34–11.96)	0.01	-	-	4.00 (0.45–35.79)	0.21				
<i>FcγRIIA-158V/F</i>												
All	108	-0.25 (0.80)	1.00 (0.72–1.39)	1	40	+1.48 (0.14)	1.5 (0.83–2.72)	0.18				
Caucasians	67	+0.43 (0.67)	1.10 (0.72–1.68)	0.67	22	+0.82 (0.41)	1.4 (0.62–3.15)	0.42				
Asians	19	-2.04 (0.04)	4.00 (1.34–11.96)	0.01	-	-	-	-				
<i>FcγRIIB-NAI</i>												
All	95	+1.51 (0.13)	1.28 (0.89–1.82)	0.18	34	+3.70 (0.0002)	3.67 (1.75–7.66)	0.0006				
Caucasians	48	+1.21 (0.23)	1.29 (0.79–2.10)	0.32	20	+2.71 (0.007)	3.6 (1.34–9.70)	0.01				
Asians	22	+0.82 (0.41)	2.14 (0.87–5.26)	0.10	-	-	-	-				

* TDT statistics was only performed where there were 10 or more informative families;

[†] OR (additive) based on the genotype of the KD patients and pseudosibbling controls derived from the 3 alternate genotypes based on the untransmitted alleles

Table 4

Results of TDT and pseudosibbling based case-control analysis of polymorphisms in activating *FcyR* genes among Kawasaki disease patients with and without coronary artery disease (CAD) in three racial/ethnic groups[†]

Genes & Polymorphisms	KD patients without CAD (n = 331)				KD patients with CAD (n = 86)			
	TDT statistics		Pseudosibbling case-control [‡]		TDT statistics		Pseudosibbling case-control [‡]	
	Informative families*	z-statistics (p-value)	OR (95% CI)	P	Informative families*	z-statistics (p-value)	OR (95% CI)	P
<i>FcyRIIA-131H/R</i>								
All	127	+1.79 (0.07)	1.33 (1.02–1.82)	0.07	43	+2.45 (0.01)	1.89 (1.10–3.33)	0.03
Caucasians	79	+2.05 (0.04)	1.52 (1.01–2.22)	0.04	20	0 (1)	1.00 (0.46–2.16)	1
Asians	17	+1.15 (0.25)	0.58 (0.23–1.48)	0.26	-	-	-	-
<i>FcyRIIA-158V/F</i>								
All	134	-0.23 (0.82)	0.96 (0.71–1.31)	0.81	40	+1.66 (0.10)	1.57 (0.88–2.80)	0.13
Caucasians	91	0 (1)	1.00 (0.69–1.55)	1	18	+0.85 (0.39)	1.44 (0.62–3.38)	0.40
Asians	21	-1.63 (0.10)	0.50 (0.21–1.17)	0.11	-	-	-	-
<i>FcyRIIB-NA1</i>								
All	113	+0.65 (0.52)	1.09 (0.80–1.49)	0.58	35	+2.41 (0.02)	2.13 (1.11–4.00)	0.02
Caucasians	63	+1.07 (0.29)	1.24 (0.81–1.85)	0.28	17	+1.34 (0.18)	1.85 (0.70–4.76)	0.19
Asians	21	+0.82 (0.41)	1.41 (0.62–3.15)	0.42	-	-	-	-

* TDT statistics was only performed where there were 10 or more informative families (several SNPs, specifically *FcyRIIA-48R/L* did not have enough informative families especially in Asians and Hispanics);

[†] OR (additive) based on the genotype of the KD patients and pseudosibbling controls derived from the 3 alternate genotypes based on the untransmitted alleles