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FcγR gene copy number in Kawasaki disease and intravenous immunoglobulin treatment response

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Abstract

Objective—Kawasaki disease (KD), response to intravenous immunoglobulin (IVIG) therapy, and associated coronary artery disease progression have been associated with genetic polymorphisms in Fc gamma receptor (*FcγR*) genes. However, it is not known whether the existing gene copy number (GCN) variability relates to KD treatment response, susceptibility, or associated sequelae.

Methods—The copy number of individuals with KD (n = 510) and their family members (n = 808) for three variable *FcγRs* was assessed using pyrosequencing. We performed the transmission disequilibrium test to examine the association of GCN for *FcγRs* (*FcγR2C*, *FcγR3A*, and *FcγR3B*) with susceptibility and used logistic regression models to determine its association with IVIG treatment outcomes.

Results—*FcγR2C* and *FcγR3B* GCN were significantly associated with KD susceptibility. IVIG response was associated with GCN variations of *FcγR3B* in Whites and *FcγR2C* in Hispanics, and gene risk score based on single nucleotide polymorphism and GCN in *FcγRs* were significantly different between IVIG responders and nonresponders among Whites. We found no significant associations between coronary artery disease and any of the *FcγR* copy numbers.

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Conflicts of interest

There are no conflicts of interest.

Conclusion—GCN of *FcγR2C* and *FcγR3B* influences IVIG treatment response and predisposes individuals to KD, providing potential insights into understanding the mechanism of the *FcγR* gene family in the IVIG pathway.

Keywords

copy number variation; *FcγR*; genetic risk; intravenous immunoglobulin; Kawasaki disease

Introduction

Kawasaki disease (KD) is the leading cause of acquired cardiovascular disease among children in developed countries, including the USA. Heart disease is the most serious sequel to KD and is characterized by ectasia and aneurysms in the coronary arteries [1]. Epidemiological studies report the cumulative incidence of coronary artery involvement as high as 25% in children without treatment, potentially resulting in ischemic heart disease, myocardial infarction, or death [2]. Treatment with intravenous immunoglobulin (IVIG) in combination with aspirin is effective in the majority of KD patients and prevents coronary artery aneurysm formation [3]. Initial treatment fails or symptoms reoccur within 10 days in up to 30% of patients [4,5]. Patients refractory to initial treatment are at a higher risk for coronary artery complications compared with those who respond. The etiology of KD is not fully known, but genetic variation within immunological pathways, including those involving the Fc gamma receptors (*FcγR*), has been proposed as a determinant of KD susceptibility and the variable response to IVIG [6–9].

Several effectors and regulatory immunological functions are initiated by the binding of IVIG to classical *FcγR* and their subsequent clustering on the surface of cells [10]. The *FcγRs* have been defined as either activating (*FcγR1*, *FcγR2A/C*, *FcγR3*) or inhibitory (*FcγR* 2B) receptors as they elicit or inhibit various immune functions including phagocytosis, cytotoxicity, degranulation, antigen presentation, and cytokine production through interactions with immune tyrosine activating or inhibitory motifs. They bind to immunoglobulin G (IgG), which is the predominant component in IVIG and form the link between the humoral and cellular parts of the immune system, as well as a crucial link between innate and adaptive immunity [11]. Altered functions due to variations in sequences [single nucleotide polymorphisms (SNPs) or gene copy numbers (GCN)] result in unbalanced immunity and subsequent inflammation, which can predispose individuals to autoimmune diseases, including KD.

Recent investigations have confirmed an increased risk of acquiring KD for individuals harboring the *FcγR2A* 131H/R (histidine encoding) polymorphism [12,13]. We have also shown that the *FcγR*2A131H (rs1801274) variant substantially increases the risk for or susceptibility to KD [odds ratio (OR): 1.4–2.75] [7]. The increased risk associated with this polymorphism appears to be consistent across ethnic groups, suggesting a direct function in KD pathogenesis. We have also reported the *FcγR3B* NA1 isotype, defined by two SNPs at positions 141 (rs403016) and 147 (rs447536) in the extracellular domain 1 (EC1), as a major risk factor for IVIG refractoriness and development of persistent coronary artery dilation. Further, we have shown that SNPs in the inhibitory *FcγR2*B at –120 T/a and – 386 G/c to be

associated with IVIG response of European descent (Whites) – these polymorphisms are known to enhance the responses to specific transcription factors [8], resulting in elevated FcγR2B expression on lymphocytes and monocytes. With mounting evidence, *FcγRs* seems to be a logical gene family involved in KD pathogenesis and IVIG resistance.

However, the known functional genetic polymorphisms in these *FcγR* genes do not account for all the variations in disease susceptibility and treatment outcomes. One complicating factor with *FcγR* genes is that some, specifically *FcγR2C*, *FcγR3A*, and *FcγR3B*, are known to have variations in GCNs that contribute to interindivi-dual differences [14]. Burns and Newburger [15] recently suggested that copy number variation for these genes might have biological implications in KD. Doses (GCNs) for these *FcγR* genes have relationships to various other autoimmune diseases [16–19]. In the current study, we extended our previous work by testing the hypotheses that doses (copy numbers) for *FcγRs* (*FcγR2C*, *FcγR3A*, and *FcγR3B*) genes determine susceptibility, IVIG treatment response, and risk for coronary artery disease (CAD) in KD patients.

Methods

Study cohort

Characterization of the participating patients and their parents in this study has been described in detail, previously [7,8]. Briefly, the study cohort was comprised of a total of 510 KD patients and 808 parents in three ethnic groups. We used the standard epidemiological criteria recommended by the American Heart Association (AHA) and the American Academy of Pediatrics (AAP) and the coronary artery involvement as defined in the AHA guidelines to define KD [20]. Standard treatment included IVIG – 2 g/kg over ~12 h as recommended in the AAP/AHA Endorsed Clinical Report [20]. Refractoriness to IVIG treatment was defined as persistent fever above 38°C extending beyond 36 h after completion of IVIG infusion or recurrent fever above 38°C after 36 h of completion of the initial IVIG infusion, also as recommended by AHA and AAP guidelines [20]. As previously described, CAD was defined by echocardiography as dilation (*Z*-score > 2.5), according to Boston *Z*-score data [21] or aneurysm defined by Japanese Ministry of Health Criteria persisting more than 6 weeks after IVIG treatment (2 g/kg).

Laboratory methods and estimation of GCN

DNA samples were extracted using the Versagene DNA purification kit (Gentra Systems Inc., Minneapolis, Minnesota, USA) from blood or saliva in Oragene collection kits (DNA Genotek Inc., Kanata, Ontario, Ottawa, Canada). We used a pyrosequencing-based methodological approach to quantify GCNs. Rather than relying on differential hybridization, this quantitative approach takes advantage of the sequence identity and allows us to simultaneously amplify two highly homo-logous genes that contain a single base difference between PCR amplicons. Relative gene quantity intensities were assessed by quantifying the single nucleotide difference using a PSQ96A (Qiagen, Limburg, the Netherlands) pyrosequencer.

Three assays were developed, comparing relative quantities of *FcγR2A* versus *FcγR2C* (2AC), *FcγR2B* versus *FcγR2C* (2BC), and *FcγR3A* versus FcγR3B (3AB). FcγR2A has been reported to be copy number invariant [14], so it was included as a negative control. The primers used for PCR amplification of gene loci for these assays were as follows: 2AC (F: 5'-AACGTTATGCCATGTGGTCA-3', R: 5'-biotin-CCCCTCTTTTTGTCATCCACTC-3'); 2BC (F: 5'-AGT GAGTCACTCCACCTCTCTGTG-3', R: 5'-biotin-TGTG TGCTGTTACTGCCTACCAG-3'); 3AB (F: 5'-TCCACC TGGGTACCAAGTCTCT-3', R: 5'-biotin-TTGAGGGTCC TTTCTCCATTTAA-3').

The reverse primers were biotinylated for PCR product purification. The sequencing primers used for these assays were as follows: 2AC (5'-CGTTATGCCATGTGGTC-3'); 2BC (5'-GGAAAATGGGGACACTA-3'); 3AB (5'-TCTC TGTGAAGACAAACATT-3').

After pyrosequencing, the relative gene intensity quantities from 2AC, 2BC, and 3AB (*FcγR2A* vs. *FcγR2C*, *FcγR2B* vs. *FcγR2C*, and *FcγR3A* vs. *FcγR3B*, respectively) for each participant sample were plotted against each other to establish clusters. These clusters were analyzed and interpreted as different forms and combinations of *FcγR* copy number variation. The majority of donors fell into a cluster centered on 2AC, 2BC, 3AB = 50, 50, 50%. These values for 2AC, 2BC, and 3AB indicate equal quantities of all genes, and therefore the cluster is made of individuals with no copy number variation. The well-characterized deletion and duplication of *FcγR2C* and *FcγR3B* were identified as the following clusters (2AC, 2BC, 3AB): 2C/3B Null (100, 100, 100%), 2C/3B one copy (66, 66, 66%), 2C/3B three copies (40, 40, 40%), 2C/3B four copies (33, 33, 33%). The rare, but previously reported GCN variation of *FcγR3A* was also observed. This GCN variation appeared to involve the 5' end of *FcγR2C* and the 3' end of FcγR2A, resulting in duplication or deletion of *FcγR2C*, but without changing the quantity of FcγR2A, as the 2C 3' end is identical to 2A at the amino acid level. The clusters representing duplication and deletion of *FcγR2C* and *FcγR3A* were identified as follows (2AC, 2BC, 3AB): 2C/3A one copy (33, 66, 33%), 2C/3A three copies (60, 40, 60%), 2C/3A four copies (66, 33, 66%). We have previously genotyped several known functional SNPs in *FcγR2A* [*131H/R*(a/g)] *FcγR2B* (–120T/a and – 386G/c), *FcγR3A* [*48L/R/H*(t/g/a) and *158V/F*(t/g)], and NA1/NA2/SH allotypes in *FcγR*3B using pyrosequencing [7,8]. The SNPs that we previously reported to be associated with KD susceptibility or response to IVIG treatment, were used to estimate the genetic risk score (GRS). Extensive quality-control procedures ensured reliable and valid GCN data. Typing samples in triplicate and along with negative controls permitted us to assess interassay reliability; only those samples whose duplicates fell in the same control were used for the analyses.

Statistical methods

Software programs to assess genetic associations typically assume that the genetic information is given as the genotypes of codominant markers. To use such software with the GCN data, it is necessary to recode the GCN counts as alleles, representing the number of copies of the GCN on each chromosome. We identified the possible state of a single chromosome as *d* for a deletion, *n* for a single copy, and *i* for a single duplication or

insertion. We assumed that both duplications and deletions are less frequent than single copies, and that multiple duplications on a single chromosome would be extremely rare. Under this assumption, the wild type of a total of two counts occurs when each chromosome has a single copy of the region (*n/n*); a total of three copies occurs when one chromosome has a single copy and the other has a single duplication (*n/i*); a total of one copy occurs when one chromosome has a single copy and the other has a deletion (*d/n*), a total of zero copies occur when both chromosomes have deletions (*d/d*), and a total of four copies occur when both chromosomes have insertions (*i/i*).

We used the coding described above for the allele counts in all KD patients and their biological parents and performed transmission disequilibrium tests (TDTs) using a family-based association test [22] to test whether any of the GCN variation counts were overtransmitted or undertransmitted from the parents to the KD patients. Briefly, the TDT is designed to detect genetic linkage only in the presence of genetic association by calculating transmission rates between unaffected parents and affected children and finding the alleles that are over-transmitted [23]. Although the TDT is relatively robust to population stratification, we also stratified the analysis by ethnic groups, as we have previously described, using well-defined ancestry informative markers (AIMs). The TDT procedures in family-based association test were limited to a minimum of 10 informative families, so not all ethnicities had large enough sample sizes. Families with Mendelian errors were excluded from the analyses, as previously described [8]. The pseudosibling controls were generated from the three untransmitted parental genotypes, and conditional logistic regression was used to estimate OR and 95% confidence intervals in the dominant model [7].

Further, using only KD patients, we conducted logistic regressions stratified across ethnicities where IVIG response and CAD progression were the dependent variables, respectively. GCN variants were coded using both dominant models for insertion and deletions in the regression models. Ethnicity was determined using principle components analysis of 155 AIMs and homogeneity of each ethnicity was checked by plotting the first three components and performing discrimination analysis, as previously described for the study participants and family [7,8].

In the previous analyses, each variable was considered one at a time; thus, we constructed a GRS on the basis of the allele count of known *FcγR* SNPs (from previous studies) and GCNs (from the current study) associated with KD susceptibility and IVIG treatment response. Previously, we have shown that *FcγR2A-131H/R*(a/g) was associated with KD susceptibility and that *FcγR*2B-120T/a and *FcγR*3B-NA1/NA2/SH allotypes were associated with IVIG treatment response. Assuming an equal and additive effect, for every statistically significant allele and GCN that was protective we assigned a + 1 value and for those that were susceptible we assigned a – 1 value. For the KD susceptibility, we summed all the protective and susceptible alleles in KD patients and their parents and compared the distributions in each ethnic group separately using the *t*-test. Likewise, a similar approach was used to compare the risk scores between the IVIG responders and nonresponders.

Results

For the three variable *FcγR* genes evaluated, GCN frequencies ranged from 5 to 14% in probands, 2–9% for deletions [one copy or less, and 8–14% for insertions (three or more copies)], as shown in Table 1. We also report *FcγR* GNC frequency for non-White populations. To our knowledge, we report the first *FcγR* GCN for non-White populations. For total copies of *FcγR2C*, *FcγR3A*, and *FcγR3B*, there were no occurrences of double deletions (zero copies), and few occurrences of double insertions (four copies) for *FcγR2C* and *FcγR3B*. GCN frequencies in parents were very similar to the probands (≤ 6%, generally ≤ 2%) within each ethnic group. The largest differences between probands and parents were observed in Hispanics; however, given that this ethnic group had the smallest sample size and distinct but considerable heterogeneous, as we reported previously [8], such differences can be expected.

GCN variations of FcγR2C

In the TDT analysis, a statistically significant association was observed between *FcγR2C* GCN variation and KD (Table 2). Using the dominant model (for deletion or insertion separately), we observed that chromosomes with extra copies of *FcγR2C* were less likely to be transmitted to KD patients from parents. Thus having more copies of *FcγR2C* seemed to reduce the odds of developing KD by approximately half. Within Whites, the results were not statistically significant, although the trend agreed with the combined analysis. We did not observe a statistically significant association between *FcγR2C* and either IVIG response or CAD across ethnicities when using a generalized linear modeling approach (Table 3).

GCN variations of FcγR3A

The lack of variability in copies of *FcγR3A* (Table 1) precluded analysis using TDT tests, regardless of model approach, so we could not determine whether *FcγR3A* GCN is associated with KD susceptibility (data not shown). Generalized linear modeling analyses with IVIG response and CAD progression suffered from the same lack of variability or indicated nonsignificant results (Table 3). Thus, we were also not able to assess the association with variability of copies of SNPs within *FcγR3A*.

GCN variations of FcγR3B

A statistically significant association was observed between *FcγR3B* and KD susceptibility in the TDT analysis (Table 2). Using the same dominant model as defined above, we observed that not having a transmission of the chromosome with extra copy of *FcγR3B* reduced the odds of developing KD by approximately two-thirds in the trios of all ethnic groups combined. Within Whites, the results were not statistically significant, although the trend agreed with the combined analysis. There seemed to be an association between deletion of *FcγR3B* and IVIG response (Table 3). In Whites, the deletion of one copy of *FcγR3B* decreased the odds of being a responder, with an OR of 0.19 (95% confidence interval: 0.05–0.83; $P = 0.02$). Numerically, of the five probands with a deletion, only one was a responder. However, having an extra copy of *FcγR3B* did not show a reverse effect with IVIG response and or any associations with CAD across ethnicities (Asians were not reported because of insufficient variability).

Linkage disequilibrium between GCN variations of *FcγR2C* and *FcγR3B*

Overall, we observed a correlation between *FcγR2C* and *FcγR3B* of 0.87 across probands and 0.84 across parents. We further determined whether those individuals outside of perfect linkage were more susceptible to CAD or response to IVIG, but our data did not show any strong patterns. The variability in frequencies of insertion/deletion combinations of *FcγR2C* and *FcγR3B* appeared random within both parents and probands and across different clinical outcomes.

Genetic risk score

For KD susceptibility, the average GRS in Whites was smaller among the KD patients than their biological parents (– 0.37±0.25 vs. – 0.32±0.27; $P = 0.03$). However, they were not significantly different in Asians or Hispanics (Table 4). For IVIG response, the average risk score among nonresponders was significantly smaller than in the responders among Whites (– 0.11±0.23 vs. – 0.18 ±0.08; $P = 0.04$), but not significantly different among Asians (– 0.26±0.16 vs. – 0.22±0.17; $P = 0.31$), and Hispanics (– 0.25±0.18 vs. – 0.24±0.19; $P = 0.46$).

Discussion

Our principal objective was to determine whether GCNs for the activating *FcγRs* influenced treatment response for KD. As a secondary objective, we sought to identify important GCN associations related to KD susceptibility for these *FcγR* genes. KD patients were less likely to have an extra copy of *FcγR2C* and *FcγR3B* genes transmitted from their parents. The pseudosibling case–control showed that the ORs for KD patients having an extra copy of these genes were 0.45 and 0.36, respectively. The regions involving these complex gene sequences are not covered in the standard genome arrays. Accordingly, we performed detailed gene specific assays for these areas. The three genes, *FcγR2A*, *2C*, and *3B* are in close alignment. *FcγR2C* is considered a pseudogene resulting from an unequal cross-over event between the 5’ part of *FcγR2B* (extracellular domain) and the 3’ part of the *FcγR2A* (cytoplasmic domain). *FcγR2A* displays no apparent variation in GCN. However, we demonstrated GCN for both *FcγR2C* and *FcγR3B*. Several previous reports suggest that these two genes display perfect linkage disequilibrium [17]. However, we identified some deviation from this relationship in our KD patients and their parents. Nevertheless, our TDT susceptibility analyses showed close to parallel relationships between these two genes and KD.

The functional impact of these GCNs still requires clarifications, particularly as the expression patterns for *FcγR2C* and *FcγR3B* vary among inflammatory cell types. *FcγR3B* has been well described and encodes two distinct receptor allotypes, known as NA1 and NA2, expressed almost exclusively on neutrophils. NA1 shows selectively increased binding affinity for IgG1 and IgG3 over NA2, suggesting that IVIG manipulates inflammation in KD through this neutrophilic receptor [7]. Tsujimoto *et al*. [24] reported that circulating neutrophils increase in number during the acute phase of KD, and IVIG treatment decreases the neutrophil numbers by accelerating their apoptosis. Although the exact function for *FcγR3B* needs exemplification, *FcγR3B* deficiency significantly diminishes the ability of

neutrophils to secrete oxidants in response to IgG-containing immune complexes. *FcγRB* copy numbers have been associated with reduced *FcγR3B* expression, neutrophil adherence to IgG-coated surfaces, and immune complex uptake [25]. These functional responses may relate to observed association for low *FcγR3B* copy number and with systemic lupus erythematosis, Sjogren's syndrome, and rheumatoid arthritis [26–28]. In concurrence, we found reduced transmission of extra *FcγR3B* copy to KD patients from their parents. This relationship occurred in the general KD population with a similar trend for our White subgroup. Thus, the data for different inflammatory diseases suggest protection by increasing *FcγR3B* dose.

FcγR2C is expressed on neutrophils like *FcγR3B*, but also on natural killer cells and monocytes, though not on T or B cells. FcγR2C is an activating IgG receptor that is implicated in antibody-mediated cellular cytotoxic killing by inflammatory cells [17]. Natural killer cell cytotoxic functionality relates directly to *FcγR2C* copy numbers, possibly explaining why *FcγR2C* copy number variation predisposes to idiopathic thrombocytopenic purpura in Whites [17]. Furthermore, decreased transmission frequency of extra *FcγR2C* copy occurs in the KD children, suggesting a dose-related response for this receptor. However, the functional relevance and cell specificity for this receptor's role in KD still require determination.

We explored whether IVIG refractoriness defined by AHA/AAP criteria or CAD progression are associated with copy number using case–control logistic regressions. Both these parameters have served as primary endpoints for past and current clinical trials in KD [29,30]. As case–control studies are highly susceptible to confounding by population heterogeneity, we stratified our cohort into AIMs-defined ethnic subgroups. In these analyses, we required that at least three individuals for each GCN allele count. This requirement led to several aborted analyses due to insufficient variability (Table 3).

We observed a significant association between *FcγR3B* and IVIG response, implying that a deletion of at least one copy reduces the probability of being a responder. Biologically, this implies that a reduction in the copies of the *FcγR3B* receptor reduces the odds of reaching therapeutic IVIG response.

Phenotyping in KD, like genotyping, remains a challenge and complicates analyses for the disease. Development of coronary artery ectasia and aneurysms is highly variable and may relate either to disease severity or differential response to IVIG. We previously demonstrated that the *FcγR3B* NA1 allotype adversely affects IVIG response and shows an increase in CAD risk [7]. Interestingly, here we show that lower *FcγR3B* copy number increases susceptibility and *FcγR3B* deletion relates to IVIG refractoriness. Thus, one may hypothesize that disease susceptibility and refractoriness represent a continuum. If IVIG refractoriness indicates severity, the data imply that *FcγR3B* dose is critical in the pathogenesis of the disease. The issue is complicated by the potent negative impact of the NA1 allotype on IVIG response that we reported earlier [7]. However, the data suggest that NA1/NA2 isoform may in fact represent a key player in KD, and/or a critical level for *FcγR3B* function is necessary to inhibit the autoimmune response conducted by neutrophils. These relationships can further be modified by genetic factors such as the *FcγR2B – 120A*

polymorphism, which alters the balance between immune activation and inhibition by these receptors in favor of positive IVIG response. Additionally, $^{Fc\gamma R2A\text{-}HR131}$, which does not exhibit copy number variation, increases KD susceptibility. Of note, *FcγR2A* and *FcγR3B* are expressed in neutrophils and these genetic associations could suggest its potential role in KD inflammatory response. Expression for these receptors on neutrophils is regulated by a cascade of signaling events initiated by protein tyrosine kinases [31]. The response to genetic variability in these *FcγRs* could be further impacted by the interaction of dietary or environmental factors with any component of the pathway. For instance, a recent hypothesis supported by epidemiological data suggests that genistein-mediated inhibition of tyrosine kinase activation affects KD susceptibility [32,33]. Dietary soy has high contents of genistein and thus large intake of soy could potentially shift the balance of the activating and inhibitory *FcγRs* [34].

In addition to the univariable analyses discussed above, we also performed the aggregate GRS using the significant genetic variants identified in this and our previous studies [7,8]. GRS is an emerging methodology used for prediction of cardiovascular diseases including arteriosclerosis and CAD [35]. For the most part, GRS has not provided substantial improvements in risk predictions [36]. This may be because of the application of GWAS data to determine GRS for relatively common diseases. Instead, we used hypothesis-derived data to develop GRS for a relatively uncommon disease in children. Almost all of our previous SNP findings, and the GCN association in this study, were based on Whites; thus, it is not surprising that the only statistically significant scores were among Whites for both susceptibility and IVIG response. Previously, we had reported that the *FcγR2B*-120T/a allele was not polymorphic in Asians and very rare in Hispanics [8], so the GRS was informed mostly based on the presence of the NA1 allotype and copy number of *FcγR3B* gene (statistically nonsignificant, see Table 4). Although, these GRS models based on a handful of variants in *FcγR* gene family cannot quite be used as a predictive model, they provide support for the *FcγR* gene family being involved in the mechanism of IVIG response and KD susceptibility, specifically among Whites. However, the GRS should be considered in the context that clinical laboratory risk scores, though successfully predictive of KD severity in Japanese populations, have failed when applied in North American cohorts of predominantly Whites [21].

The high homology across *FcγR* genes should be considered when interpreting these results. We have carefully developed pyrosequencing-based assays that are specific to each gene and the frequencies of GCNs in these genes seem to be similar to other studies that have used different methodologies [14,37]. However, whereas pyrosequencing-based assays can correctly determine the copies, the assay might represent just the segment around the assay site rather than the entire gene copy. Furthermore, we presented the total *FcγR3B* copies, even though the NA1, N2, and SH allotypes could also differ in copy numbers. We did attempt to measure the copy number of these allotypes on the basis of the relative intensities of the two SNPs that defined them. On the basis of the number of Mendelian errors observed (data not shown), several individuals appeared to have two *FcγR3B* copies on one chromosome and zero copies on the other. Accordingly, we believe that our assays were not sensitive enough to distinguish between that situation and when there is one copy on each

chromosome. Future analyses that are able to distinguish between such states are needed to determine whether associations exist between the specific allotypes and KD affection status, IVIG response, or CAD progression.

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Table 1

Frequency of copy numbers of *FcγR*2C, *FcγR*3A, and *FcγR*3B genes in Kawasaki patients and their parents in three ethnic groups

Gene copy number	All KD	All parents	White KD	White parents	Asian KD	Asian parents	Hispanic KD	Hispanic parents
Sample size	510	808	260	422	86	122	82	110
*FcγR*2C								
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.08	0.09	0.08	0.07	0.08	0.09	0.10	0.07
2	0.76	0.76	0.80	0.83	0.76	0.77	0.63	0.51
3	0.14	0.14	0.12	0.09	0.14	0.12	0.22	0.38
4	0.01	0.01	0.00	0.00	0.02	0.00	0.05	0.04
*FcγR*3A								
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.02	0.03	0.01	0.03	0.03	0.02	0.03	0.04
2	0.95	0.94	0.96	0.96	0.97	0.98	0.94	0.89
3	0.03	0.03	0.03	0.01	0.00	0.00	0.03	0.07
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
*FcγR*3B								
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	0.06	0.06	0.06	0.04	0.05	0.07	0.05	0.02
2	0.83	0.84	0.86	0.88	0.85	0.79	0.71	0.69
3	0.10	0.09	0.08	0.08	0.08	0.14	0.19	0.24
4	0.01	0.01	0.00	0.00	0.02	0.00	0.05	0.04

KD, Kawasaki disease.

Table 2

TDT-based association of gene copy number variation of total *FcγR* genes in trios of Kawasaki disease using a dominant model[a], where one chromosome has at least two copies

CNV	Susceptibility			
	Informative families	**Z-score**	**OR[b] and 95% CI**	***P*-value**
All individuals[c]				
FcγR2C	36	–2.18	0.45 (0.22–0.93)	**0.03**
FcγR3B	29	–2.79	0.36 (0.16–0.82)	**0.005**
Whites				
FcγR2C	22	–1.94	0.42 (0.17–1.04)	0.05
FcγR3B	16	–2.00	0.33 (0.11–1.03)	0.05

CI, confidence interval; CNV, copy number variation; OR, odds ratio; TDT, transmission disequilibrium test.

P-values in bold were below the significance threshold of 0.05.

[a] Dominant model where one chromosome has at least two copies.

[b] OR based on pseudosiblings.

[c] There were less than 10 informative families in Hispanic Asian populations, so data not analyzed.

Table 3

Case-control analysis of *FcγR2C*, *FcγR3A* and *FcγR3B* gene copy number variation with IVIG response and coronary artery disease in three ethnic groups

	IVIG response		CAD	
	Insertion (≥ 3)	**Deletion (≤ 1)**	**Insertion (≥ 3)**	**Deletion (≤ 1)**
Whites	125 responders vs. 53 nonresponders		39 CAD vs. 169 non-CAD	
***FcγR2C*[a]**	0.17	0.08	0.86	0.45
***FcγR3A*[a]**	0.61	IV	0.36	IV
***FcγR3B*[a]**	0.24	**0.02**	IV	0.66
Asians	45 responders vs. 6 nonresponders		12 CAD vs. 51 non-CAD	
***FcγR2C*[a]**	IV	IV	0.61	0.55
***FcγR3A*[a]**	IV	IV	IV	IV
***FcγR3B*[a]**	IV	IV	IV	IV
Hispanics	39 responders vs. 15 nonresponders		17 CAD vs. 40 non-CAD	
***FcγR2C*[a]**	0.37	**0.04**	0.32	0.47
***FcγR3A*[a]**	IV	0.51	IV	0.53
***FcγR3B*[a]**	0.47	IV	0.15	IV

CAD, coronary artery disease; IV, insufficient variability; IVIG, intravenous immunoglobulin.

P-values in bold were below the significance threshold of 0.05.

[a]Dominant model where one chromosome has at least two copies.

Table 4

Risk score of KD susceptibility and IVIG response based on *FcγR* gene SNPs and CNVs variants

		Risk score		
KD susceptibility	**Risk**	**KD patients**	**Parents**	***P*-value**
FcγR2A -131H/R (A allele)	+1			
FcγR3B gene copy number (1 copy)	–1			
Whites		–0.37±0.25	–0.32±0.27	0.03
Asians		–0.39±0.25	–0.38±0.22	0.34
Hispanics		–0.26±0.31	–0.25±0.32	0.41
IVIG response		Responders	Nonresponders	*P*-value
FcγR2B-120T/a *(A allele)*	+1			
FcγR3B neutrophil antigen 1 (presence)	–1			
FcγR3B gene copy number (1 copy)	–1			
Whites (125 R vs. 53 NR)		–0.11±0.23	–0.18±0.23	0.04
Asians (45 R vs. 6 NR)		–0.26±0.16	–0.22±0.17	0.31
Hispanics (39 R vs. 15 NR)		–0.25±0.18	–0.24±0.19	0.46

Parentheses denote the state that is associated with the risk score in the ‘Risk’ column.

CNV, copy number variation; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; NR, nonresponders; R, responders; SNP, single nucleotide polymorphism.

For KD: + 1 =protective, –1 =susceptible.

For IVIG: + 1 =responder, –1 =nonresponder.