HHS Public Access

Author manuscript

Genes Immun. Author manuscript; available in PMC 2015 June 01.

Published in final edited form as:

Genes Immun. 2014 December ; 15(8): 534–542. doi:10.1038/gene.2014.47.

High-density Genotyping of Immune Loci in Kawasaki Disease and IVIG Treatment Response in European-American Case-parent Trio Study

Aditi Shendre[1], **Howard W. Wiener**[1], **Degui Zhi**[2], **Ana I Vazquez**[2], **Michael A. Portman**[2], and **Sadeep Shrestha**[1,*]

[1]Department of Epidemiology, University of Alabama at Birmingham, Birmingham, AL

[2]Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL

[3]Department of Pediatrics, University of Washington, Seattle Children's Research Institute, Seattle, WA

Abstract

Kawasaki disease (KD) is a diffuse and acute small-vessel vasculitis observed in children and has genetic and autoimmune components. We genotyped 112 case-parent trios of European decent (confirmed by AIMS) using the ImmunoChip array and performed association analyses with susceptibility to KD and IVIG non-response. KD susceptibility was assessed using the transmission disequilibrium test whereas IVIG non-response was evaluated using multivariable logistic regression analysis. We replicated SNPs in three gene regions (*FCGR*, *CD40*/*CDH22*, and *HLA-DQB2*/*HLA-DOB*) that have been previously associated with KD and provide support to other findings of several novel SNPs in genes with potential pathway in KD pathogenesis. SNP rs838143 in the 3′ UTR of *FUT1* gene (2.7×10^{-5}) and rs9847915 in the intergenic region of *LOC730109* | *BRD7P2* (6.81×10^{-7}) were the top hits for KD susceptibility in additive and dominant models, respectively. The top hits for IVIG responsiveness were rs1200332 in the intergenic region of *BAZ1A* | *C14orf19* (1.4×10^{-4}) and rs4889606 in the intron of the *STX1B* gene (6.95×10^{-5}) in additive and dominant models, respectively. Our study suggests that genes and biological pathways involved in autoimmune diseases play an important role in the pathogenesis of KD and IVIG response mechanism.

Keywords

Mucocutaneous lymph node syndrome; Kawasaki disease; intravenous immunoglobulins therapy; Immunogenetics; Immune-related genes

*Correspondence: Sadeep Shrestha, PhD, MHS, MS, Department of Epidemiology, University of Alabama at Birmingham, 1665 University Blvd, RPHB Room 217L, Birmingham, Alabama 35294-0022, Phone: (205) 934-6459, Fax: (205) 934-8665, sshrestha@uab.edu.

Introduction

Kawasaki disease (KD) is the leading cause of acquired heart disease in children in the United States (U.S.). It is a potentially life-threatening acute vasculitis with diffuse involvement of multiple organ systems[1] and has a predilection for the coronary arteries. In the U.S. about 5500 cases were estimated in 2009 by passive surveillance alone;[2] and based on the system dynamics modeling simulations, there will be an average of 6200 new patients per year and a total of 161 776 individuals (half of them adults) with a history of KD by 2030.[3] The disease is primarily associated with the East Asian populations although there has been an increase in the incidence of KD among European-Americans over the last two decades.[4] The primary treatment of KD is the administration of intravenous immunoglobulins (IVIG) accompanied by aspirin.[5] Approximately 15 to 25% of the cases require more than a single dose of IVIG therapy to achieve complete resolution.[5] Furthermore, coronary artery aneurysms develop in 15-25% untreated cases compared to <5% among those treated, although a substantially higher percentage show coronary artery dilation.[5]

KD, also known as mucocutaneous lymph node syndrome (MCLS), is considered an autoimmune disease since walls of the blood vessels are inflamed throughout the body with damage to coronary arteries likely mediated by auto-antibodies to endothelial cells (ECs).[6] Parental KD is also considered a risk factor with the odds of KD among siblings and recurrent cases being 7 and 3 times higher among KD children and parents with history of KD, respectively compared to the general population,[7] suggesting a genetic etiology. However, genetic studies, including genome-wide association studies (GWAS) are often inconsistent and the associations are often race or ethnicity specific. Furthermore, only few candidate gene studies, including ours have assessed the gene-IVIG nonresponse association.[8-12] Our objective was to fine-map regions of immune-related genes using the Human ImmunoChip to validate the association of immune related genes with susceptibility to Kawasaki disease, search for novel SNPs, examine shared loci with other autoimmune diseases and perform genetic evaluation for IVIG treatment response among European American case-parent trios.

Results

The average age of the probands was 45.7 (± 32.3) months, with the average age for the responders being 44.8 (± 30.8) months and the non-responders being 46.6 (± 33.9) months. Overall, the study population consisted of 56.1% male probands, 48.1% males being responders and 64.2% being non-responders.

Susceptibility to Kawasaki disease

The Manhattan plots corresponding to the p-values for the SNPs in the transmission disequilibrium test (TDT) test are presented in Figure 1a for the additive model and Figure 1b for the dominant model. The Q-Q plots are illustrated in supplementary Figure S1a and S1b. The top 15 results from the FBAT analysis for the association between the SNPs and susceptibility to KD are presented in Table 1; upper panel for the additive model and lower panel for the dominant model (all results in supplementary Table 1 and Supplementary

Figure S3 shows the LD plot for regions with multiple hits in chromosomes 1, 5 and 19). In the additive model, the top SNPs were predominantly located in chromosome 19, largely located in regions of galactoside 2-alpha-L-fucosyltransferase 1 (*FUT1*) gene and Ras interacting protein 1 (*RASIP1*) gene (Table1), but not in the region of inositol-trisphosphate 3-kinase C (*ITPKC*), another gene located on chromosome 19 that has been previously linked to KD. The topmost SNP (rs838143) and two other more common SNPs (rs838142 and rs4021), all in the untranslated region (UTR) of the *FUT1* gene, were significantly over-transmitted from the parents to KD children ($p=2.70\times10^{-5}$, 4.80×10^{-5}, 1.18×10^{-4}, respectively).

In the dominant model, rs9847915 in the intergenic region between *LOC730109* and Bromodomain containing 7 Pseudogene 2 (*BRD7P2*) on chromosome 3 was the topmost SNP significantly associated ($p=6.81\times10^{-7}$) with susceptibility to KD (Table 1, lower panel). However, association of SNPs was largely observed in genes on chromosome 1, in the region of *FCGR* gene family cluster.

IVIG response

The Manhattan plots that summarize the associations between each SNP and response to IVIG treatments are presented in Figure 2a for the additive model and Figure 2b for the dominant model (Q-Q plots in supplementary Figure S2a and S2b). The top 15 associated SNPs are presented in Table 2, upper panel for additive model and lower panel for dominant model (all results in supplementary Table 2 and Supplementary Figure S3 shows the LD plot for regions with multiple hits in chromosomes 6 and 16). In the additive model, intergenic variant rs1200332 between Bromodomain adjacent to zinc finger domain, 1A (*BAZ1A*) and *C14orf19* genes on chromosome 14 was the topmost significantly associated SNP with response to IVIG treatment. The odds of not responding to IVIG treatment was 5 times higher in those with the minor allele T (OR-nonresponders: 5.49, $p=1.40\times10^{-4}$) compared to the wild-type C allele. On the other hand, individuals with a minor allele at SNPs rs6017164, rs72945401, rs7579420, rs7163190, rs3134926, rs3777914, rs1883137, and rs11641163 had higher odds of responding to IVIG and most of these responders had minor allele frequency (MAF) closer to the reported frequency for HapMap Caucasians (Table 2). Several SNPs on chromosome 6 were also found to be significantly associated with IVIG responsiveness in or around G-protein coupled receptor-6 (*GPR6*)/Wiskott-Aldrich syndrome protein (WASP)-family, member 1 (*WASF1*), protein reversionless 3-like (*REV3L*), neurogenic locus notch homolog protein 4 (*NOTCH4*)/ *C6orf10*, and tumor necrosis factor receptor family 3 interacting protein 2 (*TRAF3IP2*) genes.

The top SNPs associated with response to IVIG treatment using the dominant model were predominantly located on chromosome 16 and several of them were in linkage disequilibrium (Table 2, lower panel). The top most significant SNP was rs4889606, located in the intronic region of the syntaxin 1B (*STX1B*) gene on chromosome 16 and in linkage disequilibrium with rs9926533. This SNP was associated with a lower odds of IVIG non-response among those carrying the minor allele G (OR-nonresponders: 0.13, $p=6.95\times10^{-5}$) compared to the wild-type “A” allele. Other SNPs located on chromosome 16 were found either in the intronic or intergenic regions of SET domain-containing protein 1A (*SETD1A*),

F-box and Leucine-rich repeat protein 19 (*FBXL19*), cardiotrophin-1 (*CTF1*)/*LOC283932*, and syntaxin 1B and 4 (*STX1B*/*STX4*) or *STX4*/ zinc finger protein 668 (*ZNF668*) genes and all were associated with higher odds of response to IVIG treatment.

Discussions

In the present study, we evaluated the genetic association of susceptibility to KD and IVIG treatment response using the ImmunoChip in homogenous European-American case-parent trios. While the sample-size was relatively small, several SNPs from previous studies were replicated in our family-based study, strengthening our results. Furthermore, these replicated SNPs along with few of the top significant SNPs in our current study were found to be expression quantitative trait loci (eQTLs) for specific genes and are therefore more likely to play a functional role in the regulation of gene and expression and subsequent immune response to KD. Consequently, we discuss these SNPs in addition to some of the SNPs that were highly significantly in our study and have shown previous associations with autoimmune diseases.

Two SNPs, rs2857151 in the intergenic region of the human leucocyte antigen class II histocompatibility antigen, DQ beta 2 chain and DO beta (*HLA-DQB2* and *HLA-DOB*), and rs4813003 downstream of TNF receptor superfamily member 5 (TNFRSF5) or B-cell associated molecule CD40 (*CD40*) gene that were previously reported in GWAS to be associated with KD were replicated (Table 3).[13] SNP rs2857151 was shown to be an eQTL for several of the HLA genes, namely, *HLA-DOB*, *-DRB1*, *-DRB5*, *-DQA1*, *-DQA2*, and transporter 2, ATP-binding cassette, sub-family B (*TAP2*).[14] SNP rs4813003 was shown to be an eQTL for the CD40 gene.[14, 15] SNP rs2857151 in combination with rs4813003 and another locus in a 3-locus model were previously shown to confer the highest risk for KD whereas the same SNP with rs1801274 was shown to confer the highest risk for CALs.[13] The compound effect was hypothesized to provide evidence for the additive effect of these loci on KD. SNPs within the *TAP2* gene have also been associated with autoimmune diseases like rheumatoid arthritis (RA), ankylosing spondylitis and multiple sclerosis and the TAP2 proteins are known to play an important role in antigen presentation.[16-19] SNP rs4813003 is located in the intergenic region between the *CD40* and the cadherin 22 (*CDH22*) genes. CD40 is a receptor protein which is part of the TNFR superfamily and is expressed on B cells, monocytes, dendritic cells, ECs and epithelial cells.[20] It interacts with the CD40 ligand on the T-helper cells and mediates immune and inflammatory responses. CD40 was also recently observed to facilitate damage to ECs through an inflammatory response mounted via activated platelets and the expression of adhesion molecules.[20] On the other hand, CDH22 is a type of cadherin, part of a superfamily of transmembrane proteins, and is known to mediate cell-to-cell adhesion in a calcium dependent manner. Altered expression of CDH22 has been previously associated with cell proliferation, invasiveness and metastasis in certain cancers.[21]

Previously, we had independently shown that rs1801274 in *FCGR2A* gene is associated with KD susceptibility in European-Americans,[9, 11] confirming similar findings from a GWAS study.[22] Multiple variants in the *FCGR* gene family cluster within the ImmunoChip were also associated with KD susceptibility in our TDT analysis with a dominant inheritance,

with SNPs in both the intronic and intergenic regions associated with KD susceptibility. This gene region is consistent with our previous studies of KD.[9-11] These findings are encouraging, specifically since results from several GWAS studies have been replicated in only few studies using independent cohorts.[23, 24] Polymorphisms and copy number variation of *FCGR* genes are also associated with susceptibility for autoimmune diseases such as RA and systemic lupus erythematosus (SLE). Accordingly, an intersection could potentially exist between KD and other autoimmune disorders.

While the function of several genes in Table 1 is unknown or unconfirmed, few (*FUT1*, *RASIP1*, and *BRD7P2*) are noteworthy and could have potential biological mechanism in KD pathway. The SNPs significantly associated with KD in the additive model were located in the 3′UTR region of the *FUT1* gene, the intronic region of the *RASIP1* gene and the intergenic region of the *BRD7P2* gene. *FUT1* encodes the galactoside 2-alpha-L-fucosyltransferase 1 enzyme that is involved in the formation of the H antigen on erythroid cells, regulating the expression of ABO antigens.[25, 26] FUT1 plays a major role in the pathogenesis of RA and is involved in angiogenesis, cell adhesion and cell proliferation of various inflammatory cells.[27] This role is further supported by experiments showing inactivation of *FUT1* using short-interfering RNA where reduction in cell proliferation and tumor growth were observed as well as migration of ECs and integration with the extracellular matrix (ECM).[28-30] Macrophages are also reported to modulate the expression of *FUT1* and *FUT2* through the interleukin-6 (IL-6) signaling pathway in epithelial cells of the endometrium to enable implantation of the embryo and increased levels of macrophages are potentially associated with inflammatory response.[31] The three SNPs from our study are located in the 3′ UTR of *FUT1*, and interestingly MAF for all three was higher in HapMap Asians compared to Caucasians but the same allele was the wild-type allele in Yoruban Africans. While the functions of these SNPs are not known, future studies could evaluate the potential binding sites of miRNAs in the 3′ UTR in relation to these variants. The *RASIP1* gene encodes the RASIP1 protein that has been associated with tubulogenesis and angiogenesis in as early as the embryonic stage and is specific to the endothelium.[32] Lumen formation via RASIP1 occurs through the regulation of the Rho family of monomeric G proteins, requiring the suppression of RhoA and activation of Cdc42 and Rac1 proteins.[33] RASIP1 deficient vessels are shown to collapse as EC adhesion to the ECM is lacking.[33] RASIP1 variants have been associated with early microvascular disease, which is a predictor of incident cardiovascular disease, and Crohn's disease in conjunction with FUT2.[34, 35] Thus it may play a more important role in the pathogenesis of both KD and CALs. SNP rs9847915 in the *BRD7P2* gene was associated with susceptibility to KD in our study; the MAF for this SNP is lowest among Africans but similar for Asians and Caucasians from HAPMAP. This gene has been associated with phenotypes such as Behcet's syndrome which also involves systemic vasculitis of small vessels with an auto-immune component.[36]

To date, IVIG responsiveness has not yet been evaluated using large-scale genotyping arrays. Even with a smaller sample-size, several genes (*BAZ1A, GPR6, TRAF3IP2, REV3L, STX1B, STX4,* and *CTF1*), listed in Table 2, are noteworthy as working mechanisms of IVIG therapy have yet to be fully elucidated. Additionally, SNP rs1200332 was shown to be an

eQTL for chromosome 14 open reading frames 10 and 24 (*C14orf10* and *C14orf24*) and SNP rs10871454 as an eQTL for *ZNF668*, *STX4* and *SETD1A* among others.[14]

In the additive model, SNPs located in the intergenic region of the *BAZ1A* gene, the 3′UTR and intergenic regions of the *GPR6* gene and the intronic regions of the *TRAF3IP2* gene were significantly associated with IVIG responsiveness. The SNP rs1200332 in the intergenic region of *BAZ1A* and *C14orf19* genes has been associated with Crohn's disease, an autoimmune disease, in Puerto Ricans.[37] The MAF of rs1200332 is higher among Africans but lower and similar across Caucasians and Asians in HAPMAP. The *BAZ1A* gene encodes a subunit of the adenosine triphosphate (ATP)-dependent chromatin assembly factor (ACF) which is part of the chromatic remodeling complex and is involved in DNA damage response and repair.[38] The *GPR6* gene encodes the GPR6 protein that is expressed on vascular smooth muscles cells and ECs and is an orphan GPCR modulated by sphingosine-1 phosphate (S1P), an important regulator in the vascular system.[39] The *TRAF3IP2* gene codes for the TRAF3-interacting protein 2, also known as activator 1 (Act1), that acts as a signaling adaptor in IL-17 mediated cellular immune responses through nuclear factor κ-B (NF- κB) activation or as a negative regulator by inhibiting CD40 and B-cell activating factor receptor (BAFFR) mediated signaling.[40] *TRAF3IP2* has also been associated with auto-immune disorders like psoriasis and SLE and is speculated to bring about loss of the negative regulatory role of Act1 in SLE resulting in B-cell hyperplasia and enhanced survival of autoreactive B-cells.[40]

In the dominant model, most SNPs associated were in chromosome 16 in the intronic and intergenic regions of the *STX* gene family; the top SNP rs4889606 in the *STX1B* gene having the lowest MAF among Africans but the same minor allele being the wild-type allele among Asians from HAPMAP. In general, the interaction of syntaxin with synaptotagmin results in fusion of the vesicular and plasma membranes and subsequent exocytosis.[41] Smirnova et. al. speculated that syntaxin may be involved in the Lambert-Eaton syndrome, an autoimmune disorder associated with some small cell lung cancers resulting in antibodies against synaptotagmin and calcium channels.[42] Several SNPs within the intronic and intergenic region of the *STX4* gene were associated with increased IVIG response. The *STX4* gene encodes for STX4 which is also part of the syntaxin family of membrane integrated proteins.[43] STX4 plays an important role in the regulation of secretions from various immune cells including neutrophils, macrophages and eosinophils.[44] The addition of short interfering RNAs against STX4 has been shown to reduce IgE antibody exocytosis from plasma cells from several cell lines.[43] It was additionally shown that STX4 knock-down results in reduced secretion of tumor necrosis factor-α from macrophages.[44] SNPs in the intergenic region of the *CTF1* gene were also significantly associated with IVIG responsiveness. The *CTF1* gene encodes for the CTF1 protein which is part of the IL-6 family and is involved in cell proliferation, cell migration and collagen production. CTF1 is largely localized to the ECs and vascular smooth muscle cells with genetic variations in the gene associated with dilated cardiomyopathy or hypertension related left ventricular hypertrophy.[45-47] CTF1 has been shown to have both beneficial as well as detrimental effects based on which signaling pathway is induced since it has been associated with wound healing in myocardial infarction and prevention of apoptosis due to hypoxia as well

as plaque instability and pathophysiology of atherosclerosis.[48, 49] The role of all these genes in IVIG response could potentially be mediated through various immunity signaling pathways.

There are several strengths to our study. Sample-size was relatively small in our study and thus the findings in our study are preliminary; however, considering that KD is rare (13.7 per 100,000 among whites < 5 years)[4] and only 15 to 25% do not respond to IVIG treatment, the difficulty in assembly of trios for this study should be appreciable. The trio-based family analysis using TDT is robust to factors like confounding and variance inflation which enabled us to confirm and replicate previous findings related to KD susceptibility despite the small sample size of our study. Additionally, we determined ethnicity based on ancestry informative markers (AIMs) and performed the analysis in confirmed European-Americans, accounting for population stratification and thus ensuring a homogenous population.

Our study is not without limitations. Of note, we included equal number of IVIG responders and non-responders for the TDT analysis; thus, some of the findings could be biased towards less frequent IVIG responders in the population, as they were preferentially included in the analyses. The ImmunoChip largely includes genes that were associated with several autoimmune disorders and was therefore ideal for testing genetic associations with KD but does not represent the whole spectrum of SNPs and genes that may be involved in immune response. As a result, the density of markers found on the ImmunoChip is not as high as opposed to fine mapping of gene regions and thus the SNPs associated in our study may be tagging one or more SNPs in LD and/or possibly the true causal variants. Lastly, we largely describe SNPs in the UTR and intronic regions although we acknowledge that variants from other parts of the region can be functional and related to distant genes.[50] And even though we include discussion of certain SNPs as eQTLs for some genes, we recognize that we were limited by the annotations provided by the ImmunoChip consortium and express caution in interpreting these results since certain eQTLs are reported in relation to specific cells and therefore may not be generalizable or applicable in the context of KD.

In the current study, we took a hypothesis-free agnostic approach to show that several SNPs in immune related genes included in the ImmunoChip are associated with susceptibility to KD and IVIG response. Some of these genetic associations have not yet been reported and can be potentially involved in KD pathogenesis while some of these genes are known to be in the pathway of other known autoimmune diseases. Future studies are therefore needed to replicate these findings and further fine-mapping is required to identify biologically causal SNPs as well as their functions in relation to either KD susceptibility or IVIG response mechanisms.

Methods

Study population

The current study included 112 case-parent trios of European-American descent from a retrospective cohort of KD. The study cohort has been described in detail previously.[11] Briefly, the probands (55 IVIG responders and 55 non-responders) were identified through

clinical or hospital databases and then cross-referenced with the echocardiography databases of the respective heart centers. The probands were enrolled from children's hospitals at three locations: Seattle, WA; Oakland, CA; and Salt Lake City, UT. The study was approved by the Institutional Review Board at each of the participating institution and informed consent was obtained from the parents.

Clinical Outcomes

Kawasaki disease—Kawasaki disease was defined based on the guidelines recommended by the American Heart Association (AHA) and the American Academy of Pediatrics (AAP).[10, 51] The standard epidemiological criteria require the presence of fever for ≥ 5 days and at least 4 of 5 clinical features to make the diagnosis of KD. Participants in our study were included based on the initial criteria but we also included those with at least 2 clinical features accompanied by coronary artery involvement.

Response to IVIG—The treatment of Kawasaki disease included administration of an intravenous infusion of Immunoglobulin at 2 g/kg along with Aspirin within 10 days of the onset of fever. Nonresponse, or refractoriness to the initial IVIG therapy was characterized by the presence of persistent fever (≥ 38 °C) at ≥ 36 hours or recurrence of fever (≥ 38 °C) at ≥ 36 hours.[51]

DNA extraction, genotyping and quality control

As previously described, genomic DNA was extracted from blood or saliva samples obtained from the probands and their biological parents using the Versagene DNA purification kit (Gentra Systems, Minneapolis MN).[11] The PicoGreen assay for double stranded DNA was used for quantification and the final concentration was adjusted to 100ng/μl in Tris-EDTA.

Genotyping was performed using the ImmunoChip, an iSelect HD custom genotyping array (Illumina, San Diego CA), developed by the ImmunoChip Consortium.[52] The ImmunoChip contains 195,806 SNPs and 718 small insertions-deletions, of which 5,001 are non-synonymous coding, 1,926 are synonymous coding, 4,065 located in the untranslated region (UTR), and the rest fall either in the intronic, intergenic or undefined regions. The loci were selected on the basis of their associations with autoimmune or inflammatory diseases and ImmunoChip was designed using early 1000 Genome Pilot data (February 2010 release).[52, 53]

For genotype calling purposes, the data was analyzed using the Genome Studio Genotyping Module (Illumina, San Diego CA) using the NCBI build 36 (hg18) map. The normalized probe intensities were extracted for all samples that passed standard laboratory quality-control (QC) thresholds. Further QC included examining the SNPs for missingness by SNP and by individual, deviations from Hardy-Weinberg Equilibrium (HWE) at p-value <0.001 and minor allele frequencies < 0.05. Of a total 195,806 SNPs, there were 191,475 in the autosomal chromosomes. A total of 36 individuals were excluded as a result of missing > 10% genotypes data, of which 3 were probands and the rest were parents. Similarly, 16,220 SNPs were excluded since they were missing genotype data in > 10% of the sample

population. Additionally, deviation from HWE was observed in 477 SNPs and 68,761 SNPs had minor allele frequency < 0.05 and therefore excluded from the analysis.

Statistical methods

TDT was employed to assess susceptibility to KD using family-based association test (FBAT), as described previously.[11] Briefly, parent-child trios were evaluated for differential transmission of alleles from heterozygous parents to the affected child. Single marker analysis was conducted to test the association under the null hypothesis of no linkage and no association using both additive and dominant genetic models. The direction and frequency of transmission is provided through the Z statistic and p-values.

The genetic association of response to IVIG treatment was assessed by implementing a case-control approach (responders versus non-responders) and performing logistic regression analyses adjusting for age and first two principal components, using both additive and dominant models in PLINK (v1.07).[54] European-American ancestry of all parents and probands was confirmed using 155 AIMs in EIGENSTRAT.[10, 11, 55] Quantile-quantile (Q-Q) and Manhattan plots, based on p-values, were created using SAS 9.3 (SAS Institute Inc., Cary, NC) to evaluate deviations of the observed test statistics distribution from the expected one and to visualize the association results, respectively.

Supplementary Figures

Figure S1 Quantile-quantile (QQ) plot of the association between SNPs and susceptibility to Kawasaki disease using a) additive model and b) dominant model

Figure S2 Quantile-quantile (QQ) plot of the association between SNPs and IVIG response to Kawasaki disease using a) additive model and b) dominant model

Figure S3 Locus-specific ($-\log_{10}$(p-value)) regional association plots for KD susceptibility (additive models in chromosomes 5 and 19 and dominant models in chromosome 1) and response to IVIG treatment (additive model in chrosmosome 6 and dominant model in chromosome 16) usin NCBI build 37, hg19. The diamond in purple indicates the most strongly associated signal. Estimated recombination rates are plotted in light blue graphical lines (labeled in right y-axis) to reflect the local LD structure from the HapMap population. The spectrum of colors indicates LD of each SNP with the most strongly associated signal (r^2 values from HapMap where bright red indicates highly correlated, dark blue indicates weakly correlated and grey indicates missing r^2 values).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The parent study and this genetic sub-study conformed to the procedures for informed consent (parental permission) approved by institutional review boards at all sponsoring organizations and to human-experimentation guidelines set forth by the United States Department of Health and Human Services. We thank the participating patients and their parents. We also thank investigators (Dr.Neil E Bowles at Department of Pediatrics, University of

Utah, Salt Lake City, UT; Dr. Hitendra Patel at Children's Hospital of Oakland, Oakland, CA), pediatricians and staffs of the participating clinics. This study was supported by grants NHLBI-R21-HL90558 & The American Heart Association.

References

1. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. Arerugi. 1967; 16(3):178–222. [PubMed: 6062087]
2. DeLisi LE, Mesen A, Rodriguez C, Bertheau A, LaPrade B, Llach M, et al. Genome-wide scan for linkage to schizophrenia in a Spanish-origin cohort from Costa Rica. American journal of medical genetics. 2002; 114(5):497–508. [PubMed: 12116183]
3. Huang SK, Lin MT, Chen HC, Huang SC, Wu MH. Epidemiology of Kawasaki disease: prevalence from national database and future trends projection by system dynamics modeling. J Pediatr. 2013; 163(1):126–31 e1. [PubMed: 23312687]
4. Holman RC, Christensen KY, Belay ED, Steiner CA, Effler PV, Miyamura J, et al. Racial/ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. Hawaii medical journal. 2010; 69(8):194–7. [PubMed: 20845285]
5. Bayers S, Shulman ST, Paller AS. Kawasaki disease: part II. Complications and treatment. Journal of the American Academy of Dermatology. 2013; 69(4):513 e1–8. quiz 521-2. [PubMed: 24034380]
6. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD. Kawasaki disease: infection, immunity and genetics. Pediatr Infect Dis J. 2005; 24(11):998–1004. [PubMed: 16282937]
7. Uehara R, Yashiro M, Nakamura Y, Yanagawa H. Clinical features of patients with Kawasaki disease whose parents had the same disease. Archives of pediatrics & adolescent medicine. 2004; 158(12):1166–9. [PubMed: 15583102]
8. Weng KP, Hsieh KS, Ho TY, Huang SH, Lai CR, Chiu YT, et al. IL-1B polymorphism in association with initial intravenous immunoglobulin treatment failure in Taiwanese children with Kawasaki disease. Circulation journal : official journal of the Japanese Circulation Society. 2010; 74(3):544–51. [PubMed: 20081319]
9. Makowsky R, Wiener HW, Ptacek TS, Silva M, Shendre A, Edberg JC, et al. FcγR gene copy number in Kawasaki disease and intravenous immunoglobulin treatment response. Pharmacogenetics and genomics. 2013; 23(9):455–462. [PubMed: 23778324]
10. Shrestha S, Wiener HW, Olson AK, Edberg JC, Bowles NE, Patel H, et al. Functional FCGR2B gene variants influence intravenous immunoglobulin response in patients with Kawasaki disease. The Journal of allergy and clinical immunology. 2011; 128(3):677–80. [PubMed: 21601260]
11. Shrestha S, Wiener HW, Shendre A, Kaslow RA, Wu J, Olson AK, et al. Role of activating FcγR gene polymorphisms in Kawasaki disease susceptibility and intravenous immunoglobulin response. Circulation: Cardiovascular Genetics. 2012; 5(3):309–16. [PubMed: 22565545]
12. Portman MA, Wiener HW, Silva M, Shendre A, Shrestha S. DC-SIGN gene promoter variants and IVIG treatment response in Kawasaki disease. Pediatric rheumatology online journal. 2013; 11(1): 32. [PubMed: 24006904]
13. Yan Y, Ma Y, Liu Y, Hu H, Shen Y, Zhang S, et al. Combined analysis of genome-wide-linked susceptibility loci to Kawasaki disease in Han Chinese. Human genetics. 2013; 132(6):669–80. [PubMed: 23456091]
14. Fehrmann RS, Jansen RC, Veldink JH, Westra HJ, Arends D, Bonder MJ, et al. Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. PLoS Genet. 2011; 7(8) e1002197.
15. Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A, et al. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nature genetics. 2012; 44(5):502–10. [PubMed: 22446964]
16. Kramer U, Illig T, Grune T, Krutmann J, Esser C. Strong associations of psoriasis with antigen processing LMP and transport genes TAP differ by gender and phenotype. Genes and immunity. 2007; 8(6):513–7. [PubMed: 17581627]

17. Moins-Teisserenc H, Semana G, Alizadeh M, Loiseau P, Bobrynina V, Deschamps I, et al. TAP2 gene polymorphism contributes to genetic susceptibility to multiple sclerosis. Human immunology. 1995; 42(3):195–202. [PubMed: 7759306]
18. Singal DP, Ye M, Qiu X, D'Souza M. Polymorphisms in the TAP2 gene and their association with rheumatoid arthritis. Clinical and experimental rheumatology. 1994; 12(1):29–33. [PubMed: 8162639]
19. Wordsworth BP, Pile KD, Gibson K, Burney RO, Mockridge I, Powis SH. Analysis of the MHC-encoded transporters TAP1 and TAP2 in rheumatoid arthritis: linkage with DR4 accounts for the association with a minor TAP2 allele. Tissue antigens. 1993; 42(3):153–5. [PubMed: 8284790]
20. Wang CL, Wu YT, Liu CA, Lin MW, Lee CJ, Huang LT, et al. Expression of CD40 ligand on CD4+ T-cells and platelets correlated to the coronary artery lesion and disease progress in Kawasaki disease. Pediatrics. 2003; 111(2):E140–7. [PubMed: 12563087]
21. Zhou J, Li J, Chen J, Liu Y, Gao W, Ding Y. Over-expression of CDH22 is associated with tumor progression in colorectal cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine. 2009; 30(3):130–40. [PubMed: 19546606]
22. Khor CC, Davila S, Breunis WB, Lee YC, Shimizu C, Wright VJ, et al. Genome-wide association study identifies FCGR2A as a susceptibility locus for Kawasaki disease. Nature genetics. 2011; 43(12):1241–1246. [PubMed: 22081228]
23. Onouchi Y. Genetics of Kawasaki disease: what we know and don't know. Circulation journal : official journal of the Japanese Circulation Society. 2012; 76(7):1581–6. [PubMed: 22789975]
24. Kuo HC, Chang WC. Genetic polymorphisms in Kawasaki disease. Acta pharmacologica Sinica. 2011; 32(10):1193–8. [PubMed: 21892198]
25. Zhou H, Yu Y, Li H, Shi W, Yang H, Liu C, et al. Anti-H can trigger apoptosis and down-regulate FUT1 expression in erythroid differentiated K562 cells without complement mediation. Transplant immunology. 2012; 27(4):151–6. [PubMed: 23063462]
26. Armistead PM, Mohseni M, Gerwin R, Walsh EC, Iravani M, Chahardouli B, et al. Erythroid-lineage-specific engraftment in patients with severe hemoglobinopathy following allogeneic hematopoietic stem cell transplantation. Exp Hematol. 2008; 36(9):1205–15. [PubMed: 18550258]
27. Isozaki T, Ruth JH, Amin MA, Campbell PL, Tsou PS, Ha CM, et al. Fucosyltransferase 1 mediates angiogenesis, cell adhesion and rheumatoid arthritis synovial tissue fibroblast proliferation. Arthritis research & therapy. 2014; 16(1):R28. [PubMed: 24467809]
28. Moehler TM, Sauer S, Witzel M, Andrulis M, Garcia-Vallejo JJ, Grobholz R, et al. Involvement of alpha 1-2-fucosyltransferase I (FUT1) and surface-expressed Lewis(y) (CD174) in first endothelial cell-cell contacts during angiogenesis. Journal of cellular physiology. 2008; 215(1):27–36. [PubMed: 18205178]
29. Moore GT, Brown SJ, Winterhalter AC, Lust M, Salvaris EJ, Selan C, et al. Glycosylation changes in hFUT1 transgenic mice increase TCR signaling and apoptosis resulting in thymocyte maturation arrest. Molecular immunology. 2008; 45(8):2401–10. [PubMed: 18155296]
30. Zhang Z, Sun P, Liu J, Fu L, Yan J, Liu Y, et al. Suppression of FUT1/FUT4 expression by siRNA inhibits tumor growth. Biochimica et biophysica acta. 2008; 1783(2):287–96. [PubMed: 18023290]
31. Nakamura H, Jasper MJ, Hull ML, Aplin JD, Robertson SA. Macrophages regulate expression of alpha1,2-fucosyltransferase genes in human endometrial epithelial cells. Molecular human reproduction. 2012; 18(4):204–15. [PubMed: 22053055]
32. Xu K, Chong DC, Rankin SA, Zorn AM, Cleaver O. Rasip1 is required for endothelial cell motility, angiogenesis and vessel formation. Developmental biology. 2009; 329(2):269–79. [PubMed: 19272373]
33. Xu K, Sacharidou A, Fu S, Chong DC, Skaug B, Chen ZJ, et al. Blood vessel tubulogenesis requires Rasip1 regulation of GTPase signaling. Developmental cell. 2011; 20(4):526–39. [PubMed: 21396893]
34. Ikram MK, Sim X, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, et al. Four novel Loci (19q13, 6q24, 12q24, and 5q14) influence the microcirculation in vivo. PLoS Genet. 2010; 6(10) e1001184.

35. Franke A, McGovern DP, Barrett JC, Wang K, Radford-Smith GL, Ahmad T, et al. Genome-wide meta-analysis increases to 71 the number of confirmed Crohn's disease susceptibility loci. Nature genetics. 2010; 42(12):1118–25. [PubMed: 21102463]
36. ENSEMBL. BRD7P2 phenotypes. 2014
37. Ballester V, Vendrell R, Haritunians T, Klomhaus A, Guo X, McGovern DP, et al. Su1761 A Baz1a Haplotype With Ancient American Ancestry Contributes to IBD in Puerto Ricans. Gastroenterology. 2013; 144(5) S-470.
38. Sanchez-Molina S, Mortusewicz O, Bieber B, Auer S, Eckey M, Leonhardt H, et al. Role for hACF1 in the G2/M damage checkpoint. Nucleic Acids Res. 2011; 39(19):8445–56. [PubMed: 21745822]
39. Uhlenbrock K, Huber J, Ardati A, Busch AE, Kostenis E. Fluid shear stress differentially regulates gpr3, gpr6, and gpr12 expression in human umbilical vein endothelial cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2003; 13(2):75–84.
40. Perricone C, Ciccacci C, Ceccarelli F, Di Fusco D, Spinelli FR, Cipriano E, et al. TRAF3IP2 gene and systemic lupus erythematosus: association with disease susceptibility and pericarditis development. Immunogenetics. 2013; 65(10):703–9. [PubMed: 23836313]
41. Bennett MK, Calakos N, Scheller RH. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science. 1992; 257(5067):255–9. [PubMed: 1321498]
42. Smirnova T, Miniou P, Viegas-Pequignot E, Mallet J. Assignment of the human syntaxin 1B gene (STX) to chromosome 16p11.2 by fluorescence in situ hybridization. Genomics. 1996; 36(3):551–3. [PubMed: 8884284]
43. Gomez-Jaramillo L, Delgado-Perez L, Reales E, Mora-Lopez F, Mateos RM, Garcia-Poley A, et al. Syntaxin-4 is implicated in the secretion of antibodies by human plasma cells. J Leukoc Biol. 2014; 95(2):305–12. [PubMed: 24146186]
44. Rahman A, Decourcey J, Larbi NB, Loughran ST, Walls D, Loscher CE. Syntaxin-4 is essential for IgE secretion by plasma cells. Biochem Biophys Res Commun. 2013; 440(1):163–7. [PubMed: 24055037]
45. Pennica D, Swanson TA, Shaw KJ, Kuang WJ, Gray CL, Beatty BG, et al. Human cardiotrophin-1: protein and gene structure, biological and binding activities, and chromosomal localization. Cytokine. 1996; 8(3):183–9. [PubMed: 8833032]
46. Robador PA, Moreno MU, Beloqui O, Varo N, Redon J, Fortuno A, et al. Protective effect of the 1742(C/G) polymorphism of human cardiotrophin-1 against left ventricular hypertrophy in essential hypertension. Journal of hypertension. 2010; 28(11):2219–26. [PubMed: 20683337]
47. Erdmann J, Hassfeld S, Kallisch H, Fleck E, Regitz-Zagrose V. Genetic variants in the promoter (g983G>T) and coding region (A92T) of the human cardiotrophin-1 gene (CTF1) in patients with dilated cardiomyopathy. Hum Mutat. 2000; 16(5):448. [PubMed: 11058912]
48. Drobic V, Cunnington RH, Bedosky KM, Raizman JE, Elimban VV, Rattan SG, et al. Differential and combined effects of cardiotrophin-1 and TGF-beta1 on cardiac myofibroblast proliferation and contraction. Am J Physiol Heart Circ Physiol. 2007; 293(2):H1053–64. [PubMed: 17483238]
49. Robador PA, San Jose G, Rodriguez C, Guadall A, Moreno MU, Beaumont J, et al. HIF-1-mediated up-regulation of cardiotrophin-1 is involved in the survival response of cardiomyocytes to hypoxia. Cardiovascular research. 2011; 92(2):247–55. [PubMed: 21771897]
50. Kumar V, Westra HJ, Karjalainen J, Zhernakova DV, Esko T, Hrdlickova B, et al. Human disease-associated genetic variation impacts large intergenic non-coding RNA expression. PLoS Genet. 2013; 9(1) e1003201.
51. Newburger JW, Takahashi M, Gerber MA, Gewitz MH, Tani LY, Burns JC, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: A statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. Circulation. 2004; 110(17): 2747–2771. [PubMed: 15505111]
52. Cortes A, Brown MA. Promise and pitfalls of the Immunochip. Arthritis research & therapy. 2011; 13(1):101. [PubMed: 21345260]

53. Parkes M, Cortes A, van Heel DA, Brown MA. Genetic insights into common pathways and complex relationships among immune-mediated diseases. Nature reviews Genetics. 2013; 14(9): 661–73.

54. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3): 559–75. [PubMed: 17701901]

55. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nature genetics. 2006; 38(8):904–9. [PubMed: 16862161]

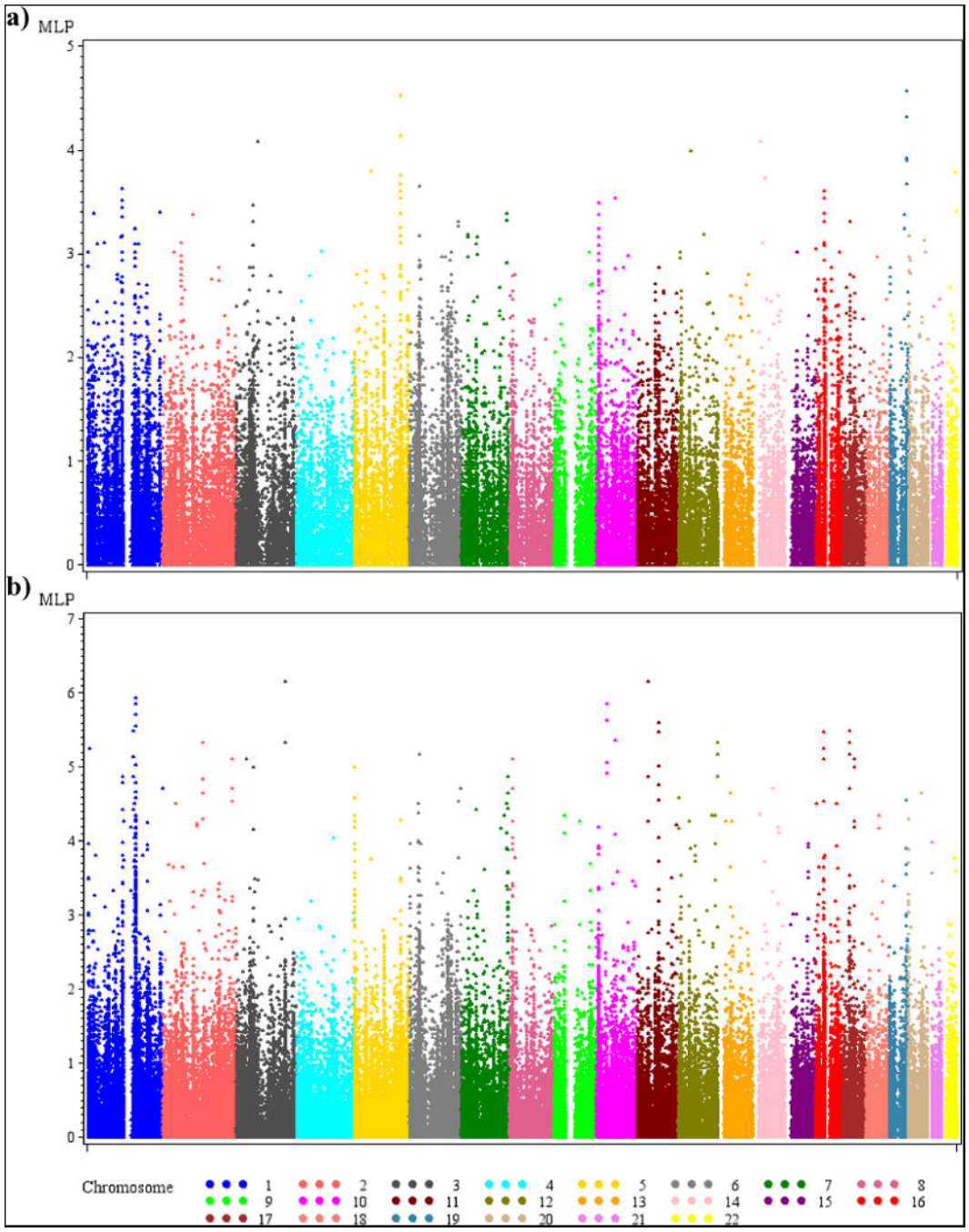

Figure 1.

Manhattan plot of the association between SNPs in the ImmunoChip and susceptibility to Kawasaki Disease using the transmission disequilibrium test (TDT) in a) additive model and b) dominant model

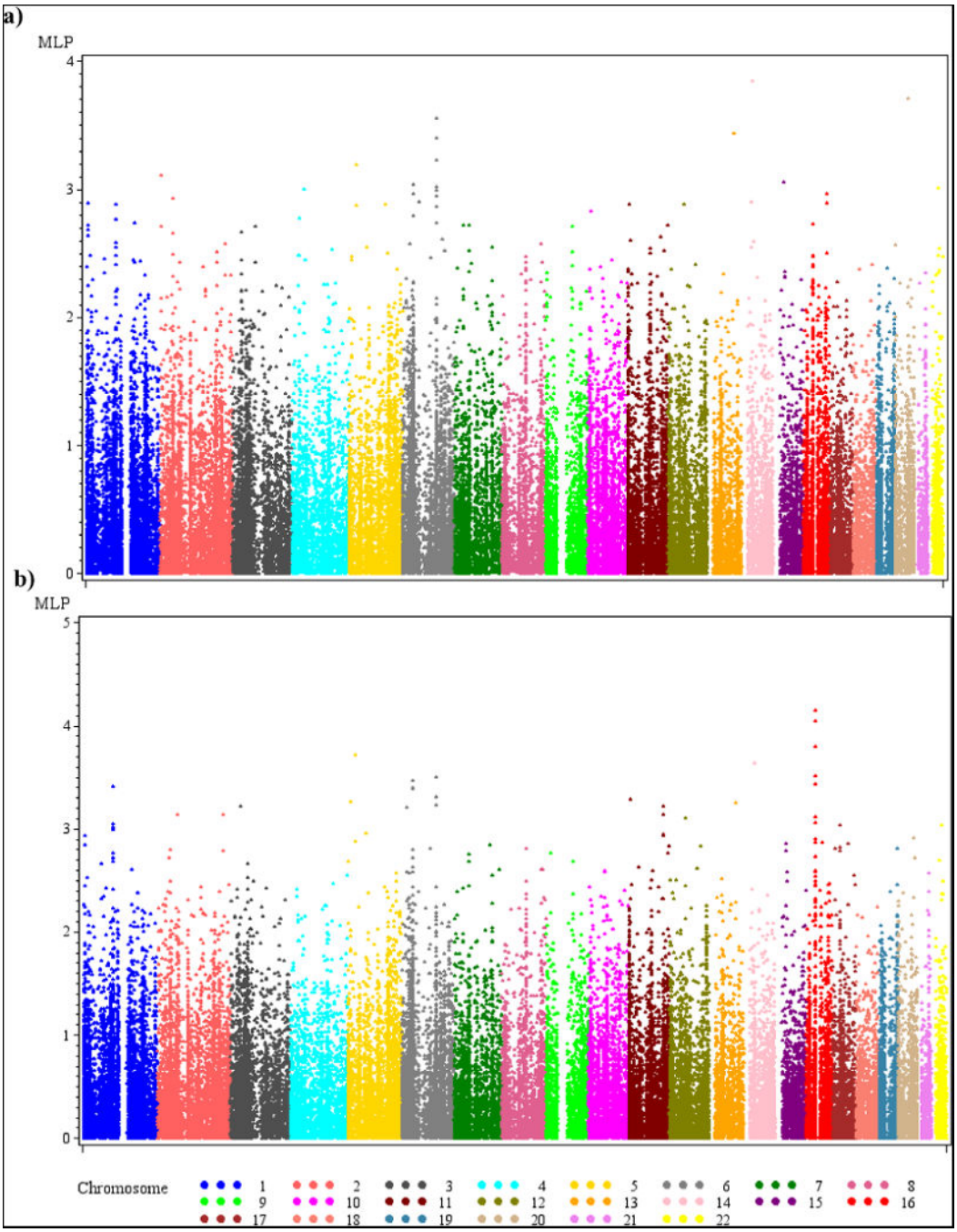

Figure 2.

Manhattan plot of the association between SNPs in the ImmunoChip and response to IVIG treatment using logistic regression in a) additive model and b) dominant model

Table 1

Top 15 significant SNPs associated with susceptibility to Kawasaki disease using the a) additive model and b) dominant model in FBAT

CHR	SNP	Position	Gene Symbol	Gene Location	Risk* allele	MAF HAPMAP/1000 Genomes	p-Value
	Additive Model						
19	rs838143	53943567	*FUT1*	3′ UTR	A	0.05	2.70 E-05
5	rs2054440	150365152	*LOC134466* | *GPX3*	Intergenic	G	0.42	3.00 E-05
19	rs838142	53943963	*FUT1*	3′ UTR	T	0.25	4.80 E-05
5	rs17111637	150361436	*LOC134466* | *GPX3*	Intergenic	A	0.35	7.30 E-05
3	rs12486347	70649298	*LOC100128160* | *FOXP1*	Intergenic	A	0.14	8.20 E-05
14	rs2273844	24173254	*GZMB*	5′ UTR	A	0.19	8.20 E-05
12	rs17539379	39414907	*CNTN1*	Intron	A	0.08	1.01 E-04
19	rs4021	53945073	*FUT1*	3′ UTR	G	0.25	1.18 E-04
19	rs463631	53926504	*RASIP1*	Intron	A	0.04	1.24 E-04
19	rs392775	53931669	*RASIP1*	Intron	T	0.04	1.24 E-04
5	rs6889028	55465663	*ANKRD55* | *LOC727984*	Intergenic	A	0.03	1.57 E-04
22	rs135058	42168027	*MPPED1*	Intron	T	0.05	1.62 E-04
5	rs2345000	150363456	*LOC134466* | *GPX3*	Intergenic	A	0.34	1.75 E-04
14	rs9919930	39686800	*FBXO33* | *LRFN5*	Intergenic	T	–	1.83 E-04
19	rs281384	53908738	*MAMSTR*	Intron	A	0.02	2.08 E-04
	Dominant Model						
3	rs9847915	161279884	*LOC730109* | *BRD7P2*	Intergenic	A	0.29	6.81 E-07
11	rs118125355	35065834	*PDHX* | *CD44*	Intergenic	–	0.01	6.82 E-07
1	rs4657067	159791329	*FCGR3A* | *FCGR2C*	Intergenic	A	–	1.15 E-06
1	rs377982	159868496	*FCGR3B* | *FCGR2B*	Intergenic	T	–	1.15 E-06
1	rs12735449	159785611	*FCGR3A*	Intron	T	–	1.36 E-06
1	rs76158727	159865216	*FCGR3B*	Intron	–	–	1.36 E-06
10	rs35980734	35301298	*PARD3* | *CUL2*	Intergenic	G	0.33	1.36 E-06
1	rs111974271	159847992	*LOC1131982* | *FCGR3B*	Intergenic	A	–	1.93 E-06
10	rs12251203	35479828	*CREM*	Intron	G	0.40	2.29 E-06
11	rs9651751	70956476	*KRTAP5-10* | *KRTAP5-11*	Intergenic	G	0.39	2.45 E-06
1	rs3964394	159803051	*FCGR3A* | *FCGR2C*	Intergenic	–	–	2.73 E-06

CHR	SNP	Position	Gene Symbol	Gene Location	Risk* allele	MAF HAPMAP/1000 Genomes	p-Value
1	rs143513265	151037507	*LCE1D* | *LCE1C*	Intergenic	–	–	3.16 E-06
17	rs149259492	23182244	*NOS2A* | *LOC201229*	Intergenic	–	0.35	3.16 E-06
16	rs762633	28515842	*SULT1A2*	5′ UTR	C	0.33	3.24 E-06
10	rs4325270	60185474	*LOC728640* | *BICC1*	Intergenic	A	0.55	4.30 E-06

*Minor allele was always used as the risk allele in the analysis

Table 2

Adjusted Odds ratio (OR) for the top 15 SNPs associated with IVIG responsiveness using a) additive model and b) dominant model

CHR	SNP	Position	Gene Symbol	Gene Location	Minor allele	Odds Ratio-IVIG nonresponders	MAF-Responders	MAF Nonresponders	MAF HAPMAP	p-Value
Additive Model										
14	rs1200332	34436886	*BAZ1A* | *C14orf19*	Intergenic	T	5.49	0.13	0.33	0.21	1.40 E-04
20	rs6017164	41815520	*GTSF1L* | *TOX2*	Intergenic	A	0.25	0.45	0.32	0.45	1.95 E-04
6	rs4354185	110408354	*GPR6*	3′ UTR	A	4.46	0.25	0.43	0.48	2.74 E-04
13	rs7999399	88031506	*SLITRK5* | *MIRH1*	Intergenic	C	3.42	0.27	0.45	0.46	3.65 E-04
6	rs4317449	110408638	*GPR6* | *WASF1*	Intergenic	T	4.22	0.25	0.42	0.48	3.90 E-04
6	rs72945401	111833015	*REV3L*	Intron	G	0.02	0.14	0.02	0.12	5.82 E-04
5	rs10473594	22511857	*CDH12*	Intron	C	4.51	0.11	0.32	0.16	6.42 E-04
2	rs7579420	3938010	*LOC728597* | *LOC727982*	Intergenic	T	0.31	0.43	0.32	0.46	7.69 E-04
15	rs7163190	32812788	*GOLGA8B* | *GJD2*	Intergenic	G	0.26	0.33	0.18	0.29	8.66 E-04
6	rs3134926	32308125	*NOTCH4* | *C6orf10*	Intergenic	C	0.27	0.40	0.25	0.20	9.12 E-04
6	rs3777914	112011860	*TRAF3IP2*	Intron	G	0.29	0.50	0.32	0.47	9.40 E-04
22	rs16995211	33781639	*LARGE* | *ISX*	Intergenic	A	5.69	0.08	0.23	0.09	9.72 E-04
4	rs719379	40537658	*APBB2*	Intron	T	3.46	0.37	0.43	0.48	9.93 E-04
6	rs1883137	112013839	*TRAF3IP2*	Intron	G	0.29	0.47	0.34	0.46	9.98 E-04
16	rs11641163	79327374	*CDYL2*	Intron	A	0.24	0.43	0.37	0.47	1.07 E-03
Dominant Model										
16	rs4889606	30918684	*STX1B*	Intron	G	0.13	0.49	0.27	0.35	6.95 E-05
16	rs9926533	30921680	*STX1B*	Intron	T	0.13	0.49	0.27	0.35	6.95 E-05
16	rs4889603	30889726	*SETD1A*	Intron	G	0.13	0.50	0.29	0.38	8.79 E-05
16	rs12917722	30855898	*FBXL19*	Intron	G	0.15	0.50	0.29	0.42	1.57 E-04
5	rs10473594	22511857	*CDH12*	Intron	C	5.82	0.11	0.32	0.16	1.87 E-04
14	rs1200332	34436886	*BAZ1A* | *C14orf19*	Intergenic	T	5.56	0.13	0.33	0.21	2.25 E-04
16	rs8046707	30823734	*CTF1* | *LOC283932*	Intergenic	A	0.18	0.45	0.28	0.38	3.00 E-04
16	rs11649653	30825988	*CTF1* | *LOC283932*	Intergenic	G	0.18	0.45	0.28	0.38	3.00 E-04
6	rs4354185	110408354	*GPR6*	3′ UTR	A	6.22	0.25	0.43	0.48	3.09 E-04
6	rs3134926	32308125	*NOTCH4* | *C6orf10*	Intergenic	C	0.16	0.40	0.25	0.20	3.38 E-04

CHR	SNP	Position	Gene Symbol	Gene Location	Minor allele	Odds Ratio-IVIG nonresponders	MAF-Responders	MAF Nonresponders	MAF HAPMAP	p-Value
16	rs2288004	30961541	*STX4* | *ZNF668*	Intergenic	C	0.17	0.50	0.27	0.36	3.65 E-04
16	rs10871454	30955580	*STX4*	Intron	T	0.17	0.48	0.27	0.36	3.65 E-04
16	rs11150604	30944521	*STX1B* | *STX4*	Intergenic	T	0.17	0.48	0.27	0.36	3.65 E-04
16	rs12448321	30955781	*STX4*	Intron	T	0.17	0.48	0.27	0.36	3.65 E-04
16	rs9939417	30960968	*STX4* | *ZNF668*	Intergenic	T	0.17	0.48	0.27	0.36	3.65 E-04

Table 3

Replication of previous genome-wide associations with susceptibility to Kawasaki Disease

CHR	SNP	Position	Gene Symbol	Gene Location	MAF HAPMAP	Model	p-Value
6	rs2857151	32871492	HLA-DQB2 | HLA-DOB	Intergenic	0.44	Dominant	0.04
20	rs4813003	44196691	CD40 | CDH22	Intergenic	0.16	Dominant	0.04