

Prediction for Intravenous Immunoglobulin Resistance by Using Weighted Genetic Risk Score Identified From Genome-Wide Association Study in Kawasaki Disease

Ho-Chang Kuo, MD, PhD*; Henry Sung-Ching Wong, MSc*; Wei-Pin Chang, PhD; Ben-Kuen Chen, PhD; Mei-Shin Wu, MSc; Kuender D. Yang, MD, PhD; Kai-Sheng Hsieh, MD; Yu-Wen Hsu, MSc; Shih-Feng Liu, MD; Xiao Liu, MSc; Wei-Chiao Chang, PhD

Background—Intravenous immunoglobulin (IVIG) is the treatment of choice in Kawasaki disease (KD). IVIG is used to prevent cardiovascular complications related to KD. However, a proportion of KD patients have persistent fever after IVIG treatment and are defined as IVIG resistant.

Methods and Results—To develop a risk scoring system based on genetic markers to predict IVIG responsiveness in KD patients, a total of 150 KD patients (126 IVIG responders and 24 IVIG nonresponders) were recruited for this study. A genome-wide association analysis was performed to compare the 2 groups and identified risk alleles for IVIG resistance. A weighted genetic risk score was calculated by the natural log of the odds ratio multiplied by the number of risk alleles. Eleven single-nucleotide polymorphisms were identified by genome-wide association study. The KD patients were categorized into 3 groups based on their calculated weighted genetic risk score. Results indicated a significant association between weighted genetic risk score (groups 3 and 4 versus group 1) and the response to IVIG (Fisher's exact P value 4.518×10^{-03} and 8.224×10^{-10} , respectively).

Conclusions—This is the first weighted genetic risk score study based on a genome-wide association study in KD. The predictive model integrated the additive effects of all 11 single-nucleotide polymorphisms to provide a prediction of the responsiveness to IVIG. (*Circ Cardiovasc Genet.* 2017;10:e001625. DOI: 10.1161/CIRCGENETICS.116.001625.)

Key Words: alleles ■ genetics ■ genome-wide association study ■ odds ratio ■ pharmacogenetics

Kawasaki disease (KD) is an acute systemic vasculitis syndrome. This disease mainly affects children who are younger than 5 years of age.¹ Since KD was first reported by Dr Kawasaki in 1967 in Japan,² it has now been recognized as a worldwide pediatric disease. Epidemiological studies indicate that the prevalence of KD is higher in East Asia than North America and Europe.³ Although the cause remains unclear, a genetic contribution to disease susceptibility is well established.⁴ Several genome-wide association studies (GWASs)⁵⁻⁷ and candidate gene association studies⁸⁻¹² have identified susceptibility loci for KD.

KD is thought to be the leading cause of acquired heart disease in children in the developed countries.¹³⁻¹⁵ High-dose intravenous immunoglobulin (IVIG) is currently acknowledged to be the standard treatment for KD patients. Receiving a single high dose of IVIG within 10 days after disease onset can significantly reduce the risk of coronary artery lesions (CALs).¹⁶ However, 10% to 20% of KD patients have recrudescence fever after the initial IVIG treatment, and these patients are at higher risk of CAL and acquired cardiac complications in adulthood.^{12,17,18}

The molecular mechanisms of IVIG for anti-inflammation in KD remain unclear. Potential mechanisms include the blockade of the Fc receptor,¹⁹⁻²¹ neutralization of the pathogenic or

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From the Chang Gung University College of Medicine, Taiwan (H.-C.K.); the Master Program for Clinical Pharmacogenomics and Pharmacoproteomics, School of Pharmacy (H.-C.K., W.-C.C.), Department of Clinical Pharmacy, School of Pharmacy (H.S.-C.W., M.-S.W., W.-C.C.), School of Health Care Administration, College of Management (W.-P.C.), and PhD Program in Biotechnology Research and Development, College of Pharmacy (W.-C.C.), Taipei Medical University, Taiwan; Department of Pediatrics and Kawasaki Disease Center (H.-C.K., K.-S.H.), Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine (S.-F.L.), and Department of Respiratory Therapy (S.-F.L.), Kaohsiung Chang Gung Memorial Hospital, Taiwan; Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan (B.-K.C.); Department of Biotechnology and Bioindustry Sciences, College of Bioscience and Biotechnology, National Cheng Kung University, Tainan, Taiwan (B.-K.C.); Department of Pediatrics, Mackay Memorial Hospital, Taipei, Taiwan (K.D.Y.); Institute of Clinical Medicine, National Yang Ming University, Taipei, Taiwan (K.D.Y.); The PhD Program for Translational Medicine, College of Medical Science and Technology, Taipei Medical University and Academia Sinica, Taiwan (Y.-W.H.); Section of Hematology/Oncology, Department of Medicine, The University of Chicago, IL (X.L.); Department of Pharmacy, Taipei Medical University-Wanfang Hospital, Taiwan (W.-C.C.); and Center for Biomarkers and Biotech Drugs, Kaohsiung Medical University, Taiwan (W.-C.C.).

*Dr Kuo and H.S.-C. Wong contributed equally to this work.

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Correspondence to Wei-Chiao Chang, PhD, 250 Wu-Hsing St, Taipei City 110, Taiwan. E-mail wcc@tmu.edu.tw

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the toxic products of unknown infectious agents, immune-modulatory effects,²² stimulation of suppressor activity, and modulation of the cytokines.²³ Several genome-wide association studies have revealed common variants that strongly associated with KD, CAL formation, and IVIG treatment.^{24–26} Here, we applied a GWAS-based weighted genetic risk score (wGRS) to develop a genetic model to predict the IVIG resistance in KD.

Methods

Patients Studied

The DNA of children who have been diagnosed as KD and received IVIG treatment at Chang Gung Memorial Hospital, Kaohsiung Medical Center from the year 2001 to 2007 were collected. The informed consent was obtained from all patients and guardians. The acquisition and subsequent use of samples were approved by the Institutional Review Board of Chang Gung Memorial Hospital (IRB No. 98-1438B), and the methods were performed in accordance with the approved guidelines. We adopted the same diagnostic criteria of KD as reported previously^{27–29} and further excluded the samples with acute fever for <5 days. A diagnosis of KD was made by a clinician using the suggested universal criteria for KD proposed by the American Heart Association. In any child with fever lasting for >5 days who fulfilled four of the following criteria: diffuse mucosal inflammation with strawberry tongue and fissure lips, bilateral nonpurulent conjunctivitis, indurative angioedema over the hands and feet, dysmorphic skin rashes, and unilateral cervical lymphadenopathy.³⁰ Every patient diagnosed as KD was treated with a single dose of IVIG infusion (2 g/kg) within a 12-hour period. Aspirin (3 to 5 mg per kg per day) was given until all signs of inflammation were resolved or regression of CAL under 2-dimensional echocardiography test by visualizing coronary arteries (including left and right) diameter on the parasternal short-axis view of the aorta. Following the guidelines of Japanese Ministry of Health guidelines, we defined CAL as an increment of the internal diameter of 3 mm (≤ 5 years old) or 4 mm (>5 years old), or 1.5× larger of internal diameter than the adjacent segment. We defined IVIG responsiveness as defervescence within 48 hours after the first administration of IVIG and no recurrence (temperature >38°C) for at least 7 days, combined with remission of inflammatory signs. KD patients who had persistence of fever beyond 48 hours were defined as IVIG resistant.

DNA Extraction and Genome-Wide Scanning

The blood cells were subject to DNA extraction for oligonucleotide arrays. Samples were first treated with 0.5% sodium dodecylsulfate lysis buffer and then protease K (1 mg/mL) for 4 hours at 60°C. DNA was extracted using Genra (Qiagen, Valencia, CA) extraction kit followed by 70% alcohol precipitation and Genra Puregene Blood Kit (Qiagen). Single-nucleotide polymorphisms (SNPs) were detected by using Affymetrix Genome-Wide Human SNP Array 6.0 platform (Affymetrix, Inc).

Statistical Analysis

Quality Control

PLINK³¹ was applied for quality control of genome-wide scan data. We excluded the SNPs with missing call rates exceeding 1.0%, a *P* value of Hardy–Weinberg equilibrium $<1 \times 10^{-5}$ and minor allele frequency <5.0%. The SNPs reference of Affymetrix Genome-Wide Human SNP Array 6.0 platform was NCBI36 (hg18). CrossMap (Version 0.1.5) was used to lift over data to NCBI37 (hg19). SHAPEIT³² and IMPUTE²³³ were applied for the haplotype phasing and genotype imputation. HapMap 3 genotype data were incorporated³⁴ with our Taiwanese data to perform principal component analysis (PCA). PCA was performed by using Genome-wide Complex Trait Analysis,³⁵ which performed PCA by the same algorithm implemented in EIGENSTRAT and output corresponding eigenvalues and eigenvectors, to identify sample substructure on autosomal genotype data.

SNP Association Analysis

To satisfy the 603698 SNPs to filtering criteria, we performed association analysis by using the mixed linear model algorithm implemented in Genome-wide Complex Trait Analysis that accounts for the polygenic effect of all SNPs during association test. Then, we calculated the fixed effect of all SNPs by excluding candidate markers (mixed linear model with candidate marker excluded), which prevented loss of power because of double fitting of the candidate markers. Manhattan plot was plotted by Haploview³⁶ software. Then we evaluated the residual population stratification by calculating genomic inflation λ value and visualized corresponding test statistics using quantile-quantile plot in R (<http://www.r-project.org/>).

Weighted Genetic Risk Scoring System

wGRS system proposed by De Jager et al³⁷ was applied to calculate the cumulative effects of candidate SNPs. In this study, the allelic odds ratios were natural logarithm transformed to become the weight of each SNP. The wGRS was calculated by multiplying the weight by the risk allele number (0, 1, or 2) and taking the sum across 11 SNPs, as shown in the following equation:

$$wGRS = \sum_{k=1}^{11} w^k X^k \quad (1)$$

where *k* is SNP, w^k is the corresponding weight of SNP ($\ln(\text{OR})$) and X^k is the number of the risk allele (0, 1, or 2). wGRS of IVIG responders and nonresponders has been compared by Wilcoxon rank-sum test with continuity correction. KD patients were then categorized by corresponding wGRS into 4 groups: group 1 ($wGRS < |\text{mean} - \text{SD}|$), group 2 ($|\text{mean} - \text{SD}| \leq wGRS < \text{median}$), group 3 ($\text{median} \leq wGRS < |\text{mean} + \text{SD}|$), and group 4 ($wGRS \geq |\text{mean} + \text{SD}|$). wGRS was also compared between groups, and relevant statistical parameters were calculated by using group 1 as a reference. The subgroup analysis was further performed to confirm the intragroup difference of wGRS between IVIG responders and nonresponders.

To further assess the performance of wGRS in the prediction of IVIG responsiveness, we conducted a receiver operating characteristic (ROC) curve analysis.³⁸ For every discrimination threshold, 95% confidence intervals (CIs) were calculated by 2000 stratified bootstrapping, which contained the same number of both groups (24 cases and 126 controls) as the original sample. The area under the ROC curve was also calculated to evaluate the accuracy of wGRS predictors. Finally, the difference between area under the ROC curves was tested by DeLong method, an asymptotically exact method to calculate the uncertainty of an AUC as described by DeLong et al.³⁹

Results

Sample Substructure Evaluation of GWAS

KD patients (n=150) treated at Kaohsiung Chang Gung Memorial Hospital were included in this study. In total, 867877 SNPs were genotyped in 24 IVIG nonresponders (male=58.33%, age=2.24 [mean]±2.82 [SD] years old) and 126 IVIG responders (male=60.32%, age=2.16±2.41 years old). After marker-level quality control, 264179 of 867877 (30.44%) markers were filtered. SNPs were defined to pass quality control with following criteria: minor allele frequency >5.0%, genotyping call rate >99%, and passed Hardy–Weinberg equilibrium test in controls ($P < 1 \times 10^{-5}$). SNPs of 603698 of 867877 (69.56%) remained for further analysis. In sample-level quality control step, all subjects passed the criteria of sample call rate >99% and were retained for further analysis. PCA was performed to assess sample structure and identify sample outliers. Inspecting the first 3 PCA axes, no significant population stratification (Figure 1A and 1B) was identified. *P* values from an allelic association test showed no inflation

and revealed a low possibility of sample substructure. (Genomic inflation factor λ value $\lambda_{GC}=1.015$; Figure 1C)

Risk Loci Affecting IVIG Responsiveness

To identify the risk loci affecting IVIG responsiveness in Taiwanese KD patients, we conducted a mixed linear model association test. Considering the modest sample size in our study, genetic relation matrix was estimated and incorporated into the mixed linear model as a polygenic effect to prevent false-positive association and increase statistical power.⁴⁰ Eight SNPs that reached genome-wide suggestive significance ($5 \times 10^{-6} < P < 5 \times 10^{-8}$) were identified (Figure 2; Table 1). Among all identified possible candidate loci, SNP rs9380548A/T that showed the strongest association signal ($P=1.03 \times 10^{-6}$) was located in an intergenic region (6p24.1) of chromosome 6 and the 3' upstream from *ADTRP*. The risk allele (A allele) showed an increasing risk (odds ratio, 1.6305) of IVIG unresponsiveness when compared with the nonrisk allele (T allele). Another SNP rs742490G/C ($P=2.67 \times 10^{-6}$) was located at intron of *EXOC2* (6p25.3), the odds ratio by which risk allele (G allele) compared with nonrisk allele (C allele) was 1.4895. rs7634A/T ($P=1.51 \times 10^{-6}$; odds ratio, 1.2676), rs1250301G/A ($P=3.41 \times 10^{-6}$; odds ratio, 1.4635) and rs2797915T/C ($P=3.41 \times 10^{-6}$; odds ratio, 1.4635) located on chromosome 10 were also found to be associated with IVIG nonresponsiveness. rs7634A/T was at 3' untranslated region of

KLF6 (10p15.2), whereas rs1250301G/A and rs2797915T/C were in an intergenic region between *ZNF438* and *ZEB1* (10p11.22). Moreover, rs4130857A/G, rs4585205T/C, and rs4602399G/T at an intergenic region between *MIR548A3* and *ZPLD1* (3q13.11) were also found to be associated with IVIG nonresponsiveness ($P=4.87 \times 10^{-6}$; odds ratio, 1.2660).

wGRS Analysis for Prediction of IVIG Responsiveness

To predict the responsiveness of KD patients to IVIG, we applied a scoring system based on wGRS proposed by De Jager et al.³⁷ In total, 11 SNPs with $P < 1 \times 10^{-5}$ were included in the wGRS algorithm. The wGRS of patients was calculated and further analyzed (Table I in the Data Supplement). The wGRS showed a significant difference (Wilcoxon rank-sum test $P=1.071 \times 10^{-10}$) between IVIG responsive and nonresponsive groups (Figure IA and IB in the Data Supplement). Patients were categorized into 4 strata: group 1 (wGRS < 0.042), group 2 ($0.042 \leq \text{wGRS} < 0.636$), group 3 ($0.636 \leq \text{wGRS} < 1.681$), and group 4 (wGRS ≥ 1.681). The cumulative effects of SNPs were evaluated (Table 2; Table I in the Data Supplement). As shown in Table 2, all patients (42 of 42) in group 1 showed good responsiveness to IVIG treatment, whereas 14 of 21 IVIG nonresponders were in the group 4. The results indicated that the patient categorized in group 4 would be estimated to have

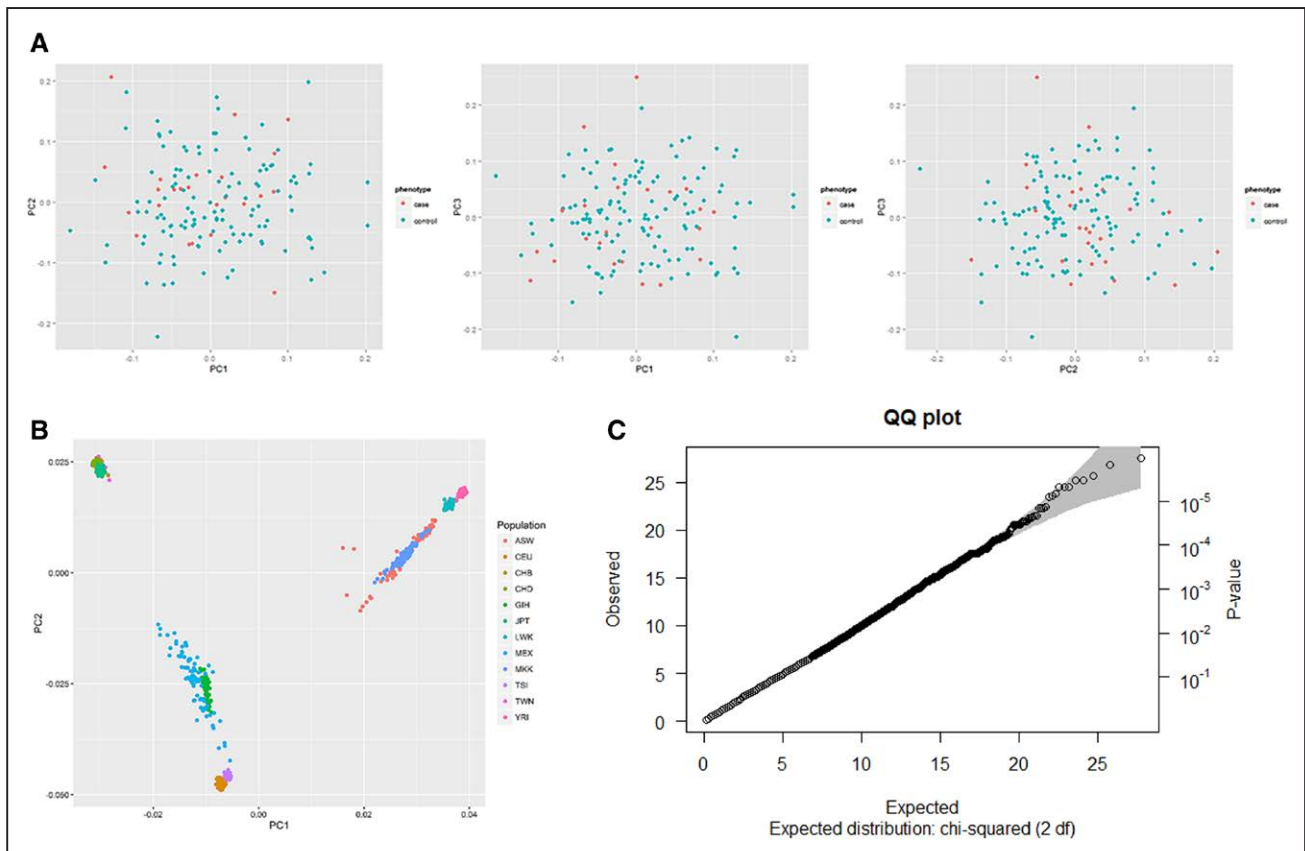


Figure 1. Evaluation of population stratification in Kawasaki disease (KD) genome-wide association study (GWAS) subjects (24 cases and 126 controls). **A**, Principal components analysis showed similar distributions of the top 3 principal components (PC1, PC2, and PC3) in cases and controls. **B**, Subjects from Taiwanese KD GWAS study and HapMap 3 database were analyzed by using Genome-wide Complex Trait Analysis v1.24 software. The top 2 principal components (PC1 and PC2) were plotted. PCA results indicated that Taiwanese KD subjects were clustered together. **C**, Allelic association using mixed linear model (MLM) was evaluated by quantile-quantile (Q-Q) plot. The genomic inflation λ value (λ_{GC}) was 1.015, revealed a low possibility of population stratification.

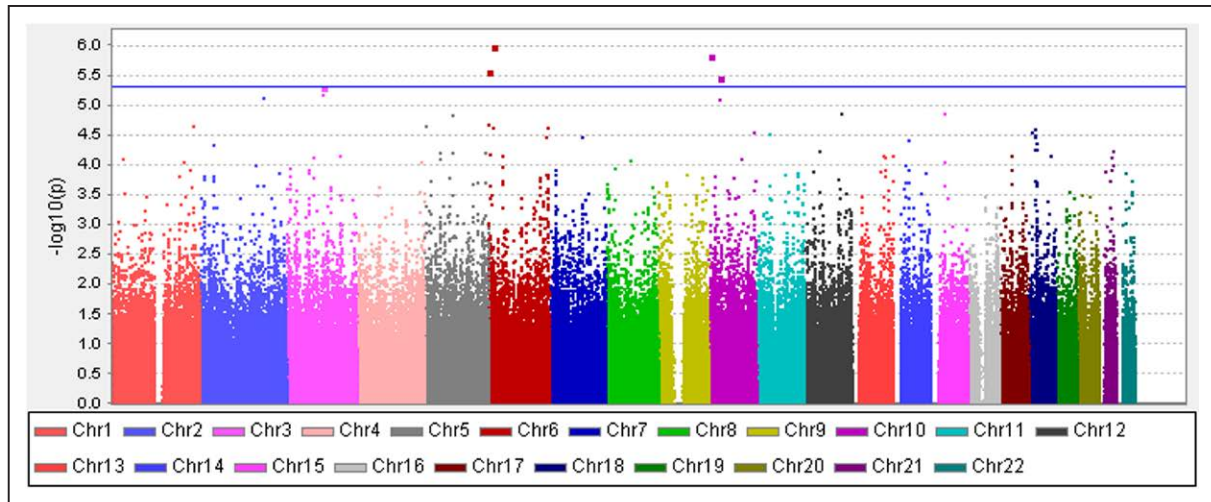


Figure 2. Manhattan plot for the Kawasaki disease intravenous immunoglobulin (IVIG) responsiveness analysis. Association values ($-\log_{10} p$) obtained using the mixed linear model association test were plotted against chromosome (based on genomic build hg19). The blue line indicated the genome-wide suggestive significance threshold ($P < 5 \times 10^{-6}$, $-\log_{10} P < 5.3$). CEU indicates Northern Europeans from Utah; CHB, Han Chinese in Beijing, China; JPT, Japanese in Tokyo; and YRI, Yoruba in Ibadan.

164.33 fold higher risk for IVIG resistance compared with those in group 1 ($P = 8.224 \times 10^{-10}$; 95% CI, 8.83–3059.50). The sensitivity and specificity of group 4 versus group 1 were 100% and 85.7%, respectively.

To assess the effect of boundaries selected on comparison groups, we repeated the association test between IVIG responsiveness and groups by different thresholds. First, we categorized the samples into 3 strata, that is, group 1 ($wGRS < |mean - SD|$), group 2 ($|mean - SD| \leq wGRS < |mean + SD|$), and group 3 ($wGRS \geq |mean + SD|$). The results were also significant, revealing a higher ratio of IVIG resistance in the group with the higher wGRS score (Table II in the Data Supplement). In addition, we recategorized the samples into 3 strata, including group 1 ($wGRS < \text{lowest tertile}$), group 2 (lowest tertile $\leq wGRS < \text{highest$

tertile), and group 3 ($wGRS \geq \text{highest tertile}$). Again, the association tests revealed that individuals with IVIG resistance had higher wGRS scores than individuals who were IVIG responsive (Table III in the Data Supplement). Together, we confirmed the correlation between IVIG responsiveness and stratification according to the wGRS.

Furthermore, we performed the analysis based on 7 SNPs after removing 3 SNPs that were in high linkage disequilibrium. As rs4130857, rs4585205, and rs4602399 on chromosome 3 and rs1250301 and rs2797915 on chromosome 10 are in highly linkage disequilibrium, we selected rs4130857 and rs1250301 (in total 7 SNPs) for the wGRS analysis. As expected, we observed a similar association compared with the results using 11 SNPs (Table IV in the Data Supplement).

Table 1. Genome-Wide Association Results

CHR	Position	Nearby Gene(s)	SNP	SNP Region	Ref/Other*	Freq†	Estimate β ‡	SE§	OR	P Value#
6	11830973	<i>ADTRP</i>	rs9380548	intergenic	A/T	0.0500	0.4889	0.1001	1.6305	1.03×10^{-06}
10	3821327	<i>KLF6</i>	rs7634	UTR-3	A/T	0.2467	0.2371	0.0493	1.2676	1.51×10^{-06}
6	568419	<i>EXOC2</i>	rs742490	intron	G/C	0.0733	0.3984	0.0849	1.4895	2.67×10^{-06}
10	31424444	<i>ZNF438-ZEB1</i>	rs1250301	intergenic	G/A	0.0700	0.3809	0.0820	1.4635	3.41×10^{-06}
10	31427323	<i>ZNF438-ZEB1</i>	rs2797915	intergenic	T/C	0.0700	0.3809	0.0820	1.4635	3.41×10^{-06}
3	103139511	<i>MIR548A3-ZPLD1</i>	rs4130857	intergenic	A/G	0.21	0.2359	0.0516	1.2660	4.87×10^{-06}
3	103144212	<i>MIR548A3-ZPLD1</i>	rs4585205	intergenic	T/C	0.21	0.2359	0.0516	1.2660	4.87×10^{-06}
3	103157445	<i>MIR548A3-ZPLD1</i>	rs4602399	intergenic	G/T	0.21	0.2359	0.0516	1.2660	4.87×10^{-06}

The single-nucleotide polymorphisms (SNPs) that reached genome-wide suggestive significant ($5 \times 10^{-6} < P < 5 \times 10^{-8}$) were listed. The genetic relationship matrix (GRM) between pairs of individuals from a set of SNPs is estimated and applied as the polygenic effect (random effect), that is, the accumulated effect of all SNPs. A mixed linear model (MLM)-based association analysis with the chromosome was implemented, on which the candidate SNP was located, and excluded from calculating the GRM. Gene and SNP region information were annotated by R Bioconductor (based on genomic build UCSC hg19). OR indicates odds ratio; and UTR, untranslated region.

*Reference allele (the coded effect allele) and the other allele for the susceptibility.

†Frequency of the reference allele.

‡Estimated SNP effect.

§Standard error.

||Odds ratio was calculated by $\exp(\beta)$.

#P value was calculated by MLM association test with the GRM be fitted together with the SNP for association test.

Table 2. wGRS Analysis

Category	wGRS*	Nonresponders/ Responders	Fisher's Exact <i>P</i> Value†	OR (95% CI)	Sensitivity	Specificity
4	≥1.681	14/7	8.224×10 ⁻¹⁰	164.33 (8.83–3059.50)	1.000	0.857
3	≥0.636–<1.681	9/48	4.518×10 ⁻⁰³	16.65 (0.94–294.67)	1.000	0.467
2	≥0.042–<0.636	1/29	1.000	4.32 (0.17–109.79)	1.000	0.592
1	<0.042	0/42		(Reference)		

P value and odds ratio (OR) were calculated after Haldane correction. CI indicates confidence interval; and wGRS, weighted genetic risk score.

*Weighted genetic risk score.

†Two-tail *P* value was calculated by Fisher's exact test.

ROC Curve Analysis of wGRS

Further analysis between groups 4 and 1 indicated a positive predictive value of 0.667, negative predictive value of 1.000, positive likelihood ratio of 6.993 (moderate evidence to rule in disease), and negative likelihood ratio of 0 (strong evidence to rule out disease). Besides, the odds ratio of IVIG resistant is estimated to be 16.65 in group 3 and 4.32 in group 2 ($P=4.518\times 10^{-03}$; 95% CI, 0.94–294.67, sensitivity = 100%, specificity = 46.7%; $P=1.000$; 95% CI, 0.17–109.79, sensitivity = 100%, specificity = 59.2%, respectively), by using group 1 as the reference. Subcategory case–control analysis was further performed to confirm the difference of intragroup wGRS in each group (Figure IC in the [Data Supplement](#)). Results revealed a significant difference between case and control in group 4 ($P=5.742\times 10^{-03}$), whereas no difference was found between groups 2 and 3 ($P=0.2398$ and $P=0.0688$, respectively).

Finally, ROC curves were constructed to evaluate the performance of wGRS in categorizing KD patients into the IVIG-responsive or nonresponsive group. The results indicated that high area under the ROC curve (91.1%) revealed a good separating ability of wGRS predictors (Figure 3A). The best predictive value of wGRS was 1.3, and the corresponding specificity and sensitivity were 88.9% and 79.2%, respectively. After adjusted for sex effects, the performance of wGRS remains well, with the best value of 2.8. The corresponding specificity and sensitivity were 81.7% and 79.2%, respectively (Figure 3B). Comparison between 2 ROCs showed a *P* value of 0.10502 (*Z* statistic of 1.621) under the null hypothesis of a true difference in AUC was equal to 0.

To infer the generalizability of our model, we compared the frequencies of 11 SNPs across different populations (Table V in the [Data Supplement](#)) according to the HAPMAP database. The frequencies of the 11 SNPs from the Taiwanese cohort were similar to other East Asian populations, including Han Chinese in Beijing, China and Japanese in Tokyo, but differed from Northern Europeans from Utah, and Yoruba in Ibadan populations.

Discussion

KD is a systemic vasculitis that mostly affected children of <5-years old, though, increased prevalence world widely. The most severe complication is CALs that will cause lifelong sequelae for children. IVIG or intravenous γ -globulin has been administrated for the treatment of KD since 1983.⁴¹ Administration of high dose IVIG significantly reduced the CAL rate from 49.0% to 18.4%. Currently, a single high dose of IVIG (2 g/kg) is considered as a gold standard in the treatment of KD. The molecular mechanisms of IVIG for the treatment of KD remain unclear. Several studies have identified epidemiological and laboratory characteristics as predictors of IVIG resistance. The biomarkers include patients' age, illness day, serum markers (including platelet count, erythrocyte sedimentation rate, hemoglobin concentration, C-reactive protein, eosinophil counts, lactate dehydrogenase, plasma level of albumin, alanine aminotransferase, clusterin, and granulocyte colony-stimulating factor), and sonographic gallbladder abnormalities.^{42–47} In addition, genetic variations were reported to associate with IVIG resistance in KD patients. The genetic markers include *Fc γ R*

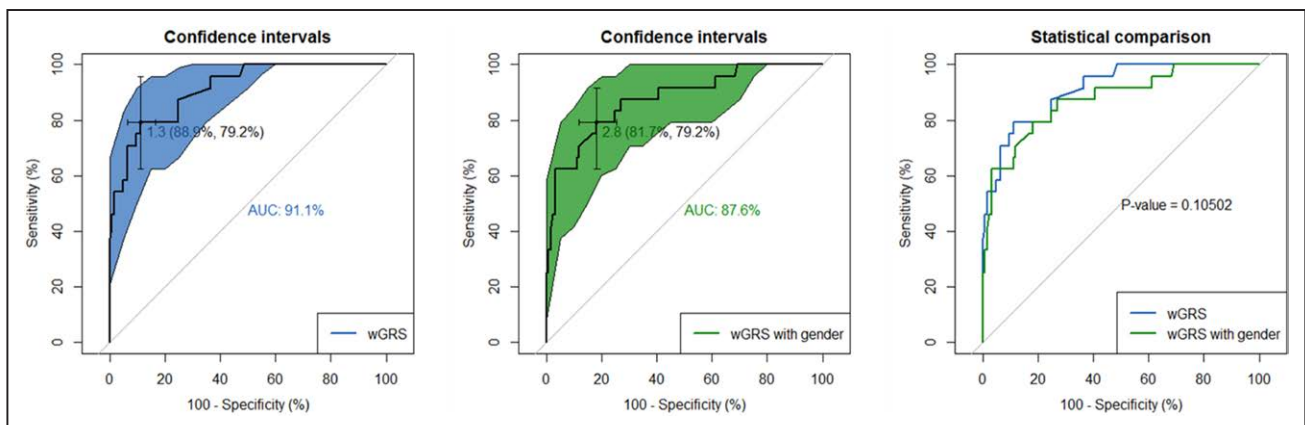


Figure 3. Receiver operating characteristic (ROC) curve analysis of weighted genetic risk score (wGRS). **A**, ROC of wGRS with 1-Specificity as x axis and sensitivity with y axis. The confidence intervals of ROC were calculated by DeLong method. The most predictive wGRS value (1.3) and the corresponding specificity and sensitivity (88.9% and 79.2%, respectively) were shown. **B**, ROC of wGRS in considered the effect of sex was shown. **C**, Comparison between ROC of wGRS and wGRS with sex.

family, *inositol 1,4,5-trisphosphate 3-kinase C (ITPKC)*, *cas-pase-3 (CASP3)*, *CD209*, *CCR2-CCR5*, *CCL3L1*, *interleukin 1 beta (IL-1B)*, and *platelet-activating factor acetylhydrolase*.⁴⁸ However, for each KD patient, the clinical value of single identified risk genetic variants is less informative because of the modest odds ratio. This limitation causes the difficulty to translate the genomics findings into clinical applications.

Chung et al applied wGRS system to categorize breast cancer patients based on their risk of chemotherapy-induced alopecia. Their study demonstrated a potential for clinical use of wGRS.⁴⁹ In this study, we also applied wGRS analysis based on the GWAS data to develop a predictive model for IVIG responsiveness. The advantage of wGRS algorithm is to identify the cumulative effects of multiple SNPs for IVIG drug responsiveness in KD patients. By using wGRS to aggregate the odds ratio of multiple genetic risk variants, the predictive accuracy of IVIG drug responsiveness can be improved largely. Indeed, our study showed the inspiring result that wGRS scoring system can significantly increase the sensitivity and specificity of prediction of IVIG responsiveness (Figure 3A and 3B).

We recognize both strengths and limitations to our study. Because of low prevalence of KD and rare cases of IVIG resistance, the sample size of cohort used in this study was small. In addition, the effect size of each SNP was modest. The wide CIs for the odds ratio suggest that replication of these findings in larger cohorts should be performed.

In short, our study combined with the wGRS algorithm and GWAS analysis (GWAS of 603 698 SNPs). We integrated the additive effects of all 11 risk SNPs from GWAS results to establish a predictive model and to identify a new algorithm model as a rational prediction information for IVIG responsiveness in KD patients.

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Disclosures

None.

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CLINICAL PERSPECTIVE

We have identified candidate single-nucleotide polymorphisms in select genetic loci to predict intravenous immunoglobulin responsiveness in Kawasaki disease patients. We also developed a scoring system that may be implemented in clinical practice to predict outcome of first-line therapy of Kawasaki disease children.

Prediction for Intravenous Immunoglobulin Resistance by Using Weighted Genetic Risk Score Identified From Genome-Wide Association Study in Kawasaki Disease

Ho-Chang Kuo, Henry Sung-Ching Wong, Wei-Pin Chang, Ben-Kuen Chen, Mei-Shin Wu, Kuender D. Yang, Kai-Sheng Hsieh, Yu-Wen Hsu, Shih-Feng Liu, Xiao Liu and Wei-Chiao Chang

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SUPPLEMENTAL MATERIAL

Supplementary Table I.

Chr.	SNP	RA ^a /NRA ^b	RAF ^c	P _{MLMA} ^d	OR ^e	Weight ^f	% of total weight
6	rs9380548	A/T	0.05	1.04×10 ⁻⁰⁶	1.630504	0.488889	13.95%
10	rs7634	A/T	0.246667	1.56×10 ⁻⁰⁶	1.267626	0.237146	6.77%
6	rs742490	G/C	0.073333	2.68×10 ⁻⁰⁶	1.489495	0.398437	11.37%
10	rs1250301	G/A	0.07	3.52×10 ⁻⁰⁶	1.463538	0.380857	10.87%
10	rs2797915	T/C	0.07	3.52×10 ⁻⁰⁶	1.463538	0.380857	10.87%
3	rs4130857	A/G	0.21	5.14×10 ⁻⁰⁶	1.265988	0.235853	6.73%
3	rs4585205	T/C	0.21	5.14×10 ⁻⁰⁶	1.265988	0.235853	6.73%
3	rs4602399	G/T	0.21	5.14×10 ⁻⁰⁶	1.265988	0.235853	6.73%
3	rs4286440	G/T	0.213333	7.09×10 ⁻⁰⁶	1.261098	0.231983	6.62%
2	rs16867137	C/T	0.093333	7.57×10 ⁻⁰⁶	1.374688	0.318227	9.08%
10	rs1250302	G/A	0.073333	8.11×10 ⁻⁰⁶	1.43344	0.360077	10.28%

Chr., chromosome location; ^aRisk allele, ^bNon-risk allele, ^cRisk allele frequency in cases and controls.

^dP_{MLMA} are calculated by the mixed linear model association test. ^eOdds ratio for risk allele. ^fWeight is calculated using natural logarithm transformation of odds ratio.

Supplementary Table II. Weighted genetic risk score (wGRS) analysis.

Category	wGRS ^a	Non-responders/Responders	Fisher Exact <i>P</i> -value ^b	OR (95% CI)	Sensitivity	Specificity
3	≥ 1.681	14/7	8.224×10 ⁻¹⁰	164.33 (8.83-3059.50)	1.000	0.857
2	≥ 0.042 to < 1.681	10/77	0.040	11.52 (0.66-201.42)	1.000	0.353
1	< 0.042	0/42		(Reference)		

^aWeighted genetic risk score. ^bTwo-tail *P*-value was calculated by Fisher's exact test. *P*-value and odds ratio were calculated after Haldane's correction.

Supplementary Table III. Weighted genetic risk score (wGRS) analysis with using tertile as category threshold.

Category	wGRS ^a	Non-responders/Responders	Fisher Exact <i>P</i> -value ^b	OR (95% CI)	Sensitivity	Specificity
3	≥ 1.063858	19/31	8.83×10 ⁻⁷	53.85 (3.14-925.85)	1.000	0.581
2	≥ 0.237146 to < 1.063858	5/52	0.082	9.11 (0.49-169.48)	1.000	0.453
1	< 0.237146	0/43		(Reference)		

^aWeighted genetic risk score. ^bTwo-tail *P*-value was calculated by Fisher's exact test. *P*-value and odds ratio were calculated after Haldane's correction.

Supplementary Table IV. Weighted genetic risk score (wGRS) analysis with using 7 SNPs.

Category	wGRS ^a	Non-responders/Responders	Fisher Exact <i>P</i> -value ^b	OR (95% CI)	Sensitivity	Specificity
4	≥ 1.027	16/6	8.224×10 ⁻¹⁰	220.85 (11.77-4143.31)	1.000	0.878
3	≥ 0.237 to < 1.027	7/62	0.053	10.44 (0.58-187.63)	1.000	0.410
2	≥ 0.050 to < 0.237	1/15	0.074	8.42 (0.33-217.74)	1.000	0.741
1	< 0.050	0/43		(Reference)		

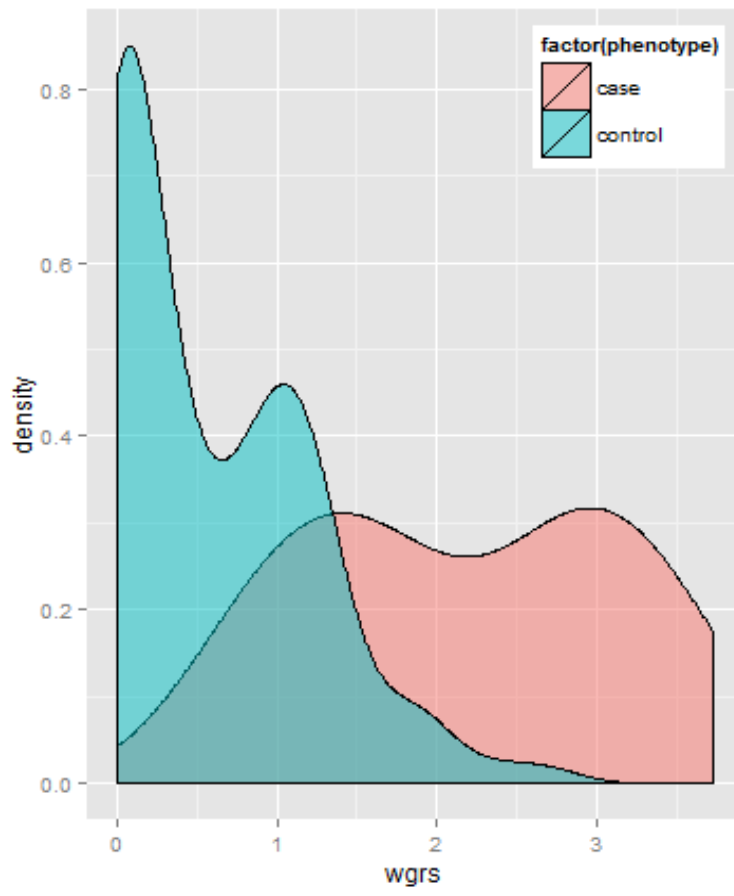
^aWeighted genetic risk score. ^bTwo-tail *P*-value was calculated by Fisher's exact test. *P*-value and odds ratio were calculated after Haldane's correction.

Supplementary Table V. Frequencies of 11 SNPs among different populations.

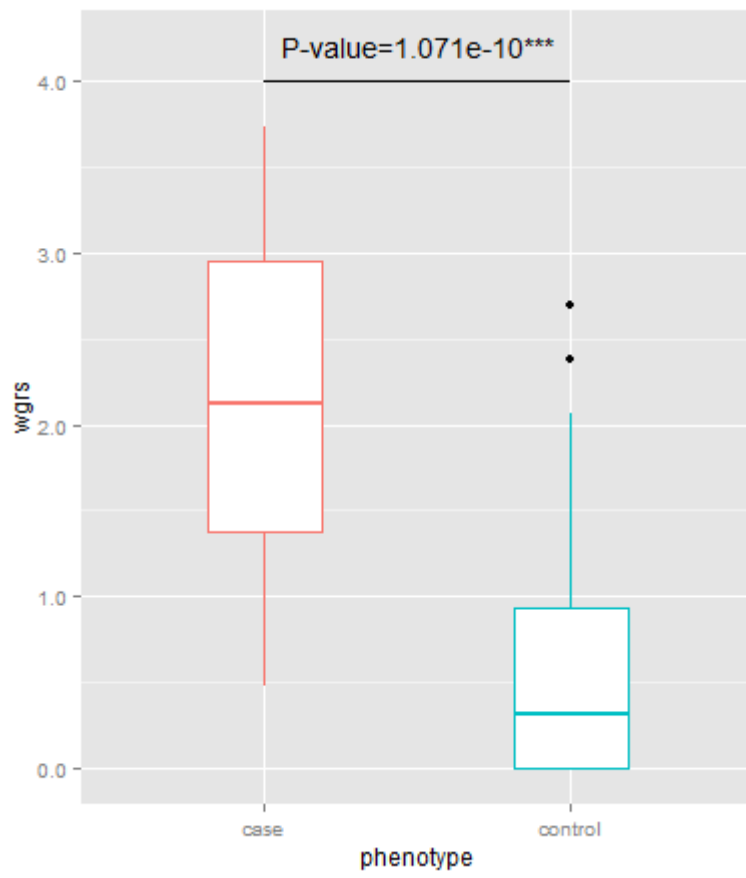
SNP	RA/NRA	RAF (TWB)	RAF (CEU)	RAF (CHB)	RAF (JPT)	RAF (YRI)
rs9380548	A/T	0.05	0	0.07	0.1	0
rs7634	A/T	0.25	0.09	0.21	0.23	0
rs742490	G/C	0.07	0.05	0.01	0.03	0.18
rs1250301	G/A	0.07	0.22	0.02	0.05	0.1
rs2797915	T/C	0.07	0.21	0.03	0.05	0.04
rs4130857	A/G	0.21	0.12	0.37	0.33	0.01
rs4585205	T/C	0.21	0.12	0.37	0.33	0.06
rs4602399	G/T	0.21	0.12	0.36	0.33	0.21
rs4286440	G/T	0.21	0.13	0.35	0.35	0.01
rs16867137	C/T	0.09	0.01	0.09	0.09	0
rs1250302	G/A	0.07	0.22	0.02	0.05	0.17

Supplementary Figure I

(A)



(B)



(C)

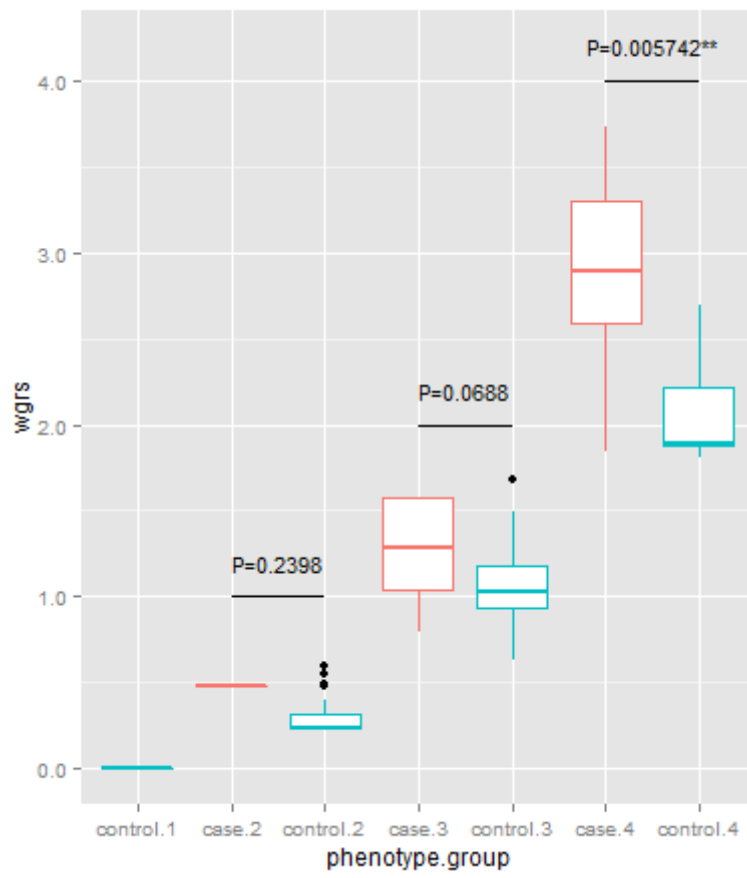


Figure legends:

Figure I: Weighted genetic risk score (wGRS) analysis. (A) Density plot of Kawasaki disease IVIG responder (case) and non-responder (control). A significant deviation from normality has been detected in control group (skewness coefficient=0.9122, 95% CI of skewness coefficient [skew.2SE] skew.2SE=2.1148, kurtosis coefficient=0.2582, 95% CI of kurtosis coefficient [kurt.2SE] kurt.2SE=0.3015, statistic of Shapiro-Wilk test for normality [normtest.W]=0.8628 and its corresponding possibility [normtest.p]= 1.948×10^{-09}), in compared to the case group (skewness coefficient=-0.0285, skew.2SE=-0.0302, kurtosis coefficient=-1.442, kurt.2SE=-0.7853, normtest.W=0.9453 and normtest.p=0.2136), which passed the normality test. A value of skew.2SE and kurt.2SE larger than one indicated a significant difference of skewness and kurtosis than zero (null hypothesis), respectively. (B) P-value of 1.071×10^{-10} showed a significant difference of wGRS between responder (case) and non-responder (control) group. In considering the deviation from normality in control group, Wilcoxon rank-sum test with continuity correction is applied. (C) Comparisons of case and control in four strata of wGRS (Group 1 to Group 4). In Group 4, responder group showed a significantly different wGRS score than non-responder group (p=0.005742). However, wGRS between case and control in strata Group 2 and Group3 showed no significant difference, with p = 0.2398 and p = 0.0688, respectively.

**P-value less than 0.01.

***P-value less than 0.001.