Cardiology in the Young

cambridge.org/cty

Associations of the *HMGB1* rs1412125 and rs2249825 polymorphisms with Kawasaki disease

Yeyi Yang[1], Ting Zhou[2], Yezhen Yang[3] and Zuocheng Yang[4]

[1]Department of Nephropathy and Rheumatology, the Third Xiangya Hospital of Central South University, Changsha, Hunan, P.R. China; [2]Department of Pediatrics, Zhuzhou Central Hospital, Zhuzhou, Hunan, P.R. China; [3]Department of Ophthalmology, the Third Xiangya Hospital of Central South University, Changsha, Hunan, P.R. China and [4]Department of Pediatrics, the Third Xiangya Hospital of Central South University, Changsha, Hunan, P.R. China

Original Article

Cite this article: Yang Y, Zhou T, Yang Y, and Yang Z (2024). Associations of the *HMGB1* rs1412125 and rs2249825 polymorphisms with Kawasaki disease. *Cardiology in the Young*, page 1 of 7. doi: 10.1017/S1047951124026854

Received: 30 May 2024
Revised: 11 September 2024
Accepted: 22 September 2024

Keywords:
Kawasaki disease; gene polymorphism; *HMGB1* gene

Corresponding author:
Zuocheng Yang; Email: yang_zcr@126.com

Abstract

Background: Kawasaki disease is an acute febrile disease causing systemic vasculitis that is common in infants and young children. This study was conducted to explore the relationships of the rs1412125, and rs2249825 single nucleotide polymorphisms of the high mobility group box 1 gene to Kawasaki disease and its complication of coronary artery injury. *Methods:* In total, 200 children with Kawasaki disease (49 with coronary artery injury) and 200 healthy controls were enrolled in this study. Polymerase chain reaction was used to amplify the target gene, and direct sequencing was performed to determine distributions at the rs1412125 T/C and rs2249825 C/G loci in the HMGB1 gene. The chi-squared test was used to compare data between groups. Linkage disequilibrium coefficients and single nucleotide polymorphism haplotype analysis were conducted, and a false-positive report probability analysis was used to assess significant associations. Expression quantitative trait loci analysis was performed to determine if single nucleotide polymorphisms affected mRNA levels via the GTEx portal. *Results:* Significant differences in the genotype TT, TC, and CC distributions ($\chi^2 = 7.918$, $P = 0.019$) and allele T and C frequencies ($\chi^2 = 6.125$, $P = 0.013$) of rs1412125 T/C locus were found between the Kawasaki disease and healthy control groups. The genotype CC was associated with a greater Kawasaki disease risk [odds ratio = 3.205, 95% confidence interval = 1.352–7.595, $\chi^2 = 7.560$, $P = 0.006$]. C allele carriers had a higher Kawasaki disease risk than did T allele carriers (odds ratio = 1.469, 95% confidence interval = 1.083–1.993, $\chi^2 = 6.125$, $P = 0.013$). The rs1412125 genotype T/C distribution ($\chi^2 = 10.906$, $P = 0.004$) and allele frequencies ($\chi^2 = 8.813$, $P = 0.003$) differed significantly between patients with and without coronary artery injury. In the dominant model, the coronary artery injury risk was 3.006 times greater for patients with the TT genotype than for those with the other genotypes (odds ratio = 3.006, 95% confidence interval = 1.540–5.867, $\chi^2 = 10.875$, $P = 0.001$). No significant difference in the rs2249825 genotype C/G distribution or allele frequencies was found between the Kawasaki disease and control groups, or between the coronary artery injury and without coronary artery injury groups. *Conclusions:* The rs1412125 polymorphism of the *HMGB1* gene is associated with Kawasaki disease and its coronary artery injury complication. The CC genotype may be a risk factor for Kawasaki disease onset, and the TT genotype may be a risk factor for coronary artery injury in Kawasaki disease.

Introduction

Kawasaki disease is an acute systemic inflammatory disease that occurs frequently in infants and young children. When left untreated, coronary artery damage occurs in 20–25% of cases. In developed countries, this disease has become the leading cause of acquired heart disease in children. The aetiology of Kawasaki disease is unknown, bacterial and viral infections may be involved in pathogenesis, and severe acute respiratory syndrome coronavirus 2 is believed to be a"priming trigger" that causes the disease.[1] Innate and adaptive immune disorders are also involved in the pathophysiological processes of Kawasaki disease, increasing the risk of cardiovascular abnormalities. In recent years, microRNA has been suggested to play an important role in the development of Kawasaki disease through differential expression and the regulation of immunity, inflammatory responses, and vascular function.[2] In a prospective cohort study conducted in Taiwan, Wei *et al.*[3] observed a potential association between caesarean section and an increased risk of Kawasaki disease development 6–18 months after birth, possibly due to changes in immune system development and the microbiome caused by caesarean section. Kawasaki disease has an ethnicity-associated distribution, and its incidence is higher in males than in females, reflecting a certain degree of genetic susceptibility.

Despite abundant research, the mechanism underlying the pathogenesis of Kawasaki disease has not been fully elucidated.

High mobility group box 1 is a non-histone deoxyribonucleic acid-binding protein that is widely present in mammalian cells and is a late inflammatory mediator. By binding to receptors such as advanced glycation end product receptor and Toll-like receptor, it activates corresponding signal transduction pathways to regulate deoxyribonucleic acid replication and transcription and maintain nucleosome stability.[4] The expression of high mobility group box 1 increased in sepsis, rheumatoid arthritis, systemic lupus erythematosus, ischaemic stroke, cancer, and other diseases, indicating the involvement of the protein in disease pathogenesis.[5] The pathogenesis of Kawasaki disease is related to inflammation and immunity, and high mobility group box 1 may be involved in it.[6] However, only one study has revealed a correlation between *HMGB1* gene polymorphism and Kawasaki disease complicated by coronary artery injury in children,[7] leaving the correlation with Kawasaki disease susceptibility inconclusive. Thus, this study investigates the associations the rs1412125 and rs2249825 polymorphisms of the *HMGB1* gene with genetic susceptibility to Kawasaki disease and coronary artery injury complications.

Materials and method

Subjects

This study was conducted with data from 200 children with Kawasaki disease who were hospitalised in the Department of Pediatrics, Third Xiangya Hospital of Central South University, and 200 healthy children (control group) who underwent physical examination in the hospital's health management centre between 2016 and 2022. It was approved by the Medical Ethics Committee of the Third Xiangya Hospital, Central South University (no. 2016-S155). Written informed consent was obtained from the children's parents or guardians.

The inclusion criteria for subjects with Kawasaki disease were (1) fulfilment of the 2017 American Heart Association's diagnostic criteria for Kawasaki disease[8] with the exclusion of other diseases; (2) coronary artery injury, diagnosed using the American Heart Association's criteria (echocardiography Z score ≥ 2.0);[8] (3) hospitalisation; and (4) receipt of parent's or guardian's informed consent. The exclusion criteria for this group were (1) previous history of cardiovascular disease; (2) septicaemia, scarlet fever, juvenile idiopathic arthritis, exudative erythema multiforme, or other febrile exanthematous diseases; (3) use of glucocorticoids, immunosuppressants, or gamma globulin in the past 2 weeks; and (4) incomplete clinical data.

Specimen collection

Venous blood samples (2 mL) were collected from the participating children into disposable blood collection vessels containing anticoagulant (EDTA-Na_2) and left for 30 min. After natural coagulation, the blood was centrifuged at 2500rpm for 15 min, the supernatant was discarded, the sediment was retained, and the samples were transferred to frozen storage tubes and stored at −80°C. Deoxyribonucleic acid was extracted within 1 year of sample collection using a genomic deoxyribonucleic acid extraction kit (Promega, USA) according to the manufacturer's instructions.

Single nucleotide polymorphism selection

Single nucleotide polymorphisms with minor allele frequencies >0.05 in the Chinese Han population, according to the Ensembl database (https://www.ensembl.org/index.html), that had been reported to be potential functional susceptibility sites regulating gene function or expression were selected. According to these criteria, we selected the single nucleotide polymorphisms rs1412125 (−1615T/C; genomic number 31,041,595), representative of the gene promoter region, and rs2249825 (3814C/G; genomic number 31,037,903), located in intron 1 of the *HMGB1* gene, to elucidate the possible susceptibility of the single nucleotide polymorphism on this gene.[9,10]

Primer sequence design and verification

Primers were designed using Primer Premier 5.0 software,[11] and the complete *HMGB1* sequence was retrieved from GenBank. The primers were synthesised by Hunan Qingke Biological Co., Ltd Their sequences were rs1412125 T/C, forward 5′-CCAA CAACCAATTCCTCCAA-3′ and reverse 5′-GATGCCACTGA AAGTATCTTAA-3′ (polymerase chain reaction product fragment length = 500 bp); and rs2249825 C/G, forward 5′-TTCTT ATGCTCCTCCCGAC-3′ and reverse 5′-CTGACCTTTGGTT TGGT TG-3′ (polymerase chain reaction product fragment length = 463 bp). The polymerase chain reaction system components were 1.0 μL deoxyribonucleic acid template, 12.5 μL 2× Taq Plus Master Mix II, 1.0 μL upstream primer, 1.0 μL downstream primer, and 9.0 μL double-distilled water (total reaction volume = 24.5 μL). Polymerase chain reaction was performed with pre-denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 20 s, and extension at 72°C for 30 s; and terminal extension at 72°C for 10 min. The samples were then stored at 4°C.

Gene sequencing

The target gene sequence was determined by direct sequencing.[12] Target gene fragments were detected and sent to Biosune Biotechnology Co., Ltd (Shanghai, China) for sequencing, and the results were analysed using SnapGene software (Chromas 2.6.5).

Statistical analysis

The HMGB1 single nucleotide polymorphism genotypes and allele frequencies in the Kawasaki disease and healthy control groups were determined by direct counting. The consistency of the genotype frequencies with Hardy–Weinberg equilibrium was examined. SPSS22.0 software was used for statistical analysis, and the χ^2 test and Fisher's exact test were used to compare data between groups. Odds ratios and 95% confidence intervals were calculated. P values < 0.05 were considered to be significant. The calculation of linkage disequilibrium coefficients (D′) and single nucleotide polymorphism haplotype analysis were performed using default settings on the SHEsis website (http://analysis.bio-x.cn/myAnalysis.php). A false-positive report probability analysis was performed to evaluate significant associations, as described elsewhere.[13] Expression quantitative trait loci were analysed to explore whether the single nucleotide polymorphisms caused mRNA level changes using the GTEx portal (http://www.gtexportal.org/home/).

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press

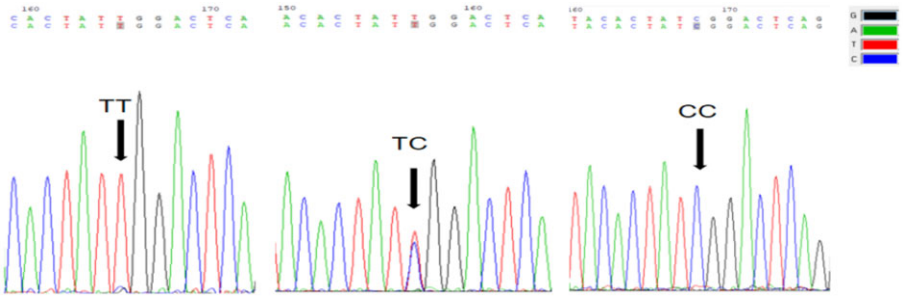

Figure 1. Sequence map of locus rs1412125 T/C of HMGB1 gene.

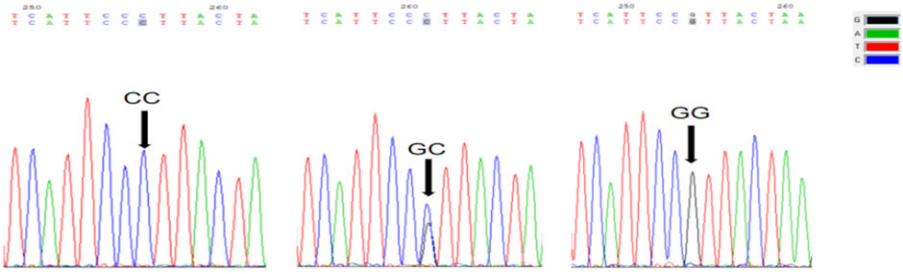

Figure 2. Sequence map of locus rs2249825 C/G of HMGB1 gene.

Results

Participant characteristics

The Kawasaki disease group comprised 123 males and 77 females with an average age of 2.53 ± 2.12 years. Children with Kawasaki disease were divided into coronary artery injury and no coronary artery injury groups. Forty-nine children were allocated to the coronary artery injury group and 151 were allocated to the without coronary artery injury group. The control group comprised 118 males and 82 females with an average age of 2.15 ± 2.09 years. The age and sex distribution did not differ between the Kawasaki disease and healthy control groups.

HMGB1 single nucleotide polymorphism genotype detection

The rs1412125 T/C locus of the *HMGB1* gene was determined to have TT, TC, and CC genotypes, and the rs2248825 C/G locus was determined to have GG, GC, and CC genotypes (Figures 1 and 2).

Hardy–Weinberg equilibrium

The single nucleotide polymorphism rs1412125 T/C was in Hardy–Weinberg equilibrium in the Kawasaki disease ($\chi^2 = 0.3173$, $P = 0.5732$) and healthy control ($\chi^2 = 3.7862$, $P = 0.0517$) groups. rs2248825 C/G was also in equilibrium (Kawasaki disease, $\chi^2 = 0.1270$, $P = 0.7216$; healthy control, $\chi^2 = 1.0923$, $P = 0.2960$). Thus, the samples were representative of the Chinese Han population.

rs1412125 T/C genotype and allele frequencies

Significant differences in the distribution of the genotypes TT, TC, and CC ($\chi^2 = 7.918$, $P = 0.019$) and the frequencies of the alleles T and C ($\chi^2 = 6.125$, $P = 0.013$) of rs1412125 T/C were detected between the Kawasaki disease and healthy control groups (Table 1). Greater risks of Kawasaki disease development were associated with the genotype CC (vs. TT and TC; odds ratio = 3.205, 95% confidence interval = 1.352–7.595, $\chi^2 = 7.560$, $P = 0.006$) and the allele C (vs. T; odds ratio = 1.469, 95% confidence interval = 1.083–1.993, $\chi^2 = 6.125$, $P = 0.013$). In a recessive model (CC vs. TT + TC), the risk of Kawasaki disease was 2.816 times greater for the genotype CC than for the other genotypes (odds ratio = 2.816, 95% confidence interval = 1.216–6.518, $\chi^2 = 6.283$, $P = 0.012$; Table 1). The distribution of the genotype TC ($\chi^2 = 10.906$, $P = 0.004$) and frequencies of the alleles C ($\chi^2 = 8.813$, $P = 0.003$) of rs1412125 differed significantly between the coronary artery injury and without coronary artery injury groups. In the dominant model (TT vs. CC + TC), the risk of coronary artery injury was 3.006 times greater for the genotype TT than for the other genotypes (odds ratio = 3.006, 95% confidence interval = 1.540–5.867, $\chi^2 = 10.875$, $P = 0.001$; Table 2).

rs2248825 C/G genotype and allele frequencies

The distribution of the genotypes GG, GC, and CC and frequencies of the alleles G and C of rs2248825 C/G did not differ between the Kawasaki disease and control groups ($\chi^2 = 0.320$, $P = 0.852$ and $\chi^2 = 0.038$, $P = 0.845$, respectively) or between the coronary artery injury and without coronary artery injury groups ($\chi^2 = 1.242$, $P = 0.538$ and $\chi^2 = 0.907$, $P = 0.341$, respectively; Tables 1 and 2).

Linkage disequilibrium and haplotypes

The D' and r^2 values for rs1412125 and rs2249825 were 1.000 and 0.433, respectively (Figure 3). The r^2 value reflects weak linkage imbalance. TC was the main haplotype of rs1412125 and rs2249825 in the Kawasaki disease and healthy control groups. The risk of Kawasaki disease was 2.043 times greater in children with the haplotype CC than in those with the other haplotypes (95% confidence interval = 1.352–3.087, $P < 0.05$) and 0.681 times lesser in children with the haplotype TC than in those with the other haplotypes (95% confidence interval = 0.502–0.924, $P < 0.05$; Table 3).

Expression quantitative trait loci analysis

Samples with the rs1412125 genotype C had significantly higher *HMGB1* mRNA levels in the tissues of the cerebral hemisphere, oesophageal mucosa, and terminal ileum than did samples with the

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press

Table 1. Genotype and allele frequencies of the polymorphic sites rs1412125 and rs2249825 in the *HMGB1* gene for the Kawasaki disease and control groups

Alleles/Genotypes	Healthy group (%)	KD group (%)	*P*	OR (95% CI)	*P*
rs1412125					
T	297 (74.25)	265 (66.25)	**0.013**	1.000	–
C	103 (25.75)	135 (33.75)		1.469 (1.083–1.993)	**0.013**
TT	105 (52.50)	86 (43.00)	**0.019**	1.000	–
TC	87 (43.50)	93 (46.50)		1.305 (0.868–1.963)	0.201
CC	8 (4.00)	21 (10.50)		3.205 (1.352–7.595)	**0.006**
Dominant (CC + TC)/TT	95/105	114/86	0.057	0.683 (0.460–1.012)	0.057
Recessive (TT + TC)/CC	192/8	179/21	**0.012**	2.816 (1.216–6.518)	**0.012**
rs2249825					
C	337(84.25)	339 (84.75)	0.845	1.000	–
G	63 (15.75)	61 (15.25)		0.963 (0.656–1.412)	0.845
CC	140 (70.00)	143 (71.50)	0.852	1.000	–
GC	57 (28.50)	53 (26.50)		0.910 (0.586–1.414)	0.676
GG	3 (1.50)	4 (2.00)		1.305 (0.287–5.939)	0.729
Dominant (GG + GC)/CC	60/140	57/143	0.741	1.075 (0.699–1.654)	0.741
Recessive (CC + GC)/GG	197/3	196/4	0.703	1.340 (0.296–6.066)	0.703

KD = Kawasaki disease; OR = odds ratio; CI = confidence interval.

Table 2. rs1412125 and rs2249825 polymorphisms of *HMGB1* gene and risk of coronary artery injury

Alleles/ Genotypes	Without coronary artery injury group (%)	Coronary artery injury group (%)	P	OR (95% CI)	P
rs1412125					
T	188 (62.25)	77 (78.57)	**0.003**	1.000	–
C	114 (37.75)	21 (21.43)		0.450 (0.263–0.768)	**0.003**
TT	55 (36.42)	31(63.27)	**0.004**	1.000	–
TC	78 (51.66)	15(30.61)		0.341 (0.168–0.692)	**0.002**
CC	18 (11.92)	3 (6.12)		0.296 (0.081–1.084)	0.055
Dominant (CC + TC)/TT	96/55	18/31	**0.001**	3.006 (1.540–5.867)	**0.001**
Recessive (TT + TC)/CC	133/18	46/3	0.250	0.482 (0.136–1.712)	0.250
rs2249825					
C	253 (83.77)	86 (87.76)	0.341	1.000	–
G	49 (16.23)	12 (12.24)		0.720 (0.366–1.418)	0.341
CC	105 (69.54)	38 (77.55)	0.538	1.000	–
GC	43 (28.48)	10 (20.41)		0.643 (0.294–1.404)	0.265
GG	3 (1.99)	1 (2.04)		0.921 (0.093–9.127)	0.944
Dominant (GG + GC)/CC	46/105	11/38	0.280	1.513 (0.711–3.221)	0.280
Recessive (CC + GC)/GG	148/3	48/1	0.975	1.028 (0.104–10.114)	0.975

OR = odds ratio; CI = confidence interval.

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press

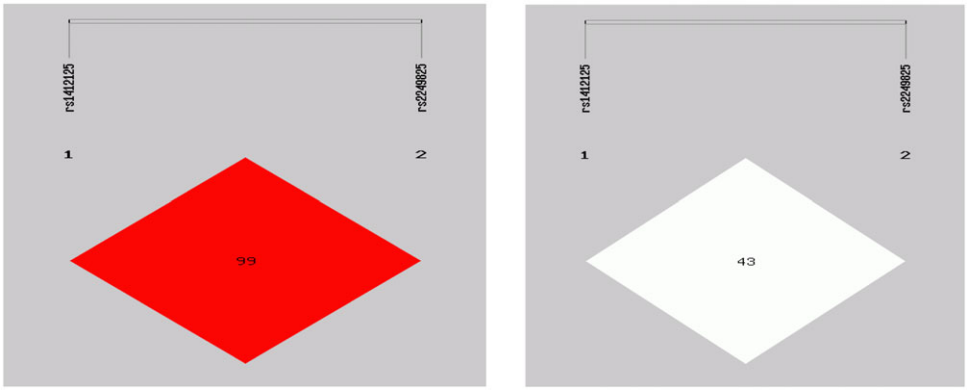

Figure 3. Detection of linkage disequilibrium at HMGB1 polymorphisms.

rs1412125 genotype T. In contrast, samples with the rs1412125 genotype T had higher *HMGB1* mRNA levels in the tissues of the pituitary gland and spleen than did samples with the rs1412125 C genotype. No significant difference between these genotypes was detected for the coronary artery (Figure 4). These results may be explained by the lack of change in mRNA expression in normal tissues, but changes in this expression due to immune inflammation with Kawasaki disease onset.

False-positive report probability analysis

We preset 0.2 as the false-positive report probability analysis threshold. At the prior probability of 0.1, all significant findings for the *HMGB1* single nucleotide polymorphism rs1412125 T/C remained noteworthy (Table 4).

Discussion

The human gene *HMGB1* is located at 13q12.3; it has a length of 9006 bp and contains a promoter of at least 1700-bp length and about five exons (2600 bp). The high mobility group box 1 protein encoded by the gene belongs to the high mobility group protein superfamily; is a non-histone deoxyribonucleic acid-binding protein that regulates gene transcription in cells. This single-stranded polypeptide consists of 215 amino-acid residues with two deoxyribonucleic acid-binding regions, the A and B boxes, and a long negatively charged acidic (C) terminus. The B box is composed of amino-acid residues 1–85 and is the main region of cytokine activity. Its initial 40 peptides induce the production of tumour necrosis factor-α, interleukin-6, and other cytokines, causing an inflammatory response. The A box is composed of amino-acid residues 88–162 and has an antagonistic effect on the inflammatory response. The C terminus contains multiple aspartate and glutamic-acid residues, which combine with advanced glycation end product receptor to exert effects.[14] rs1412125 (−1615T/C) and rs2249825 (3814 C/G), examined in this study, are located in the promotor region and intron 1, respectively.

High mobility group box 1 is a proinflammatory cytokine distributed widely in the nucleus and cytoplasm that participates in nucleosome structure maintenance and gene transcription regulation. When immune cells are activated by external stimulation, high mobility group box 1 is released into the extracellular system to activate vascular endothelial cells and neutrophils by activating nuclear factor-κB, which results in the release of TNF-α, IL-1β, and other proinflammatory cytokines.[15] Thus, high mobility group box 1 plays important roles in the pathogenesis of many diseases, such as atherosclerosis, coronary heart disease, ischaemia-reperfusion injury, rheumatoid arthritis, systemic lupus erythematosus, Henoch-Schönlein purpura, and ulcerative colitis.[15] *HMGB1* polymorphisms are associated with a variety of diseases. rs1412125 has domain homology with Drosophila CUT, a transcription suppressor whose mutation leads to the loss of inhibitory function and overexpression of high mobility group box 1, resulting in enhancer effects.[16] rs2249825 may also provide transcriptional control in the regulation of high mobility group box 1 expression, and its allele G may create a new binding site for the v-myb oncogene and enhance high mobility group box 1 expression.[10]

Mutations in the promoter region and intron 1 may silence or enhance transcription for the regulation of gene expression.[10] Batnozic Varga *et al.*[16] found that the *HMGB1* polymorphisms rs41369348, rs1045411, rs2249825, and rs1412125 were associated with the occurrence of generalised purpura rash, and that rs1412125 was associated with immunoglobulin a vasculitis nephritis. Song *et al.*[17] reported that the 30-day mortality rate of patients with community-acquired pneumonia who had rs1412125 and rs2249825 mutations was significantly higher than that of their counterparts with wild-type alleles, suggesting that these single nucleotide polymorphisms are associated with pneumonia susceptibility, severity, and inflammation. Qiu *et al.*[18] found that people with the allele C of rs2249825 had higher high mobility group box 1 expression levels and were more likely to develop sepsis, suggesting that this allele confers a sepsis risk. Wang *et al.*[9] found that the rs2249825 polymorphism of the *HMGB1* gene was associated with the pathogenesis of rheumatoid arthritis, and that its allele G was protective. Qu *et al.*[19] found that patients with the genotype CG + GG were more likely to develop postoperative atrial fibrillation after coronary artery bypass grafting, but that HMBG1 expression was higher in patients with the genotype CG + GG, indicating that the allele G of rs2249825 is a risk factor for this condition.

Kawasaki disease is a common paediatric vascular inflammatory disease. It causes severe intravascular inflammation and endothelial damage, leading to serious complications such as coronary artery injury. During bacterial infection or Kawasaki disease, high-intensity inflammatory mediators are stimulated and high mobility group box 1 is released as a result of tissue injury. Qian *et al.*[20] found that the serum high mobility group box 1 level in children with Kawasaki disease increased in the acute stage of

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press

Table 3. Haplotype analysis of HMGB1 gene loci rs1412125 and rs2249825

Haplotype	Control Group n(%)	KD Group n(%)	χ^2	P	OR (95%CI)
CC	40.0 (10.0)	74.0 (18.5)	11.825	<0.05	2.043 (1.352–3.087)
CG	63.0 (15.7)	61.0 (15.2)	0.038	>0.05	0.963 (0.656–1.412)
TC	297.0 (74.2)	265.0 (66.2)	6.125	<0.05	0.681 (0.502 ~ 0.924)

KD = Kawasaki disease; OR = odds ratio; CI = confidence interval.

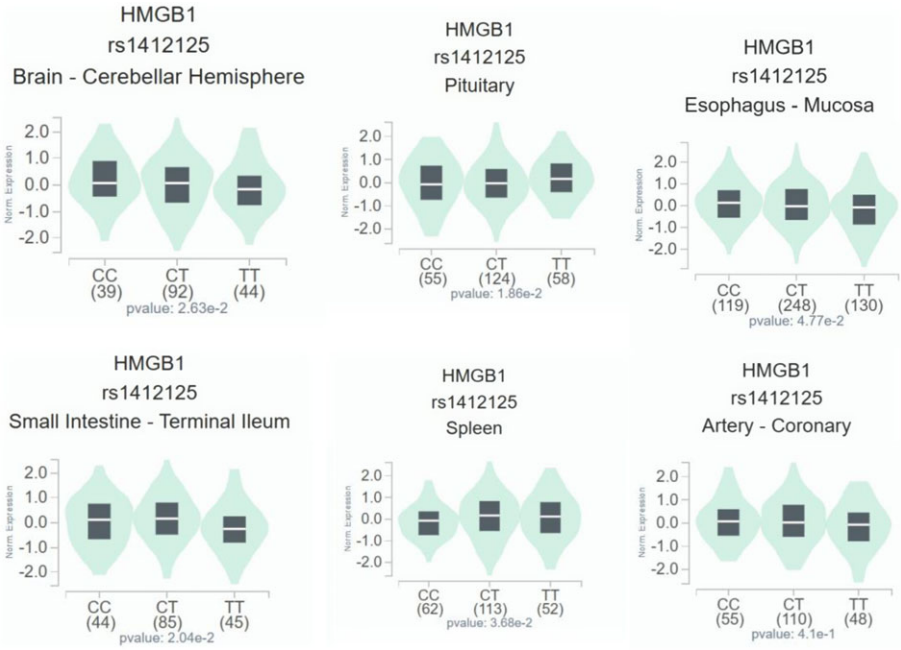

Figure 4. Functional implication of HMGB1 gene rs1412125 polymorphisms based on the public database GTEx portal. The genotype of rs1412125 and expressions of HMGB1 in different tissues.

Table 4. False-positive report probability analysis for the significant findings from HMGB1 gene

				Prior probability				
Genotype	OR (95% CI)	P^a	Statistical power[b]	0.25	0.10	0.01	0.001	0.0001
rs1412125 T/C								
T versus C	1.469 (1.083–1.993)	**0.013**	0.087	0.001	0.003	0.028	0.225	0.744
TT versus CC	3.205 (1.352–7.595)	**0.006**	0.141	0.001	0.002	0.019	0.162	0.658
TT/TC versus CC	2.816 (1.216–6.518)	**0.012**	0.125	0.001	0.003	0.032	0.249	0.768
coronary artery injury								
T versus C	0.450 (0.263–0.768)	**0.003**	0.148	0.000	0.001	0.010	0.095	0.512
TC versus TT	0.341 (0.168–0.692)	**0.002**	0.234	0.000	0.001	0.009	0.086	0.486
CC/TC versus TT	3.006 (1.540–5.867)	**0.001**	0.233	0.000	0.001	0.006	0.056	0.373

OR = odds ratio; CI = confidence interval.
[a]χ^2 test was used to calculate the genotype frequency distributions.
[b]Statistical power was calculated using the number of observations in the subgroup and the OR and *P* values in this table.

the disease and then decreased gradually in the subacute and recovery stages and was significantly higher in children with coronary artery injury than in those without. Eguchi *et al.*[6] examined the serum high mobility group box 1 levels of 36 patients with Kawasaki syndrome and found that these levels were significantly increased in the intravenous immunoglobulin-resistant group. Namba *et al.*[21] found that the ratio of high mobility group box 1 to histidine-rich glycoprotein was significantly higher in intravenous immunoglobulin-resistant than in intravenous immunoglobulin-sensitive patients in the subacute

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press

phase of Kawasaki disease. In the intravenous immunoglobulin-sensitive group, the high mobility group box 1 level was significantly lower in the subacute phase than that in the acute phase.[21] These results suggest that high mobility group box 1 is involved in the pathogenesis of Kawasaki disease and may be a useful tool for assessing the severity of this disease, as well as a potential marker of poor response to intravenous immunoglobulin treatment.

Ahn *et al.*[7] reported that the *HMGB1* rs117077167 C/A and rs1412125 A/G polymorphisms were not associated with susceptibility to Kawasaki disease in the Korean population. In their recessive model (GG vs. AA + GA) of rs1412125, however, the risk of Kawasaki disease with coronary artery injury was 4.98 times greater and that of intravenous immunoglobulin resistance was 4.11 times greater in patients with the genotype GG than in those with the genotypes AA and GA.[7] In the present study, the rs1412125 genotype distribution and allele frequencies differed significantly between patients with Kawasaki disease and healthy controls. The risk of Kawasaki disease was 2.816 times greater for patients with the genotype CC than for those with the genotypes TT and TC, and 1.469 times greater for patients with the allele C than for those with the allele T. These results indicate that the genotype CC and allele C of rs1412125 are Kawasaki disease risk factors. Moreover, the genotype and allele C and T frequencies differed significantly between the coronary artery injury and without coronary artery injury groups. In the dominant model, the risk of coronary artery injury was 3.006 times greater for patients with the genotype TT than for those with the genotypes CC and TC, suggesting that this genotype is a coronary artery injury risk factor. In contrast, the rs2249825 genotype distribution and allele frequencies did not differ between the Kawasaki disease and healthy control groups, and this polymorphism was not associated with coronary artery injury. Differences between these results and those reported by Ahn *et al.*[7] may be related to the ethnic difference in the study populations. A multicentre study conducted with a larger sample may provide more complete information about the role and mechanism of the *HMGB1* polymorphism in Kawasaki disease.

Acknowledgements. We are very grateful for Shan Tan, MPH, for her contribution to the statistics of this article.

Financial support. None.

Competing interests. None.

References

1. Ünlü AM, Holm M, Krusenstjerna-Hafstrøm T, et al. Changes in Kawasaki disease incidence and phenotype during the COVID-19 pandemic. Dan Med J 2023; 70 *(6)*: A10220600.
2. Xiong Y, Xu J, Zhang D, et al. MicroRNAs in Kawasaki disease: An update on diagnosis, therapy and monitoring. Front Immunol 2022; 13: 1016575.
3. Wei CF, Chen MH, Lin CC, et al. Association between Cesarean section delivery and increased risk of childhood Kawasaki disease. J Formos Med Assoc 2024; 123 *(8)*: 843–848.
4. Ren W, Zhao L, Sun Y, et al. HMGB1 and toll-like receptors: potential therapeutic targets in autoimmune diseases. Mol Med 2023; 29 *(1)*: 117.
5. Tang D, Kang R, Zeh HJ, et al. The multifunctional protein HMGB1: 50 years of discovery. Nat Rev Immunol 2023; 23 *(12)*: 824–841.
6. Eguchi T, Nomura Y, Hashiguchi T, et al. An elevated value of high mobility group box 1 is a potential marker for poor response to high-dose intravenous immunoglobulin treatment in patients with Kawasaki syndrome. Pediatr Infect Dis J 2009; 28 *(4)*: 339–334.
7. Ahn JG, Bae Y, Shin D, et al. HMGB1 gene polymorphism is associated with coronary artery lesions and intravenous immunoglobulin resistance in Kawasaki disease. Rheumatology (Oxford) 2019; 58 *(5)*: 770–775.
8. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American heart association. Circulation 2017; 135 *(17)*: e927–e999.
9. Wang LH, Wu MH, Chen PC, et al. Prognostic significance of high-mobility group box protein 1 genetic polymorphisms in rheumatoid arthritis disease outcome. Int J Med Sci 2017; 14 *(13)*: 1382–1388.
10. Wang Y, Li XP, Yin JY, et al. Association of HMGB1 and HMGB2 genetic polymorphisms with lung cancer chemotherapy response. Clin Exp Pharmacol Physiol 2014; 41 *(6)*: 408–415.
11. Zha L, Li S, Liu X, et al. Association of miR-146a gene polymorphism at loci rs2910164 G/C, rs57095329 A/G, and rs6864584 T/C with susceptibility to Kawasaki disease in Chinese children. Pediatr Cardiol 2019; 40 *(3)*: 504–512.
12. Yao M, He Q, Yang M, et al. Association of miR-181c/d gene locus rs8108402 C/T polymorphism with susceptibility to Kawasaki disease in Chinese children. Front Pediatr 2022; 10: 89977.
13. Zhuo Z, Miao L, Hua W, et al. Genetic variations in nucleotide excision repair pathway genes and hepatoblastoma susceptibility. Int J Cancer 2021; 149 *(9)*: 1649–1658.
14. Tripathi A, Shrinet K, Kumar A. HMGB1 protein as a novel target for cancer. Toxicol Rep 2019; 6: 253–261.
15. Yang H, Wang H, Andersson U. Targeting inflammation driven by HMGB1. Front Immunol 2020; 11: 484.
16. Batnozic Varga M, Held M, Wagner J, et al. The association of HMGB1 and RAGE gene polymorphisms with IgA vasculitis. Biochem Genet 2024; 62 *(3)*: 2268–2278.
17. Song W, Tan H, Wang S, Zhang Y, Ding Y. Association of high mobility group box protein B1 gene polymorphisms with pneumonia susceptibility and severity. Genet Test Mol Biomarkers 2019; 23 *(1)*: 3–11.
18. Qiu P, Wang L, Ni J, et al. Associations between HMGB1 gene polymorphisms and susceptibility and clinical outcomes in Chinese Han sepsis patients. Gene 2019; 687: 23–29.
19. Qu C, Wang XW, Huang C, et al. High mobility group box 1 gene polymorphism is associated with the risk of postoperative atrial fibrillation after coronary artery bypass surgery. J Cardiothorac Surg 2015; 10 *(1)*: 88.
20. Qian B, Huang H, Cheng M, et al. Mechanism of HMGB1-RAGE in Kawasaki disease with coronary artery injury. Eur J Med Res 2020; 25 *(1)*: 8.
21. Namba T, Yashiro M, Fujii Y, et al. Decreased levels of histidine-rich glycoprotein and increased levels of high-mobility group box 1 are risk factors for refractory Kawasaki disease. Mod Rheumatol 2023; 33 *(3)*: 599–607.

https://doi.org/10.1017/S1047951124026854 Published online by Cambridge University Press