High mobility group box 1 gene polymorphism in Kawasaki disease

Jong Gyun Ahn

Department of Medicine

The Graduate School, Yonsei University

High mobility group box 1 gene polymorphism in Kawasaki disease

Directed by Professor Dong Soo Kim

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Jong Gyun Ahn

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This certifies that the Doctoral Dissertation of Jong Gyun Ahn is approved.

Thesis Supervisor: Dong Soo Kim

Thesis Committee Member#1: Jeon-Soo Shin

Thesis Committee Member#2: Hyon-Suk Kim

Thesis Committee Member#3: Jun Yong Choi

Thesis Committee Member#4: Dongjik Shin

The Graduate School
Yonsei University

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ABSTRACT

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The Graduate School, Yonsei University

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Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology that affects infants and young children. High mobility group box 1 (*HMGB1*) is a damage-associated molecular pattern (DAMP) molecule that is implicated in the pathogenesis of many inflammatory diseases. Recent reports of elevated serum *HMGB1* level during the acute phase of KD and its relationship to poor-response to intravenous immunoglobulin (IVIG) treatment suggest a possible association of *HMGB1* polymorphisms with KD. Whole genome sequencing of the *HMGB1* gene was performed to identify causative variants. Two tagging single

nucleotide polymorphisms (SNPs) of the *HMGB1* gene (rs1412125 and rs117077167) were selected using linkage disequilibrium analysis. The tagging SNPs were genotyped using the TaqMan Allelic Discrimination assay in a total of 468 subjects (265 KD patients and 203 controls). Susceptibility, coronary artery lesions (CAL), and IVIG treatment response of KD were then analyzed with *HMBG1* genetic variations. The *HMGB1* SNPs were not associated with KD susceptibility. However, in KD patients, there was a significant association of rs1412125 with CAL formation in the recessive model (GG vs. AA + GA: OR = 4.98, 95% CI = 1.69–14.66, P = 0.005). In addition, rs1412125 was associated with IVIG resistance in the recessive (GG vs. AA + GA: OR = 4.11, 95% CI = 1.38–12.23, P = 0.017) and allelic models (G vs. A: OR = 1.80, 95% CI = 1.06–3.06, P = 0.027). The rs1412125 in *HMGB1* might be a relevant risk factor for the development of CAL and IVIG resistance in KD patients.

Key words: Kawasaki disease; *HMGB1*; SNP; coronary artery lesion; IVIG resistance

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I. INTRODUCTION

Kawasaki disease (KD) is an acute febrile vasculitis of childhood with the classic complication of coronary artery lesions (CAL). The etiology of KD remains unknown, although it is thought that symptoms of KD are related to hyperactivation of the immune system triggered by infection with microorganisms in patients with genetic susceptibility.[1]

High mobility group box 1 (*HMGB1*) is a highly conserved, non-histone chromosomal protein that plays a role as a DNA chaperone regulating DNA replication, V(D)J recombination, transcription, DNA repair, and the stabilization of nucleosome formation by DNA binding and bending activities.[2] In response to injurious or infectious stimuli, *HMGB1* also acts as a damage-associated molecular pattern (DAMP) to induce inflammation, proliferation and migration of immune cells.[2,3] With the recent numerous studies on *HMGB1* and its role as a DAMP molecule in conjunction with other inflammatory mediators, *HMGB1* has been implicated as a critical molecular

target in inflammatory mechanisms in the pathogenesis of many autoimmune and inflammatory diseases.[4] Two recent studies have reported an association between serum *HMGB1* levels and KD. Hoshina et al. reported that children in the early acute phase of KD showed the highest levels of *HMGB1*, and levels decreased significantly during the late acute phase and convalescent phase of the disease.[5] In the study of Eguchi et al., serum *HMGB1* levels were significantly higher in poor-responders to IVIG than good-responders to IVIG.[6] The evidence suggests that *HMGB1* may play an important role in the pathogenesis of KD. So far, there are no data on the association between single nucleotide polymorphisms (SNPs) of *HMGB1* and clinical outcomes such as development of CAL in KD patients. We hypothesized that SNPs of *HMGB1* could influence clinical outcomes, including CAL formation and response to IVIG treatment, in patients with KD.

II. MATERIALS AND METHODS

1. Study subjects

KD patients were recruited from the Department of Pediatrics at Severance Children's Hospital, Seoul, Korea from 2012 to 2015. Diagnosis of KD was made by pediatricians according to the criteria of the Japanese Kawasaki Disease Research Committee.[7] Incomplete or atypical KD cases were excluded. Control group consisted of healthy children without history of KD (Figure 1), who had visited outpatient clinic of the hospital for their symptoms other than

KD. CAL was defined by echocardiography as aneurysm proposed by the Japanese Ministry of Health Criteria or dilation (Z-score > 2.5), according to American Heart Association (AHA) criteria.[8,9] IVIG resistance was defined as the presence of persistent or recurrent fever (≥ 38°C) at ≥ 36 hours after IVIG treatment completion.[8]

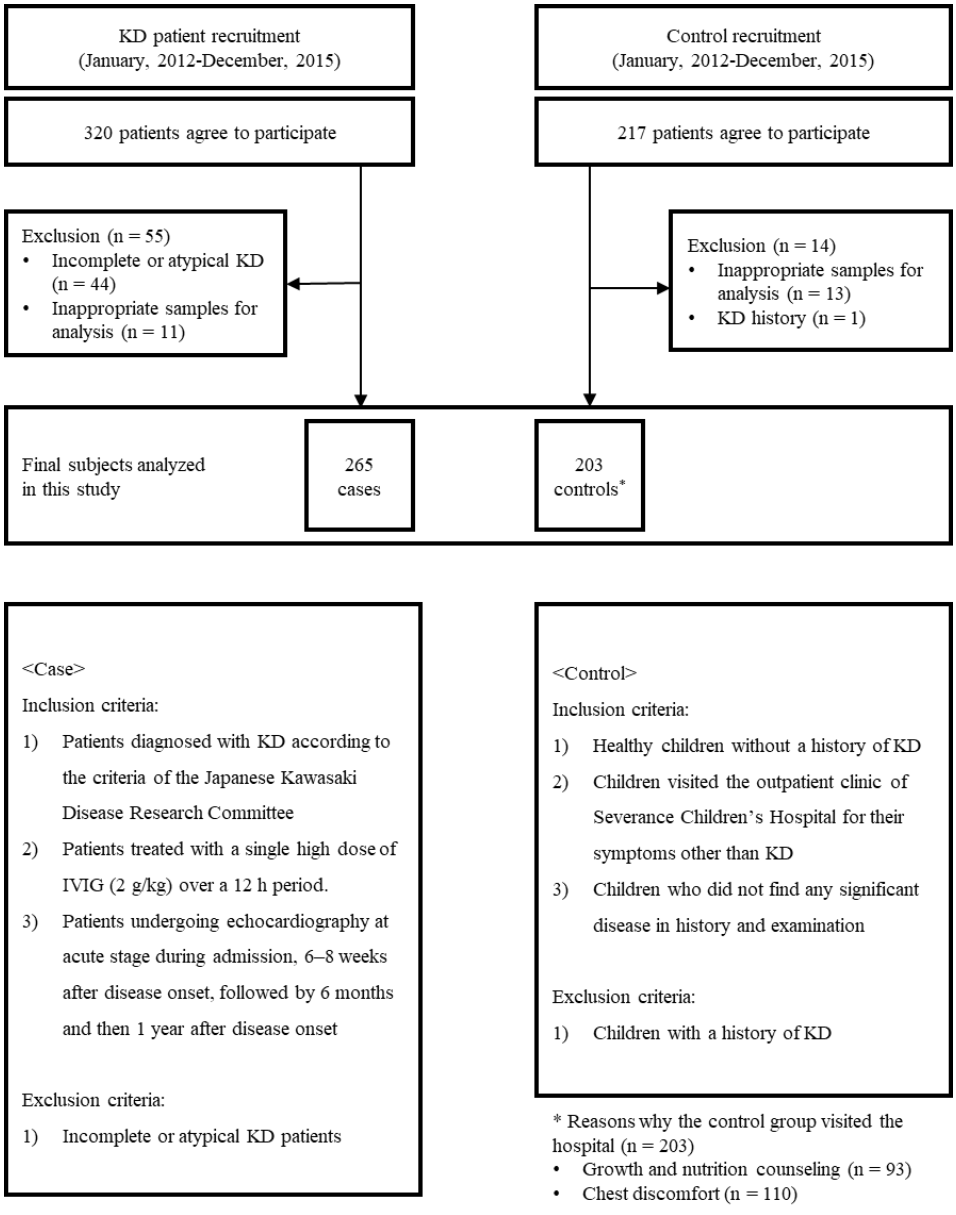

Figure 1. Flow chart for selection of study subjects

2. Discovery of variations and tag SNP selection

Genomic DNA was extracted from blood cells using a QIAmp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The quantity of DNA was measured using an Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA).

For causative SNP discovery, we sequenced the *HMGB1* gene in 24 subjects (12 KD and 12 controls). Genomic sequencing data for the *HMGB1* gene were obtained from the GenBank database (http://www.ncbi.nlm.nih.gov/). Polymerase chain reaction (PCR) primers that amplified the exon and promoter regions of the gene were designed using Primer[3] software (http://carbon.bineer.co.kr/primer3plus). PCR reaction mixtures consisted of 2.5 mM $MgCl_2$, 10× reaction buffer, 2.5 mM of each dNTP, 0.5 pM of each primer, 0.25 U of Taq DNA polymerase (SolgTM) and 10 ng of genomic DNA in a 30 $\mu\ell$ reaction volume. Amplification conditions for all reactions were as follows: initial denaturation at 96℃ for 5 min; 35 cycles at 96℃ for 30 s, 62℃ for 30 s and 72℃ for 60 s; and a final extension cycle of 72℃ for 10 min. PCR products were purified by treatment with MEGA quick-spin total Fragment DNA Purification kit (iNtRON Biotechnology, Korea). Purified PCR products were sequenced using BigDye Terminator chemistry on an ABI Prism 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. The PolyPhred program (http://droog.gs.washington.edu/polyphred/) was used to identify DNA polymorphisms.[10] The tagging SNPs were selected using Haploview 4.2

software (Availability: http://www.broad.mit.edu/mpg/haploview/).

3. Genotyping

Genotyping was carried out using the TaqMan® fluorogenic 5'-nuclease assay (Applied Biosystems, Foster City, CA, USA). Briefly, PCR was performed using on a QuantStudio™6 Flex Real-Time PCR System (Applied Biosystems, USA). Fifteen nanograms of DNA were amplified in a total volume of 10 μl containing TaqMan Universal PCR Master Mix (Applied Biosystems) including 40x TaqMan SNP Genotyping Assay. The thermal cycle conditions were as follows: denaturing at 95℃ for 10 min, followed by 45 cycles of denaturing at 95℃ for 15 s and annealing and extension at 60℃ for 1 min. After PCR, fluorescence was measured and analyzed using QuantStudio™ 6 FlexReal-Time PCR software v1.2 (Applied Biosystems).

4. Statistical analysis

All statistical analyses were performed using R software, version 3.4.0, on a Windows 10 platform. The statistical differences between KD patients and controls in genotype and allele frequency were assessed using the chi-square test or Fisher's exact test. Statistical differences in genotype and allele frequency of KD children with/without CAL formation and patients with IVIG resistance/responsiveness were assessed using the chi-square test. The Bonferroni test was used to correct for multiple tests. Binary multiple logistic

regression was performed for the odds ratio (OR), 95% confidence interval (CI), and corresponding *P* values, with adjustment for age and gender.

5. Ethical considerations

The study protocol was reviewed and approved by the Yonsei University Health System Institutional Review Board, Seoul, Korea (4-2008-0055). The study was conducted in accordance with good clinical practices (national regulations and ICH E6) and the principles of the Helsinki Declaration. Written informed consent was obtained from the parents or legal guardians of the patients prior to sample collection following a detailed explanation of schedules and contents of the study.

III. RESULTS

1. Basic and clinical characteristics of the subjects

Study subjects included 265 KD patients and 203 controls (Table 1). Average ages were 35.31 months in cases and 116.96 months in controls. Of the 265 KD patients, 45 (17.0%) developed CAL, and 38 (14.3%) were resistant to initial IVIG treatment. Fourteen (5.3%) had both CAL formation and IVIG resistance.

Table 1. Characteristics of children with Kawasaki disease and normal controls

Characteristics	KD Cases (n = 265)	Controls (n = 203)
Male gender	172 (64.9%)	77 (37.9%)
Mean (SD) age (months)	35.31±28.07	116.96±33.77
CAL formation	45 (17.0%)	
IVIG resistance	38 (14.3%)	
CAL formation + IVIG resistance	14 (5.3%)	

KD: Kawasaki disease; CAL: coronary artery lesions; IVIG: intravenous immunoglobulin; SD: standard deviation.

2. Identification of variations and linkage disequilibrium analysis in *HMGB1*

A total of two SNPs were identified by the entire *HMGB1* gene sequencing for causative SNP discovery. These SNPs were identical to those in the dbSNP database and no novel SNPs were identified (https://www.ncbi.nlm.nih.gov/snp). Based on the results of linkage disequilibrium analyses, two tagging SNPs were selected for genotyping analysis (Figure 2).

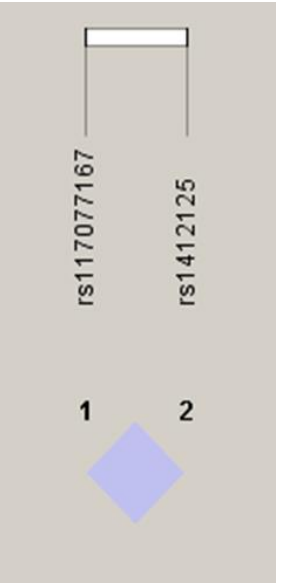

Figure 2. *HMGB1* gene linkage disequilibrium and haplotype block structure in KD.

3. No Association between *HMGB1* Polymorphisms and Susceptibility to KD

The distribution of *HMGB1* genotypes between the KD patients and healthy controls is shown in Table 2. None of the tagging SNPs were significantly associated with the genotype or allele frequency of KD children and controls under five genetic models (codominant, dominant, recessive, overdominant, or log-additive models).

Table 2. Genotype and allele frequencies of the *HMGB1* gene in patients with Kawasaki disease and normal controls

SNP	Genotype	KD (%) (n = 265)	Control (%) (n = 203)	Model	*P* value
rs1412125	AA	159 (60)	124 (61.1)	Codominant	0.287
	GA	90 (34)	69 (34)	Dominant	0.413
	GG	16 (6)	10 (4.9)	Recessive	0.288
				Overdominant	0.182
				Log-additive	0.781
rs117077167	CC	250 (94.3)	194 (95.6)	Codominant	0.796
	CA	15 (5.7)	9 (4.4)	Log-additive	0.796
SNP	Allele	KD (%) (n = 265)	Control (%) (n = 203)		*P* value
rs1412125	A	408 (77)	317 (78.1)		0.69
	G	122 (23)	89 (21.9)		
rs117077167	C	515 (97.2)	397 (97.8)		0.556
	A	15 (2.8)	9 (2.2)		

4. Association of *HMGB1* Polymorphisms with CAL Formation in KD Patients

The SNP rs1412125 was found to be associated with CAL formation in the recessive model. The frequency of individuals carrying the GG genotype of *HMGB1* (rs1412125) was 15.6% for KD with CAL and 4.1% for KD without

CAL. KD patients with the GG genotype showed a higher rate of CAL development (OR = 4.98, 95% CI = 1.69–14.66, *P* = 0.005) (Table 3). The SNP rs117077167 was not statistically associated with the development of CAL in KD patients.

Table 3. Genotype and allele frequencies of *HMGB1* SNP rs1412125 in Kawasaki disease patients with or without coronary artery lesion formation

SNP	Genotype	CAL (%) (n = 45)	Without (%) (n = 220)	Dominant *P* value	Recessive *P* value
rs1412125	AA	26 (57.8)	133 (60.5)	0.722	**0.005***
	GA	12 (26.7)	78 (35.5)		
	GG	7 (15.6)	9 (4.1)		
SNP	Allele	CAL (%) (n = 45)	Without (%) (n = 220)		Allelic *P* value
rs1412125	A	64 (71.1)	344 (78.2)		0.147
	G	26 (28.9)	96 (21.8)		

*Significant (*P* < 0.05) values are in bold.

5. Association of *HMGB1* Polymorphisms with IVIG Resistance in KD Patients

The SNP rs1412125 was associated with IVIG resistance in the recessive (OR = 4.11, 95% CI = 1.38–12.23, *P* = 0.017) and allelic models (OR = 1.80, 95% CI = 1.06–3.06, *P* = 0.027) (Table 4.). The frequency of patients carrying

the GG genotype of *HMGB1* (rs1412125) was 15.8% for IVIG resistant KD and 4.4% for IVIG responsive KD. KD children with the GG genotype showed a higher rate of IVIG unresponsiveness. The prevalence of the rs1412125 G allele was significantly higher in IVIG non-responders (32.9%) than in IVIG responders (21.4%). The SNP rs117077167 had no significant association with IVIG resistance in KD patients.

Table 4. Genotype and allele frequencies of *HMGB1* SNP rs1412125 in Kawasaki disease patients resistant and responsive to intravenous immunoglobulin treatment

SNP	Genotype	Resistant (%) (n = 38)	Responsive (%) (n = 227)	Dominant *P* value	Recessive *P* value
rs1412125	AA	19 (50.0)	140 (61.7)	0.164	**0.017***
	GA	13 (34.2)	77 (33.9)		
	GG	6 (15.8)	10 (4.4)		
SNP	Allele	Resistant (%) (n = 38)	Responsive (%) (n = 227)		Allelic *P* value
rs1412125	A	51 (67.1)	357 (78.6)		**0.027***
	G	25 (32.9)	97 (21.4)		

*Significant ($P < 0.05$) values are in bold.

IV. DISCUSSION

In this study, we investigated the association of *HMGB1* SNPs with KD in the Korean population. There were no differences in the distribution of *HMGB1* genotypes between KD patients and controls, indicating that *HMGB1* polymorphisms do not play a role in the susceptibility to KD. However, the *HMGB1* SNP rs1412125 was associated with IVIG resistance as well as development of CAL in KD patients. KD patients with the GG genotype for *HMGB1* (rs1412125) showed higher rates of CAL development and IVIG unresponsiveness. In addition, the rs1412125 G allele was significantly higher in the IVIG resistant group than in the IVIG responsive group. Our results suggest that the polymorphism of the *HMGB1* gene may play an important role in KD pathogenesis involved in the occurrence of CAL complications and resistance to IVIG treatment.

HMGB1 is a ubiquitous nuclear DNA binding protein located on chromosome 13.[11] It acts in the nucleus as a DNA chaperone under physiologic conditions.[2,12] However, *HMGB1* can be actively secreted from immune cells like macrophages, monocytes and dendritic cells or passively released from necrotic, damaged cells or from apoptotic cells when exposed to infection and injury.[11,13,14] Extracellular *HMGB1* functions through specific receptors to activate the NF-κB signaling pathway inducing the production of cytokines and chemokines.[15,16] These findings indicate that *HMGB1* plays an important role in regulating inflammatory, injurious, and infectious responses.

The role of *HMGB1* in KD has been investigated in recent studies. Hoshina et al. showed that serum *HMGB1* concentrations are higher in KD early acute phase compared to the late acute phase and the convalescent phase.[5] Even in the latter phase, *HMGB1* concentrations were higher than in normal controls and were comparable to levels in sepsis patients. It is known that *HBGB1* is elevated not only in KD, but also in other systemic diseases, such as infectious diseases, ischemia, immune disorders, neurodegenerative diseases, metabolic disorders, and cancer.[17] Therefore, *HMBG1* may be related to severity or complications rather than to the diagnostic specificity of KD. No association between *HMGB1* SNPs and susceptibility to KD in this study supports this hypothesis.

KD is characterized by multisystem involvement and inflammation of all the medium-sized arteries including the coronary artery. Various kinds of proinflammatory cytokines and chemokines are increased through the prominent immunologic cascade during the acute febrile phase of KD, when inflammatory cell infiltration into KD vascular tissue leads to vascular damage.[1,18,19] Although the specific immunologic pathways that develop CAL in KD remain unclear, ongoing coronary artery inflammation may be a risk of CAL complication.[20] Active immune cells and damaged cells are the sources of increased *HMGB1* release in serum during the acute febrile phase. We speculate that increased levels of *HMGB1* in the serum of acute phase KD function as DAMP in conjunction with other inflammatory factors and then activate the ongoing coronary artery inflammatory response, leading to CAL formation. In

this study, *HMGB1* SNP rs1412125 was associated with CAL complication. This result suggests that the polymorphism of the *HMGB1* gene may play a crucial role in pathogenesis of CAL in KD.

High-dose IVIG is the primary treatment for KD. However, approximately 10-20% of patients are resistant to initial IVIG and have an increased risk of developing CAL.[21-23] Like the mechanism of action of IVIG in KD treatment, the immunological mechanism for IVIG resistance has not yet been clarified. The possible mechanisms of the beneficial effect of IVIG include modulating cytokine production, augmenting regulatory T-cell activity, neutralizing toxins or other pathogens, downregulating antibody synthesis, and providing anti-idiotypic antibodies.[24] Extracellular *HMGB1* not only acts as a DAMP with cytokine and chemokine activities but also has several effects on T lymphocytes.[4,25] Regarding regulatory T-cells, *HMGB1* has been shown to reduce the expression of CTLA4 (cytotoxic T-lymphocyte antigen 4) and FoxP3 (forkhead box P3), and to suppress the release of IL-10, inducing decreased regulatory T-cell activity,[26,27] which is in contrast to the anticipated mechanism of action of IVIG in treatment of KD. Eguchi et al. showed that mean *HMGB1* levels were higher in IVIG poor responders than in IVIG good responders and were correlated with leukocyte counts, a known predictor of unresponsiveness to IVIG.[6] These findings suggest that *HMGB1* may be promising candidate as a prognostic marker for IVIG unresponsiveness in KD. Recent studies have shown that genetic factors in the host, such as polymorphisms in the Fc gamma

receptors,[28,29] may play a role in the mechanisms for both IVIG response and resistance. In this study, we demonstrated that *HMGB1* SNP rs1412125 was associated with IVIG unresponsiveness. The current findings demonstrate that the variant in the *HMGB1* gene plays a crucial role in IVIG resistance.

Interestingly, in this study, rs1412125 was associated with both CAL formation and IVIG resistance in the recessive model. The GG genotype of *HMGB1* (rs1412125) increased the risk for CAL development and IVIG resistance. Many studies have shown that poor-responder to initial IVIG are at increased risk of CAL development.[30-32] The consistent association of *HMGB1* SNP rs1412125 with the two clinical outcomes in this study suggests that the polymorphism of the *HMGB1* gene may be a potential and independent predictor of those in KD patients.

We note that our study had several limitations. First, the modest sample size may not have had sufficient power to detect the small genetic effect of *HMGB1* in KD. Future analysis in other populations with a larger sample size is required to verify the result of the present study. Second, the control group was not age matched. However, as future development of KD in young, healthy children cannot be ruled out, older subjects without a history of KD may be a better choice as the control group.

V. CONCLUSION

We identified a significant association between the recessive model of the *HMGB1* SNP rs1412125 and risk of developing KD with CAL and IVIG resistance in Korean children. To our knowledge, this study is the first to investigate the potential clinical relevance of all known SNPs within the entire *HMGB1* gene in relation to the development of CAL and IVIG unresponsiveness in KD patients. Although verification by larger-scale studies is needed, our results suggest that variants in the *HMGB1* gene may be useful as potential markers for the prediction of CAL formation and poor response to IVIG.

REFERENCES

1. Kim KY, Kim DS. Recent Advances in Kawasaki Disease. Yonsei Med J 2016;57:15-21.
2. Lee SA, Kwak MS, Kim S, Shin JS. The role of high mobility group box 1 in innate immunity. Yonsei Med J 2014;55:1165-76.
3. Wang H, Yang H, Czura CJ, Sama AE, Tracey KJ. HMGB1 as a late mediator of lethal systemic inflammation. Am J Respir Crit Care Med 2001;164:1768-73.
4. de Souza AW, Westra J, Limburg PC, Bijl M, Kallenberg CG. HMGB1 in vascular diseases: Its role in vascular inflammation and atherosclerosis. Autoimmun Rev 2012;11:909-17.
5. Hoshina T, Kusuhara K, Ikeda K, Mizuno Y, Saito M, Hara T. High mobility group box 1 (HMGB1) and macrophage migration inhibitory factor (MIF) in Kawasaki disease. Scand J Rheumatol 2008;37:445-9.
6. Eguchi T, Nomura Y, Hashiguchi T, Masuda K, Arata M, Hazeki D, et al. An elevated value of high mobility group box 1 is a potential marker for poor response to high-dose of intravenous immunoglobulin treatment in patients with Kawasaki syndrome. Pediatr Infect Dis J 2009;28:339-41.
7. Ayusawa M, Sonobe T, Uemura S, Ogawa S, Nakamura Y, Kiyosawa N, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). Pediatr Int 2005;47:232-4.

8. 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;110:2747-71.

9. Group JCSJW. Guidelines for diagnosis and management of cardiovascular sequelae in Kawasaki disease (JCS 2008)--digest version. Circ J 2010;74:1989-2020.

10. Nickerson DA, Tobe VO, Taylor SL. PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. Nucleic Acids Res 1997;25:2745-51.

11. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 2002;418:191-5.

12. Einck L, Bustin M. The intracellular distribution and function of the high mobility group chromosomal proteins. Exp Cell Res 1985;156:295-310.

13. Rendon-Mitchell B, Ochani M, Li J, Han J, Wang H, Yang H, et al. IFN-gamma induces high mobility group box 1 protein release partly through a TNF-dependent mechanism. J Immunol 2003;170:3890-7.

14. Kazama H, Ricci JE, Herndon JM, Hoppe G, Green DR, Ferguson TA.

Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. Immunity 2008;29:21-32.

15. Taguchi A, Blood DC, del Toro G, Canet A, Lee DC, Qu W, et al. Blockade of RAGE-amphoterin signalling suppresses tumour growth and metastases. Nature 2000;405:354-60.

16. Park JS, Arcaroli J, Yum HK, Yang H, Wang H, Yang KY, et al. Activation of gene expression in human neutrophils by high mobility group box 1 protein. Am J Physiol Cell Physiol 2003;284:C870-9.

17. Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, et al. HMGB1 in health and disease. Mol Aspects Med 2014;40:1-116.

18. Matsubara T, Ichiyama T, Furukawa S. Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease. Clin Exp Immunol 2005;141:381-7.

19. Franco A, Touma R, Song Y, Shimizu C, Tremoulet AH, Kanegaye JT, et al. Specificity of regulatory T cells that modulate vascular inflammation. Autoimmunity 2014;47:95-104.

20. Shulman ST, Rowley AH. Kawasaki disease: insights into pathogenesis and approaches to treatment. Nat Rev Rheumatol 2015;11:475-82.

21. Tremoulet AH, Best BM, Song S, Wang S, Corinaldesi E, Eichenfield JR, et al. Resistance to intravenous immunoglobulin in children with Kawasaki disease. J Pediatr 2008;153:117-21.

22. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, 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:e927-e99.

23. Jang H, Kim KY, Kim DS. Clinical Outcomes of Low-Dose Methotrexate Therapy as a Second-Line Drug for Intravenous Immunoglobulin-Resistant Kawasaki Disease. Yonsei Med J 2018;59:113-8.

24. Burns JC, Franco A. The immunomodulatory effects of intravenous immunoglobulin therapy in Kawasaki disease. Expert Rev Clin Immunol 2015;11:819-25.

25. Sundberg E, Fasth AE, Palmblad K, Harris HE, Andersson U. High mobility group box chromosomal protein 1 acts as a proliferation signal for activated T lymphocytes. Immunobiology 2009;214:303-9.

26. Zhu XM, Yao YM, Liang HP, Xu CT, Dong N, Yu Y, et al. High mobility group box-1 protein regulate immunosuppression of regulatory T cells through toll-like receptor 4. Cytokine 2011;54:296-304.

27. Zhang Y, Yao YM, Huang LF, Dong N, Yu Y, Sheng ZY. The potential effect and mechanism of high-mobility group box 1 protein on regulatory T cell-mediated immunosuppression. J Interferon Cytokine Res 2011;31:249-57.

28. 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. J Allergy Clin Immunol 2011;128:677-80.

29. Shendre A, Wiener HW, Zhi D, Vazquez AI, Portman MA, Shrestha S. High-density genotyping of immune loci in Kawasaki disease and IVIG treatment response in European-American case-parent trio study. Genes Immun 2014;15:534-42.

30. Burns JC, Capparelli EV, Brown JA, Newburger JW, Glode MP. Intravenous gamma-globulin treatment and retreatment in Kawasaki disease. US/Canadian Kawasaki Syndrome Study Group. Pediatr Infect Dis J 1998;17:1144-8.

31. Durongpisitkul K, Soongswang J, Laohaprasitiporn D, Nana A, Prachuabmoh C, Kangkagate C. Immunoglobulin failure and retreatment in Kawasaki disease. Pediatr Cardiol 2003;24:145-8.

32. Uehara R, Belay ED, Maddox RA, Holman RC, Nakamura Y, Yashiro M, et al. Analysis of potential risk factors associated with nonresponse to initial intravenous immunoglobulin treatment among Kawasaki disease patients in Japan. Pediatr Infect Dis J 2008;27:155-60.

ABSTRACT(IN KOREAN)

가와사끼병에서 High mobility group box 1 유전자의 polymorphism

<지도교수 김동수>

연세대학교 대학원 의학과

안 종 균

가와사끼병은 주로 영유아에서 호발하는 급성 전신성 혈관염으로 원인은 아직 불명이다. High mobility group box 1 (*HMGB1*)은 여러 염증성 질환의 병인에 관여하는 DAMP(Damage-associated molecular pattern)이다. 최근 연구들은 가와사끼병의 급성기에 혈중 *HMGB1* 수치가 상승되어 있고, 특히 면역글로불린 저항성 가와사키병에서 *HMGB1* 수치가 의미 있게 높아 있음을 보여준다. 이번 연구는 *HMGB1*의 유전적 변이와 가와사끼병의 상관관계를 알기 위하여 진행되었다.

HMGB1 유전자의 단일염기 다형성(single nucleotide polymorphism, SNP)을 확인하기 위해 *HMGB1* 유전자의 전장유전체 염기서열분석(whole genome sequencing)을 수행하였다. 연관불평형(linkage disequilibrium)분석을 이용하여 *HMGB1* 유전자의 두 가지 tagging SNP (rs1412125, rs117077167)을 선택하였다. TaqMan Allelic Discrimination방법을 사용하여 265명의 가와사끼병 환자와 203명의 대조군에서 두 가지 tagging SNP에 대한 유전자형을 분석하였다. *HMBG1* 유전자 변이와 가와사끼병의 감수성, 관상동맥 합병증 및 면역글로불린 치료 반응에 대한 상관관계를 분석하였다. *HMGB1* 유전자의 SNP은 가와사끼병의 감수성과 관련이 없었다. 그러나 rs1412125은 열성모델(recessive model)에서 가와사끼병 환자의 관상동맥 합병증과 연관성이 있었다(GG vs. AA + GA: OR = 4.98, 95% CI = 1.69–14.66, P = 0.005). 또한 rs1412125는 열성모델(GG vs. AA + GA: OR = 4.11, 95% CI = 1.38–12.23, P = 0.017) 및 대립유전자 모델(allelic model)(G vs. A: OR = 1.80, 95% CI = 1.06–3.06, P = 0.027)에서 면역글로불린 저항성과 연관성이 있었다. *HMGB1* 유전자의 rs1412125는 가와사끼병 환자에서 관상동맥 합병증 및

면역글로불린 저항성에 영향을 미치는 유전적 위험요인일 수 있다.

핵심되는 말: 가와사끼병; *HMGB1*; 단일염기 다형성; 관상동맥 합병증; 면역글로불린 저항성