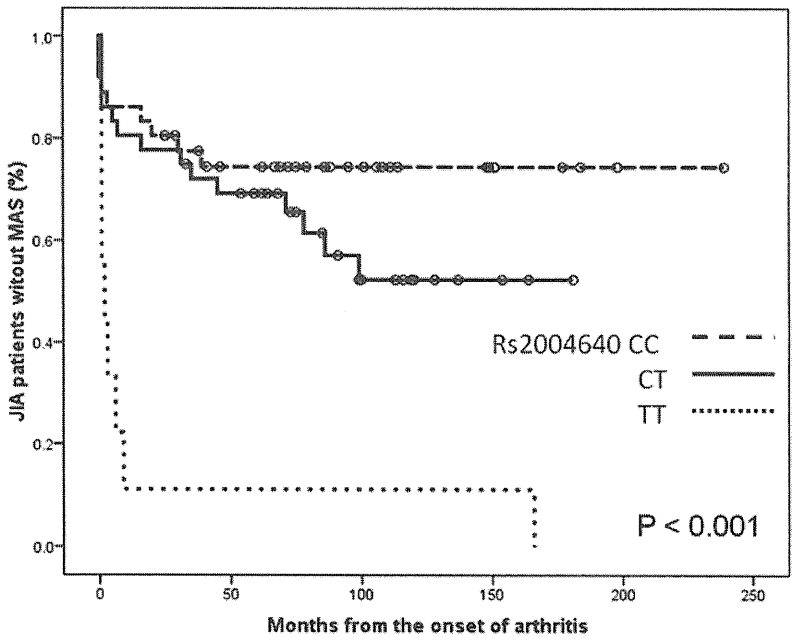

Figure 1. Kaplan-Meier analysis of survival without macrophage activation syndrome and rs2004640 genotypes. JIA: juvenile idiopathic arthritis.

Table 5. Comparison of IRF5 haplotypes in patients with systemic juvenile idiopathic arthritis (JIA) with or without MAS. The order of SNP (single-nucleotide polymorphisms) in the haplotype is rs729302-rs2004640-rs2280714.

Haplotype	Haplotype Frequencies in Patients without MAS	Haplotype Frequencies in Patients with MAS	p	OR	95% CI
A-G-C	0.438	0.303	0.06	1.0	—
C-G-T	0.330	0.182	0.04	0.92	0.35–2.39
A-T-C	0.017	0.015	0.93	1.19	0.08–17.6
A-T-T	0.201	0.485	< 0.001	4.61	1.73–12.3

IRF5: interferon regulatory factor 5; MAS: macrophage activation syndrome.

ence of fairly frequent polymorphisms in multiple genes involved in the regulation of innate and adaptive immunity may be one of the major determinants in the initiation of rheumatic diseases[19]. To develop innate and adaptive immune responses, the activation of a TLR signaling pathway is essential. The transcription factor IRF5 is generally involved downstream of the TLR signaling pathway for induction genes for proinflammatory cytokines such as IL-6, IL-12, and TNF-α[11,20]. As mentioned, proinflammatory cytokines such as IFN-γ and TNF-α are responsible for the clinical and laboratory abnormalities seen in MAS[4,5]. Thus, we examined the association of polymorphisms in the *IRF5* gene with susceptibility to MAS in patients with systemic JIA.

Our investigation revealed that the rs2004640 T allele and the ATT haplotype in the *IRF5* gene were associated with MAS developing in patients with systemic JIA. All the patients with the TT genotype at rs2004640 had MAS, and they had an early onset of MAS compared to those with non-TT genotypes (Figure 1). While the ATT haplotype of the *IRF5* gene was associated with susceptibility to MAS in patients with systemic JIA in our study, it was also reported that this is the common haplotype conferring increased risk of SLE[12]. The T alleles of both rs2004640 and rs2280714 were associated with higher levels of IRF5 messenger RNA expression[12]. Further, the other IRF5 haplotype was associated with high serum IFN-α activity in patients with SLE[21]. Although we did not address the association between proinflammatory cytokine activity and genotype/haplotype in the *IRF5* gene, there may be the potential role of IRF5-associated immune response in the pathogenesis of MAS. Further research is needed to determine the influence of gene polymorphisms in the *IRF5* gene on proinflammatory cytokine activities.

Although several drugs, such as tolmetin and tocilizumab, and viral infections were considered the triggering caus-

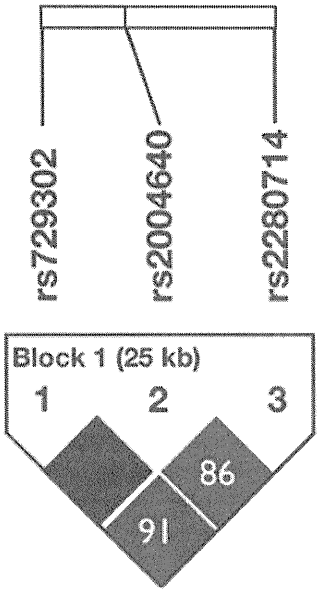

Figure 2. Haplotype blocks of interferon regulatory factor 5 gene polymorphisms.

es of MAS in our cohort, the triggers were not determined in most cases. All patients with MAS recovered from their severe complication. We could not find any association between IRF5 genotypes/haplotypes and the characteristics of clinical symptoms and severity of MAS in this study, presumably because of a small study population. We need a larger cohort to determine this association.

There are some limitations to our study. The incidence of MAS (40.7%) was significantly higher than generally seen (about 10%)[4,5]. Our hospital is one of the pediatric rheumatology centers in Japan and we have treated many patients with severe systemic JIA. The incidence of MAS is so high partly because of the characteristics of our hospital. In addition, there are ethnic differences in the incidence by the subtypes of JIA. Specifically, systemic JIA accounts for about 20% of JIA in Japan but for only about 10% in Europe and the United States[22]. Therefore there may also be ethnic differences in susceptibility to MAS.

A second issue is that we could not carry out a validation study. Although the genetic association study should be validated, the incidence of MAS complicating systemic JIA is too low to validate this association in a single institution. Therefore it is important for the association between the IRF5 genotype/haplotype and MAS susceptibility to be confirmed by other groups.

We found a strong association between polymorphisms in the *IRF5* gene and susceptibility to MAS in patients with systemic JIA. This finding suggests a potentially important role of the IRF5-associated immune response in the pathogenesis of MAS.

ACKNOWLEDGMENT

We are grateful to T. Kaneko and S. Morita for advice on statistics. We also thank R. Tanoshima for secretarial assistance.

REFERENCES

1. Cassidy JT, Petty RE. Juvenile idiopathic arthritis. In: Cassidy JT, Petty RE, editors. Textbook of pediatric rheumatology. Philadelphia: WB Saunders; 2005:291-303.
2. De Benedetti F. Targeting interleukin-6 in pediatric rheumatic diseases. Curr Opin Rheumatol 2009;21:533-7.
3. Pascual V, Allantaz F, Arce E, Punaro M, Banchereau J. Role of interleukin-1 (IL-1) in the pathogenesis of systemic onset juvenile idiopathic arthritis and clinical response to IL-1 blockade. J Exp Med 2005;201:1479-86.
4. Kelly A, Ramanan AV. Recognition and management of macrophage activation syndrome in juvenile arthritis. Curr Opin Rheumatol 2007;19:477-81.
5. Ravelli A, Martini A. Macrophage activation syndrome. In: Lehman TJ, Cimaz R, editors. Pediatric rheumatology. Amsterdam: Elsevier; 2008:55-63.
6. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.
7. Grom AA, Villanueva J, Lee S, Goldmuntz EA, Passo MH, Filipovich A. Natural killer cell dysfunction in patients with systemic-onset juvenile rheumatoid arthritis and macrophage activation syndrome. J Pediatr 2003;142:292-6.
8. Vastert SJ, van Wijk R, D'Urbano LE, de Vooght KM, de Jager W, Ravelli A, et al. Mutations in the perforin gene can be linked to macrophage activation syndrome in patients with systemic onset juvenile idiopathic arthritis. Rheumatology 2010;49:441-9.
9. Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. Curr Opin Rheumatol 2010;22:561-6.
10. Zhang K, Biroschak J, Glass DN, Thompson SD, Finkel T, Passo MH, et al. Macrophage activation syndrome in patients with systemic juvenile idiopathic arthritis is associated with MUNC13-4 polymorphisms. Arthritis Rheum 2008;58:2892-6.
11. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, et al. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature 2005;434:243-9.
12. Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, Bauer JW, et al. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nat Genet 2006;38:550-5.
13. Han SW, Lee WK, Kwon KT, Lee BK, Nam EJ, Kim GW. Association of polymorphisms in interferon regulatory factor 5 gene with rheumatoid arthritis: a metaanalysis. J Rheumatol 2009;36:693-7.
14. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004;31:390-2.
15. Ravelli A, Magni-Manzoni S, Pistorio A, Besana C, Foti T, Ruperto N, et al. Preliminary diagnostic guidelines for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. J Pediatr 2005;146:598-604.
16. Gonzalez JR, Armengol L, Sole X, Guino E, Mercader JM, Estivill X, et al. SNPassoc: an R package to perform whole genome association studies. Bioinformatics 2007;23:644-5.
17. Lake SL, Lyon H, Tantisira K, Silverman EK, Weiss ST, Laird NM, et al. Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. Hum Hered 2003;55:56-65.

18. Shimane K, Kochi Y, Yamada R, Okada Y, Suzuki A, Miyatake A, et al. A single nucleotide polymorphism in the IRF5 promoter region is associated with susceptibility to rheumatoid arthritis in the Japanese population. Ann Rheum Dis 2009;68:377-83.
19. Gutierrez-Roelens I, Lauwerys BR. Genetic susceptibility to autoimmune disorders: clues from gene association and gene expression studies. Curr Mol Med 2008;8:551-61.
20. Yanai H, Chen HM, Inuzuka T, Kondo S, Mak TW, Takaoka A, et al. Role of IFN regulatory factor 5 transcription factor in antiviral immunity and tumor suppression. Proc Natl Acad Sci USA 2007;104:3402-7.
21. Niewold TB, Kelly JA, Flesch MH, Espinoza LR, Harley JB, Crow MK. Association of the IRF5 risk haplotype with high serum interferon-alpha activity in systemic lupus erythematosus patients. Arthritis Rheum 2008;58:2481-7.
22. Yokota S, Mori M, Imagawa T, Takei S, Murata T, Tomiita M, et al. Proposal for juvenile idiopathic arthritis guidance on diagnosis and treatment for primary care pediatricians and nonpediatric rheumatologists. Mod Rheumatol 2007;17:353-63.

BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2010.03814.x

Influence of polymorphisms within the methotrexate pathway genes on the toxicity and efficacy of methotrexate in patients with juvenile idiopathic arthritis

Masakatsu Yanagimachi,[1] Takuya Naruto,[1] Takuma Hara,[1] Masako Kikuchi,[1] Ryoki Hara,[1] Takako Miyamae,[1] Tomoyuki Imagawa,[1] Masaaki Mori,[1] Tetsuji Kaneko,[2] Satoshi Morita,[2] Hiroaki Goto[1] & Shumpei Yokota[1]

[1]Department of Pediatrics and [2]Department of Biostatistics and Epidemiology, Yokohama City University Graduate School of Medicine, Kanazawa-ku, Yokohama 236-0004, Japan

Correspondence
Dr Masakatsu Yanagimachi, Department of Pediatrics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
Tel.: +81 45 787 2800
Fax: +81 45 787 0461
E-mail: m-yanagi@mvi.biglobe.ne.jp

Keywords
5-aminoimidazole-4-carboxamide ribonucleotide transformylase, articular-type juvenile idiopathic arthritis, γ-glutamyl hydrolase, methotrexate

Received
12 June 2010

Accepted
16 September 2010

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Methotrexate (MTX), which causes adverse effects, such as liver and/or renal dysfunction, is the most common disease-modifying antirheumatic drug used for the treatment of rheumatoid arthritis and articular-type juvenile idiopathic arthritis (JIA).
- Pharmacogenetic studies analysing the MTX pathway genes would aid in the development of more personalized therapy.
- Results regarding the influence of gene polymorphisms on the toxicity and efficacy of MTX are conflicting, and there are marked differences between racial groups in pharmacogenetics.

WHAT THIS STUDY ADDS

- The non-TT genotype at γ-glutamyl hydrolase (*GGH*) T16C is associated with a high risk of liver dysfunction due to MTX, even after adjustment for duration of MTX treatment.
- Longer time interval from disease onset to MTX treatment and rheumatoid factor positivity are associated with lower efficacy of MTX in Japanese patients, as reported previously in Caucasian patients with JIA.

AIMS

We investigated whether several polymorphisms within the methotrexate (MTX) pathway genes were related to the toxicity and efficacy of MTX in 92 Japanese patients with articular-type juvenile idiopathic arthritis (JIA).

METHODS

Eight gene polymorphisms within the MTX pathway genes, namely, *RFC*, *BCRP*, *MTHFR* (two), *FPGS*, γ-glutamyl hydrolase (*GGH*; two) and *ATIC*, were genotyped using TaqMan assays. Liver dysfunction was defined as an increase in alanine transaminase to five times the normal upper limit. Non-responders to MTX were defined as patients refractory to MTX and were therefore treated with biologics.

RESULTS

The non-TT genotype at *GGH* T16C was associated with a high risk of liver dysfunction ($P = 0.028$, odds ratio = 6.90, 95% confidence interval 1.38–34.5), even after adjustment for the duration of MTX treatment. A longer interval from disease onset to treatment (8.5 and 21.3 months, $P = 0.029$) and rheumatoid factor positivity ($P = 0.026$, odds ratio = 2.87, 95% confidence interval 1.11–7.39) were associated with lower efficacy of MTX.

CONCLUSIONS

The non-TT genotype at *GGH* T16C was associated with a high risk of liver dysfunction, presumably because the C allele of *GGH* C16T may reduce the activity of GGH. The time interval before MTX treatment and rheumatoid factor positivity were associated with the efficacy of MTX treatment. The pharmacogenetics of the MTX pathway genes affects the toxicity and efficacy of MTX in Japanese JIA patients.

Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common forms of paediatric chronic arthritis, with an incidence of approximately 9.7 per 100 000 children (aged 15 years and under) in Japan [1, 2]. Methotrexate (MTX) is the most common disease-modifying antirheumatic drug used for the treatment of articular-type JIA, namely the polyarticular- and oligoarticular-onset types of JIA [2]. Methotrexate is effective in about 75% of cases of the articular-type JIA, but causes adverse effects, such as liver and/or renal dysfunction [2, 3]. The effects of polymorphisms within the MTX pathway genes on the toxicity and efficacy of MTX in patients with rheumatoid arthritis (RA) and JIA have been studied [4–6].

The influence of polymorphisms within the MTX pathway genes encoding solute carrier family 19 member 1 (SLC19A1), also known as reduced folate carrier (RFC), 5,10-methylenetetrahydrofolate reductase (MTHFR), folypolyglutamate synthetase (FPGS), γ-glutamyl hydrolase (GGH), 5-aminomidazole-4-carboxamide ribonucleotide transformylase (ATIC) and breast cancer resistance protein (BCRP/ABCG2) on the toxicity and efficacy of MTX in patients with RA, JIA and other diseases has been studied [4–9]. However, results regarding the influence of these polymorphisms on the toxicity and efficacy of MTX are conflicting, and there are marked differences in pharmacogenetics between racial groups [10]. Therefore, we investigated whether polymorphisms within the MTX pathway genes were related to the toxicity and efficacy of MTX in 92 patients with articular-type JIA in Japan.

Patients and methods

Study population

Patients were eligible if they met the International League of Association for Rheumatology classification criteria for articular-type JIA [11]. A total of 92 children (74 girls and 18 boys; 12 with seronegative polyarticular onset, 46 with seropositive polyarticular onset and 34 with oligoarticular onset) in this study were treated at the Yokohama City University Hospital between December 2007 and December 2009.

All 92 patients had been treated with MTX for at least 3 months without biologics. Initially, MTX was administered orally at a dosage of 4–5 mg m^{-2} per week. Then the dosage was adjusted depending on tolerability and response (maximal dosage, 10 mg m^{-2} $week^{-1}$) [2]. Prednisolone was used concomitantly with MTX in 89 patients (96.7%). Folic acid supplementation was performed in nine patients (9.9%). Clinical data were collected from a patient's medical record without any knowledge of the individual's polymorphisms.

The study was performed in accordance with the Declaration of Helsinki, and approval for it was obtained from the Yokohama City University School of Medicine Ethics Committee. Each patient or his/her guardians gave written informed consent to participate in this study.

Definitions of toxicity and efficacy

For the evaluation of toxicity, liver dysfunction was defined as an increase in serum alanine transaminase (ALT) level to five times the normal upper limit before the addition of biologics.

Responders to MTX were defined as follows: (i) patients in whom the medication was terminated because they had remission of symptoms; (ii) patients who continued the treatment with stable doses of MTX; and (iii) patients who continued MTX treatment with the concomitant use of acceptable doses of prednisolone, without the addition of biologics, such as anti-tumour necrosis factor therapy [12] and anti-interleukin-6 receptor antibody therapy [13, 14].

Non-responders to MTX were defined as patients who were refractory to MTX and thus treated with biologics. Treatment with biologics was conducted according to the following criteria: (i) patients with a history of treatment with nonsteroidal anti-inflammatory drugs and MTX; and (ii) patients who had the active disease for at least 3 months after MTX treatment (up to 10 mg m^{-2} $week^{-1}$). Active disease was characterized by five or more swollen joints and three or more joints with limited range of movement accompanied by pain and/or tenderness, or the use of high doses of corticosteroids (>0.25 mg kg^{-1} daily), with accompanying unacceptable side-effects [12, 13].

Clinical predictors

Clinical predictors that may influence a patient's disease state and the toxicity and efficacy of MTX were selected on the basis of previous reports [5, 6, 15, 16]. The following factors were included: sex; age at disease onset; duration of MTX treatment; time interval from disease onset to MTX treatment; rheumatoid factor (RF) status; anti-cyclic citrullinated peptide (anti-CCP) status; and concomitant use of prednisolone and folic acid.

Genetic predictors

Genomic DNA was isolated from peripheral blood using the QIAamp DNA Mini kit (Qiagen K.K., Tokyo, Japan).

The following eight single nucleotide polymorphisms (SNPs) within the MTX pathway genes encoding RFC, MTHFR, FPGS, GGH, ATIC and BCRP were selected according to previous reports [4–9]. Genotyping for the SNPs of *RFC* G80A (rs1051266), *MTHFR* A1298C (rs1801131), *MTHFR* C677T (rs1801133), *FPGS* A1994G (rs10106), *GGH* C452T (rs11545078), *GGH* T16C (rs1800909), *ATIC* C347G (rs2372536) and *BCRP* C421A (rs2231142) was performed using the TaqMan assay (Applied Biosystems, Foster City, CA, USA). TaqMan SNP Genotyping Assays were used for *MTHFR* A1298C and *MTHFR* C677T, and Custom TaqMan SNP Genotyping Assays were used for *RFC* G80A, *FPGS*

A1994G, *GGH* C452T, *GGH* T16C, *ATIC* C347G and *BCRP* C421A [9] (see Supplementary data 1). These SNPs were analysed in real-time PCRs by the AB7500 Real Time PCR system (Applied Biosystems), in the conditions recommended by the manufacturer. Allele discrimination was performed using SDS software version 1.4 (Applied Biosystems).

Statistical analysis

For continuous predictors, such as age and duration of MTX treatment, Student's unpaired *t*-test was used to assess the association between clinical predictors and the toxicity and efficacy. For categorical predictors, such as genetic predictors and sex, a χ^2 test and Fisher's exact test were used to assess the association between predictors and the toxicity and efficacy. Possible confounding effects among the predictors were adjusted using a multiple logistic regression model.

Haplotype phases and haplotype frequencies were estimated using the Expectation-Maximization algorithm (minimum haplotype frequency >0.05). All statistical analyses were carried out using the SAS system version 9 (SAS Institute Inc., Cary, NC, USA).

Results

Distribution of the polymorphisms within the MTX pathway genes

The genotype frequencies for the eight SNPs under study were in Hardy–Weinberg equilibrium ($P > 0.05$). Each result was consistent with the findings of a previous report (see Supplementary data 2) [17].

The toxicity of MTX

Of 92 patients, 10 developed liver dysfunction. Methotrexate treatment of longer duration was a risk factor for liver dysfunction (104.3 months with liver dysfunction, 53.6 months without, $P = 0.005$). No other clinical variables were associated with liver dysfunction (Table 1). None of the patients with folic acid supplementation had liver dysfunction. However, this correlation of folic acid supplementation preventing liver dysfunction was not statistically significant, presumably because of the small study population.

Regarding the association between liver dysfunction and genetic predictors, the TT genotype at *GGH* T16C was a low risk factor for liver dysfunction [$P = 0.031$, odds ratio (OR) = 0.20, 95% confidence interval (CI) 0.03–0.98; Table 2 and Supplementary data 3]. In contrast, the non-TT genotype at *GGH* T16C was a high risk factor for liver dysfunction ($P = 0.031$, OR = 5.10, 95% CI 1.02–25.6), which is of significant clinical interest. This association was statistically significant even after adjustment for duration of MTX treatment ($P = 0.028$, OR = 6.90, 95% CI 1.38–34.5). None of the other SNPs was associated with liver dysfunction.

The *MTHFR* haplotypes and *GGH* haplotypes showed no significant association with liver dysfunction (data not shown).

The efficacy of MTX

Of 92 patients, 67 were non-responders to MTX. Delayed MTX treatment from disease onset (21.3 months with non-responders *vs.* 8.5 months with responders, $P = 0.029$) and RF positivity ($P = 0.026$, OR = 2.87, 95% CI 1.11–7.39) were risk factors for lower efficacy of MTX (Table 3). No other clinical variables were associated with efficacy.

Regarding the association between the efficacy of MTX and genetic predictors, there was no gene polymorphism significantly associated with efficacy (Table 4). The *MTHFR* haplotypes and *GGH* haplotypes showed no significant association with efficacy (data not shown).

In 64 patients treated with MTX within 1 year of disease onset, the CC genotype at *ATIC* C347G tended to be associated with lower efficacy. However, this was not statistically significantly after adjustment for the time interval and RF ($P = 0.106$, OR = 0.38, 95% CI 0.12–1.23) (Table 5).

Discussion

Several studies have shown the influence of polymorphisms within the MTX pathway genes on the toxicity and

Table 1

Association between clinical predictors and liver dysfunction

	ALT >5.0 times normal ($n = 10$)	ALT ≤5.0 times normal ($n = 82$)	*P* value
Age at onset (years, mean)	9.5	7.4	0.138
Sex (male)	20.0%	19.5%	0.971
Time interval from onset to treatment (months, mean)	17.7	17.9	0.987
Prednisolone	90.0%	97.6%	0.204
Folic acid	0.0%	11.0%	0.270
Duration of MTX treatment (months, mean)	104.3	52.6	0.005
MTX efficacy	30.0%	26.8%	0.832

ALT, alanine transaminase.

Table 2

Association between genetic predictors and liver dysfunction

Genotype	Allele model* OR†	*P* value	Dominant model* OR†	*P* value	Recessive model* OR†	*P* value
***RFC* G80A**	1.51	0.414	0.21	0.121	0.59	0.627
***BCRP* C421A**	1.05	0.930	0.80	0.840	0.99	0.988
***MTHFR* C677T**	1.45	0.451	1.12	0.896	2.28	0.214
***MTHFR* A1298C**	0.89	0.852	1.08	0.539	0.74	0.655
***FPGS* A1994G**	0.54	0.249	4.88	0.068	0.70	0.600
***GGH* T16C**	0.42	0.118	0.83	0.475	0.20	0.031
***GGH* C452T**	0.61	0.506	–	–	0.61	0.502
***ATIC* C347G**	1.40	0.560	0.48	0.814	1.17	0.336

M, major allele; and m, minor allele. Major alleles are the A allele at *RFC* G80A, C allele at *BRCP* C421A, C allele at *MTHFR* C677T, A allele at *MTHFR* A1298C, G allele at *FPGS* A1994G, T allele at *GGH* T16C, C allele at *GGH* C452T and C allele at *ATIC* C347G. *Allele model: M *vs.* m; dominant model, (MM or Mm) *vs.* mm; recessive model, MM *vs.* (Mm or mm). †Non-adjusted odds ratio.

Table 3

Association between clinical predictors and methotrexate efficacy

	Responder ($n = 25$)	Non-responder ($n = 67$)	*P* value
Age at onset (years, mean)	6.6	7.9	0.180
Sex (male)	12.0%	22.4%	0.264
Time interval from onset to treatment (months, mean)	8.5	21.3	0.029
Prednisolone	96.0%	97.0%	0.807
Folic acid	4.0%	11.9%	0.254
C-reactive protein at start of treatment (mg dl^{-1}, mean)	2.8	3.3	0.685
Anti-cyclic citrullinated peptide [>4.5 (U ml^{-1})]	32.0%	55.2%	0.062
Rheumatoid factor [>14 (IU ml^{-1})]	40.0%	65.7%	0.026

Table 4

Association between genetic predictors and methotrexate efficacy

Genotype	Allele model* OR†	*P* value	Dominant model* OR†	*P* value	Recessive model* OR†	*P* value
***RFC* G80A**	1.01	0.979	1.32	0.572	1.61	0.435
***BCRP* C421A**	1.28	0.496	0.24	0.151	0.99	0.979
***MTHFR* C677T**	0.75	0.399	0.79	0.708	0.42	0.115
***MTHFR* A1298C**	1.05	0.918	0.36	0.282	0.87	0.775
***FPGS* A1994G**	0.95	0.900	1.37	0.726	1.01	0.984
***GGH* T16C**	1.01	0.986	2.83	0.294	1.24	0.654
***GGH* C452T**	1.15	0.805	–	–	1.15	0.805
***ATIC* C347G**	0.65	0.237	1.08	0.931	0.50	0.139

*Allele model: M *vs.* m.; dominant model, (MM or Mm) *vs.* mm; recessive model, MM *vs.* (Mm or mm). †Non-adjusted odds ratio.

efficacy of MTX in patients with RA [4, 8, 9]. However, results are conflicting, and there are marked differences between racial groups in pharmacogenetic studies [10]. We could find only two studies on the pharmacogenetics of MTX in patients with JIA in Caucasian patients [5, 6], but not one in an Asian population. This is the first reported study on pharmacogenetics of MTX in patients with JIA in an Asian population.

First, we found that the non-TT genotype at *GGH* T16C was associated with a high risk of liver dysfunction. This should be taken into consideration in treating patients carrying the non-TT genotype at *GGH* T16C with MTX in order to prevent liver dysfunction.

Once inside the cell, MTX undergoes FPGS-catalysed polyglutamation by the addition of two to seven glutamic acid groups. The polyglutamated form is not

Table 5

Association between *ATIC* 347CC genotype and methotrexate efficacy in patients with the early phase of juvenile idiopathic arthritis

(a)	OR†	95% Confidence interval	*P* value
ATIC 347CC genotype	0.32	0.11–0.93	0.033

(b)	OR‡	95% Confidence interval	*P* value
ATIC 347CC genotype	0.38	0.12–1.23	0.106
Rheumatoid factor [>14 (IU ml^{-1})]	0.22	0.07–0.72	0.012
Time interval*	0.85	0.70–1.04	0.12

*Time interval, time interval from disease onset to methotrexate treatment. †Non-adjusted odds ratio. ‡Adjusted odds ratio.

readily transported across the cell membrane, and thus, the intracellular half-life of MTX is increased. This polyglutamation process is reversed via GGH-catalysed removal of the glutamic acid groups. Therefore, the amount of intracellular MTX-polyglutamates (MTX-PGs) depends on the net rate of polyglutamation determined by the opposing activities of FPGS and GGH [8].

It was reported that *GGH* T16C, which results in a Cys6Arg substitution, was associated with the efficacy of MTX in patients with RA. The variant C allele may cause a loss of GGH activity, resulting in decreased efflux of MTX and thus increased intracellular MTX-PG levels [8]. This result was consistent with ours. Although we did not address the MTX-PG levels in hepatic cells, it is possible that the C allele at *GGH* T16C was associated with reduced GGH activity and thereby increased the MTX-PG levels in hepatic cells. As a result, the risk of liver dysfunction rises. The AA genotype at *FPGS* A1994G tended to be associated with liver dysfunction ($P = 0.068$, OR = 4.88, 95% CI 0.78–30.9). Future research using large study populations to address the effects of the combination of *GGH* and *FPGS* polymorphisms on MTX toxicity is needed.

The MTX dosage was probably associated with the toxicity and efficacy of the drug. In this cohort study, some patients underwent MTX treatment at other hospitals and had liver dysfunction before being referred to our institution. For these patients, we did not have access to previous medical records concerning the exact dosage of MTX at the time of liver dysfunction. As a general rule, non-responders to MTX received higher dosages of MTX (up to 10 mg m^{-2}) before the introduction of biologics than the responders. We therefore used MTX efficacy as the clinical predictor instead of MTX dosage. The MTX efficacy tended to be associated with liver dysfunction ($P = 0.083$), although the effect of MTX dosage on the toxicity and efficacy of this drug should be evaluated directly in the future.

Second, we found that the longer time interval from disease onset to MTX treatment and RF positivity were associated with lower efficacy of MTX. This was consistent with previous research results. Time to treatment was reported as an important factor in the response to MTX in patients with JIA [6], and RF positivity was associated with worse disease activity [18, 19].

Paediatric rheumatologists have recently been able to use MTX for patients with earlier phases of JIA, because MTX has become well known as a first-line drug in the treatment of RA and JIA [2, 3]. Therefore, we analysed the subgroup of early JIA patients. In those who were treated with MTX within 1 year of disease onset, the CC genotype at *ATIC* C347G tended to be associated with the lower efficacy of MTX. Methotrexate-polyglutamates inhibit ATIC, the last enzyme in the *de novo* purine synthesis pathway. Methotrexate achieves part of its anti-inflammatory effect through inhibition of ATIC, which results in the release of the anti-inflammatory agent, adenosine [9].

It was reported that RA patients with the G allele at *ATIC* C347G, resulting in a Thr116Ser substitution, were likely to have a good response to MTX [9]. Although the effect of the C347G polymorphism on ATIC enzyme activity is unknown, *ATIC* C347G may be in linkage disequilibrium with an unknown functional variant, which is associated with the activity of the purine synthesis pathway and with the level of adenosine production. Future basic and clinical prospective studies on a large number of patients are needed to elucidate this association.

There are some limitations to the present study. The incidence of RF positivity in the patient population studied was higher than generally seen (~10%) [18], presumably because our institution is one of the very few paediatric rheumatology centres in Japan, and many severe cases with RF positive are referred to our institution for highly specialized treatment with biologics [13, 20]. The efficacy rate of MTX in this study (28%) was significantly lower than those in previous Japanese reports [2, 3]. This may be due to the use of a new second-line choice of biologics, as well as the characteristics of our institution and the lower limit of the maximal MTX dosage (10 mg m^{-2}) for the treatment of JIA in Japan [2].

In summary, we found an association between the non-TT genotype at *GGH* T16C and liver dysfunction due to MTX. We also found an influence of time interval from disease onset to MTX treatment on the efficacy of MTX in Japanese patients with JIA. Our study showed the importance of early use of MTX for patients with JIA as well as the possibility of more personalized therapy for patients with JIA based on pharmacogenetic study of the MTX pathway genes.

Competing Interests

There are no competing interests to declare.

This work was supported by a grant from Grand-in-Aid for Scientific Research from Japan Society for the Promotion of Science (No. 16790583). We are grateful to Mr C. W. P. Reynolds and Teddy Kamata for their careful linguistic assistance with this manuscript.

REFERENCES

1 Cassidy JT, Petty RE. Juvenile idiopathic arthritis. In: Textbook of Pediatric Rheumatology, 5th edn. eds Cassidy JT, Petty RE. Philadelphia, PA: WB Saunders, 2005; 206–60.

2 Mori M, Naruto T, Imagawa T, Murata T, Takei S, Tomiita M, Itoh Y, Fujikawa S, Yokota S. Methotrexate for the treatment of juvenile idiopathic arthritis: process to approval for JIA indication in Japan. Mod Rheumatol 2009; 19: 1–11.

3 McKendry RJ, Dale P. Adverse effects of low dose methotrexate therapy in rheumatoid arthritis. J Rheumatol 1993; 20: 1850–6.

4 Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y, Akama H, Kitamura Y, Kamatani N. Polymorphisms in the methylenetetrahydrofolate reductase gene were associated with both the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analyses. Pharmacogenetics 2002; 12: 183–90.

5 Schmeling H, Biber D, Heins S, Horneff G. Influence of methylenetetrahydrofolate reductase polymorphisms on efficacy and toxicity of methotrexate in patients with juvenile idiopathic arthritis. J Rheumatol 2005; 32: 1832–6.

6 Albers HM, Wessels JA, van der Straaten RJ, Brinkman DM, Suijlekom-Smit LW, Kamphuis SS, Girschick HJ, Wouters C, Schilham MW, le Cessie S, Huizinga TW, Ten Cate R, Guchelaar HJ. Time to treatment as an important factor for the response to methotrexate in juvenile idiopathic arthritis. Arthritis Rheum 2009; 61: 46–51.

7 Laverdière C, Chiasson S, Costea I, Moghrabi A, Krajinovic M. Polymorphism G80A in the reduced folate carrier gene and its relationship to methotrexate plasma levels and outcome of childhood acute lymphoblastic leukemia. Blood 2002; 100: 3832–4.

8 van der Straaten RJ, Wessels JA, de Vries-Bouwstra JK, Goekoop-Ruiterman YP, Allaart CF, Bogaartz J, Tiller M, Huizinga TW, Guchelaar HJ. Exploratory analysis of four polymorphisms in human GGH and FPGS genes and their effect in methotrexate-treated rheumatoid arthritis patients. Pharmacogenomics 2007; 8: 141–50.

9 Dervieux T, Furst D, Lein DO, Capps R, Smith K, Walsh M, Kremer J. Polyglutamation of methotrexate with common polymorphisms in reduced folate carrier, aminoimidazole carboxamide ribonucleotide transformylase, and thymidylate synthase are associated with methotrexate effects in rheumatoid arthritis. Arthritis Rheum 2004; 50: 2766–74.

10 Toffoli G, De Mattia E. Pharmacogenetic relevance of MTHFR polymorphisms. Pharmacogenomics 2008; 9: 1195–206.

11 Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, He X, Maldonado-Cocco J, Orozco-Alcala J, Prieur AM, Suarez-Almazor ME, Woo P. International League of Associations for Rheumatology. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol 2004; 31: 390–2.

12 Mori M, Takei S, Imagawa T, Imanaka H, Maeno N, Kurosawa R, Kawano Y, Yokota S. Pharmacokinetics, efficacy, and safety of short-term (12 weeks) etanercept for methotrexate-refractory polyarticular juvenile idiopathic arthritis in Japan. Mod Rheumatol 2005; 15: 397–404.

13 Yokota S, Imagawa T, Mori M, Miyamae T, Aihara Y, Takei S, Iwata N, Umebayashi H, Murata T, Miyoshi M, Tomiita M, Nishimoto N, Kishimoto T. Efficacy and safety of tocilizumab in patients with systemic-onset juvenile idiopathic arthritis: a randomised, double-blind, placebo-controlled, withdrawal phase III trial. Lancet 2008; 371: 998–1006.

14 Nishimoto N, Ito K, Takagi N. Safety and efficacy profiles of tocilizumab monotherapy in Japanese patients with rheumatoid arthritis: meta-analysis of six initial trials and five long-term extensions. Mod Rheumatol 2010; 20: 222–32.

15 Gossec L, Dougados M, Goupille P, Cantagrel A, Sibilia J, Meyer O, Sany J, Daurès JP, Combe B. Prognostic factors for remission in early rheumatoid arthritis: a multiparameter prospective study. Ann Rheum Dis 2004; 63: 675–80.

16 Wessels JA, van der Kooij SM, le Cessie S, Kievit W, Barerra P, Allaart CF, Huizinga TW, Guchelaar HJ. A clinical pharmacogenetic model to predict the efficacy of methotrexate monotherapy in recent-onset rheumatoid arthritis. : Pharmacogenetics Collaborative Research Group. Arthritis Rheum 2007; 56: 1765–75.

17 International HapMap Consortium. The International HapMap Project. Nature 2003; 426: 789–96.

18 Petty RE, Cassidy JT. Polyarthritis. In: Textbook of Pediatric Rheumatology, 5th edn. eds Cassidy JT, Petty RE. Philadelphia, PA: WB Saunders, 2005; 261–73.

19 Adib N, Silman A, Thomson W. Outcome following onset of juvenile idiopathic inflammatory arthritis: II. predictors of outcome in juvenile arthritis. Rheumatology 2005; 44: 1002–7.

20 Yokota S, Mori M, Imagawa T, Murata T, Tomiita M, Itoh Y, Fujikawa S, Takei S. Guidelines on the use of etanercept for juvenile idiopathic arthritis in Japan. Pediatric Rheumatology Association of Japan. Mod Rheumatol 2010; 20: 107–13.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Supplementary data 1
TaqMan® SNP Genotyping Assays.
Supplementary data 2
Distribustion of gene polymorpshisms under the study.
Supplementary data 3
Distribustion of gene polymorpshisms in patiets with or without liver dysfunction.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

J Clin Immunol (2011) 31:946–951
DOI 10.1007/s10875-011-9583-x

Association of *IRF5* Polymorphisms with Susceptibility to Hemophagocytic Lymphohistiocytosis in Children

Masakatsu Yanagimachi · Hiroaki Goto · Takako Miyamae · Keisuke Kadota · Tomoyuki Imagawa · Masaaki Mori · Hidenori Sato · Ryu Yanagisawa · Tetsuji Kaneko · Satoshi Morita · Eiichi Ishii · Shumpei Yokota

Received: 2 May 2011 / Accepted: 9 August 2011 / Published online: 4 September 2011

Abstract
Introduction Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome and has a varied genetic background. The polymorphism of *interferon regulatory factor 5* gene (*IRF5*) was reported to be associated with susceptibility to macrophage activation syndrome. IRF5 acts as a master transcription factor in the activation of pro-inflammatory cytokines. We assessed associations of *IRF5* gene polymorphisms with susceptibility to secondary HLH.
Methods Three *IRF5* single nucleotide polymorphisms (rs729302, rs2004640, and rs2280714) were genotyped using TaqMan assays in 82 secondary HLH patients and 188 control subjects.
Results There was a significant association of the GT/TT genotype at rs2004640 with secondary HLH susceptibility ($p<0.01$). The *IRF5* haplotype (rs729302 A, rs2004640 T, and rs2280714 T) was associated with secondary HLH susceptibility ($p<0.01$).
Conclusions These findings indicate that *IRF5* is a genetic factor influencing the susceptibility to secondary HLH and that the IRF5-associated immune response contributes to the pathogenesis of HLH.

Keywords Interferon regulatory factor 5 · polymorphisms · hemophagocytic lymphohistiocytosis

Electronic supplementary material The online version of this article (doi:10.1007/s10875-011-9583-x) contains supplementary material, which is available to authorized users.

M. Yanagimachi · H. Goto (✉) · T. Miyamae · K. Kadota · T. Imagawa · M. Mori · S. Yokota
Department of Pediatrics,
Yokohama City University Graduate School of Medicine,
Yokohama, Japan
e-mail: hgoto39@med.yokohama-cu.ac.jp

H. Sato
CNV Laboratory, DNA Chip Research Institute,
Yokohama, Japan

R. Yanagisawa
Department of Pediatrics, Shinshu University School of Medicine,
Matsumoto, Japan

T. Kaneko · S. Morita
Department of Biostatistics and Epidemiology,
Yokohama City University Graduate School of Medicine,
Yokohama, Japan

E. Ishii
Department of Pediatrics,
Ehime University Graduate School of Medicine,
Ehime, Japan

E. Ishii
The HLH Study Committee,
Japanese Pediatric Leukemia/Lymphoma Study Group,
Nagoya, Japan

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome that is accompanied by serious morbidity [1, 2]. The incidence of HLH is estimated to be about 1.2 cases per million individuals per year [3]. HLH is characterized by prolonged fever, cytopenias, hepatosplenomegaly, and hemophagocytosis in reticuloendothelial systems. The characteristic laboratory findings include hypertriglyceridemia, hyperferritinemia, hypofibrinogeneima, and increased soluble CD25 [1–4]. These manifestations and laboratory values are described as the result of hypercytokinemia caused by an

ineffective immunological response mediated by histiocytes (macrophages and dendritic cells), natural killer (NK) cells, and cytotoxic T cells (CTL) [1, 5–7]. Increased levels of several pro-inflammatory cytokines, such as interleukin-6 (IL-6), interferon (IFN)-γ, and tumor necrosis factor (TNF)-α have been demonstrated in patients with HLH [8–10]. HLH is classified into primary (genetic) or secondary (acquired) HLH. There are two subtypes of primary HLH, namely, familial HLH (FHL) and other immunodeficiencies such as Chediak–Higashi syndrome, Griscelli syndrome type 2, Hermansky–Pudlak syndrome type 2, and the X-linked lymphoproliferative syndrome [2, 11]. Mutations of *perforin* (*PRF1*), *UNC13D*, *STX11*, and *STXBP2* genes are responsible for 30–70% of FHLH cases [12–16]. It is thought that other unknown genetic defects remain as causes of FHL. Secondary HLH may occur under conditions of severe infections, malignancies, or autoimmune diseases [1, 2]. Many viruses, bacteria, and other infectious agents have been reported to trigger infection-associated HLH (IHLH) [17]. Epstein–Barr virus (EBV) is the most studied virus that trigger IHLH [18]. EBV-associated HLH (EBV-HLH) has a higher prevalence in East Asian countries [18]. Therefore, there may be a genetic variation in susceptibility to EBV-HLH.

Genetic factors other than *PRF1*, *UNC13D*, *STX11*, and *STXBP2* might influence susceptibility even to secondary HLH. Macrophage activation syndrome (MAS) is one form of secondary HLH [1, 2]. MAS occurs in patients with autoimmune diseases, especially with systemic-onset juvenile idiopathic arthritis (systemic JIA) [19, 20]. We recently reported that the *interferon regulatory factor 5* (*IRF5*) gene polymorphism is associated with susceptibility to MAS in systemic JIA patients [21]. IRF5 is a member of the IRF family of transcription factors and is known to have a crucial role in the Toll-like receptor signaling pathway [22, 23]. The activation of the Toll-like receptor is central to innate and adaptive immunity. IRF5 acts as a master transcription factor in the activation of pro-inflammatory cytokine genes especially in the virus-mediated immunological signaling pathway [23]. In *IRF5* knockout mice, a severely impaired induction of IL-6, IL-12, and TNF-α was observed [22].

In the present study, we hypothesized that polymorphisms in the *IRF5* gene may be associated with susceptibility to secondary HLH. We found a close relationship between the *IRF5* gene polymorphism/haplotype and susceptibility to secondary HLH.

Patients and Methods

Study Population

Patients with secondary HLH except for MAS were diagnosed based on the diagnostic criteria used in the HLH-94 Study (for patients who developed HLH before October 2006) and HLH-2004 Study (after October 2006) [4, 24]. The patients who showed known genetic mutations were excluded as primary HLH in this study. Patients under 1 year were also excluded to reduce the possible inclusion of undiagnosed primary HLH because the onset of FHL is below 1 year of age in 70–80% of the cases [25].

Patients with MAS were diagnosed as having systemic JIA based on the International League of Associations for Rheumatology classification criteria for systemic JIA [26]. Because the HLH-94/2004 diagnostic criteria may not always be appropriate when diagnosing MAS in systemic JIA patients who are under inflammatory conditions, patients with systemic JIA were diagnosed as having MAS based on the preliminary diagnostic guidelines for MAS complicating systemic JIA [27], as follows: (1) clinical criteria including central nervous dysfunction, hemorrhage or hepatomegaly and (2) laboratory criteria including decreased platelet counts ($<26.2\times10^9$/l), elevated levels of asparate aminotransferase (>59 U/l), decreased white blood cell counts ($<4.0\times10^9$/l), and hypofibrinogenemia (<2.5 g/l). The diagnosis of MAS requires the presence of two or more of these criteria.

For the diagnosis of EBV-HLH, EBV load in peripheral blood was quantified by real-time PCR as described in our previous study [28]. Patients were diagnosed as having EBV-HLH if they had EBV loads of over 1,000 genome copies per milliliter in whole blood and fulfilled the diagnostic criteria used in the HLH-94/HLH-2004 Study.

A total of 82 patients, 39 males and 43 females, were enrolled in the present study. Among the 82 patients, 48, including 33 having systemic JIA with MAS, were diagnosed as having secondary HLH at Yokohama City University Hospital between November 2000 and December 2009. The remaining 34 patients, who were diagnosed as having secondary HLH between March 2007 and December 2010, were registered in the HLH-2004 as a study of Japanese Pediatric Leukemia/Lymphoma Study Group. In these patients, 32 were diagnosed as having EBV-HLH. The 188 control subjects were recruited from apparently healthy adult volunteers.

Notably, the 33 MAS patients were identical to those analyzed in our previous study, where the significance of *IRF5* polymorphisms was evaluated among systemic JIA patients with or without MAS. In this study, to evaluate the significance of *IRF5* polymorphisms in the susceptibility to secondary HLH as a whole, data were reanalyzed in comparison with healthy controls using the different study population.

This study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Yokohama City University School of Medicine and each member of the Japan Leukemia/Lymphoma Study Group. Written informed consent was obtained from each patient or his/her guardians as well as the control subjects.

Table I Characteristics entire secondary HLH Study Group and subgroups

	N	Age	Gender
All patients with secondary HLH	82	4.7 (1–16)	39 (47.6%)
Subgroups of HLH patients			
MAS	33	4.8 (1–16)	16 (48.5%)
Non-MAS HLH	49	4.6 (1–15)	23 (46.9%)
EBV-HLH	32	4.3 (1–15)	16 (50.0%)

HLH hemophagocytic lymphohistiocytosis, *MAS* macrophage activation syndrome, *Non-MAS HLH* secondary HLH including EBV-HLH but not MAS, *EBV-HLH* Epstein–Barr virus-associated HLH

Genotyping

Three SNPs—rs729302, rs2004640, and rs2280714—in the *IRF5* gene were selected as described in our previous study [21]. Patients with HLH and control subjects were genotyped using TaqMan SNP Genotyping Assays as described previously [21].

Statistical Analysis

The SNPassoc package using R-language, version 2.8 (The R Foundation for Statistical Computing, http://www.R-project.org) was employed to evaluate the associations between HLH and the SNPs by logistic regression analysis [29]. Haplotype phases and haplotype frequencies were estimated using the Expectation–Maximization algorithm as implemented in the haplostat package (minimum haplotype frequency, >0.05; www.docstoc.com.) [30]. The associations between genotypes under study and laboratory values were analyzed by the Jonckheere–Terpstra test. The following laboratory values were included: levels of hemoglobin, neutrophils, platelets, triglycerides, fibrinogen, ferritin, transaminases, and lactate dehydrogenase. The association between HLH and the *IRF5* haplotypes was evaluated by logistic regression analysis.

Results

Patient characteristics are summarized in Table I. The mean age of the 82 patients with secondary HLH was 4.7 years (1–16 years) at onset. The numbers of patients with MAS and non-MAS HLH were 33 and 49, respectively. In those with non-MAS HLH, 32 with EBV-HLH were included.

The genotype frequencies for the three SNPs of the HLH patients, including their subgroups, and the control subjects were in Hardy–Weinberg equilibrium ($p>0.05$). These results were consistent with the findings of a recent Japanese population study (Table II) [31].

Table II Association of polymorphisms in the *IRF5* gene with susceptibility to secondary HLH

SNP subject subset	*n*	MAF	Allelic association			
			OR	(95% CI)	*p* value	p_c
		rs729302				
All patients with secondary HLH	82	0.20	1.05	0.96–1.15	0.26	n.s.
Subgroups of HLH patients						
MAS	33	0.18	1.04	0.96–1.12	0.32	n.s.
Non-MAS HLH	49	0.20	1.03	0.95–1.12	0.46	n.s.
EBV-HLH	32	0.23	1.00	0.93–1.10	0.90	n.s.
Control subjects	188	0.24	1.0	–	–	
		rs2004640				
All patients with secondary HLH	82	0.49	0.88	0.82–0.95	<0.01	0.006
Subgroups of HLH patients						
MAS	33	0.50	0.92	0.86–0.99	0.02	n.s.
Non-MAS HLH	49	0.49	0.91	0.84–0.98	0.01	0.030
EBV-HLH	32	0.55	0.95	0.88–1.01	0.11	n.s.
Control subjects	188	0.35	1.0	–	–	
		rs2280714				
All patients with secondary HLH	82	0.34	1.1	1.02–1.19	0.02	0.0465
Subgroups of HLH patients						
MAS	33	0.32	1.07	1.00–1.14	0.06	n.s.
Non-MAS HLH	49	0.35	1.07	0.99–1.14	0.09	n.s.
EBV-HLH	32	0.36	1.04	0.98–1.12	0.22	n.s.
Control subjects	188	0.44	1.0	–	–	

IRF5 interferon regulatory factor 5, *SNP* single nucleotide polymorphism, *MAF* minor allele frequency (the C allele at rs729302, T rs2004640, C rs2280714), p_c corrected combined *p* value using the Bonferroni method

Table III Association of polymorphisms in the *IRF5* gene with susceptibility to secondary HLH

SNP	MM/Mm vs. mm			MM vs. Mm/mm		
	OR	(95% CI)	*p* value	OR	(95% CI)	*p* value
rs729302	2.62	0.75–9.19	0.137	1.19	0.69–2.03	0.59
rs2004640	0.43	0.22–0.84	0.18	0.47	0.26–0.83	<0.01
rs2280714	2.54	1.08–5.97	0.03	1.59	0.93–2.71	0.096

Minor allele: the C allele at rs729302, T rs2004640, C rs2280714

SNP single nucleotide polymorphism, *M* major alleles, *m* minor allele

rs2004640 and rs2280714 were associated with susceptibility to secondary HLH as a whole even after Bonferroni correction (Table II). The T allele at rs2004640 was a risk factor for susceptibility to not only secondary HLH as a whole (p_c=0.006, OR=1.13, 95% CI=1.05–1.23) but also to non-MAS HLH (p_c=0.030, OR=1.10, 95% CI=1.02–1.19; Table II). Moreover, the GT/TT genotype at rs2004640 presented a risk for secondary HLH in general (p_c=0.028, OR=2.15, 95% CI=1.21–3.82; Table III). This genotype was also associated with non-MAS HLH (p_c=0.04, OR=2.28, 95% CI=1.12–4.66; Electronic Supplementary Material (ESM) Table 1).

Additionally, a statistically significant association of the ATT haplotype of the *IRF5* gene (rs729302–rs2004640–rs2280714) with susceptibility to secondary HLH was shown (p<0.001, OR=1.92, 95% CI=1.21–3.04; Table IV). This haplotype was also associated with susceptibility to subtypes of the MAS and non-MAS HLH, respectively, but not to EBV-HLH (ESM Table 2).

With regard to the laboratory values in the 34 patients with non-MAS HLH registered in the HLH-2004 Study, the low platelet count was associated with the C allele at rs2280714 (p=0.026, Jonckheere–Terpstra test). Other laboratory values were not associated with the IRF5 gene polymorphisms studied (data not shown).

Discussion

HLH is a clinically heterogeneous syndrome presumably because it is associated with a variety of genetic background. Even in primary HLH, there remain about 30% of FHL patients with unknown responsible genes [13]. With regard to secondary HLH, there may be several HLH-susceptible genes. Although mutations of *PRF1*, *UNC13D*, *STX11*, and *STXBP2* genes can be causable for the pathogenesis of FHL, a particular HLH-susceptible gene may contribute to the pathogenesis of secondary HLH cooperatively with other HLH-susceptible genes and may have the potential of influencing the severity of HLH.

In the present study, we revealed that the T allele at rs2004640 and the ATT haplotype in *IRF5* gene are associated with susceptibility to secondary HLH as well as to MAS in systemic JIA patients. The ATT haplotype in the *IRF5* gene was also associated with an increased risk of SLE [32]. The T alleles at both rs2004640 and rs2280714 were related to higher levels of IRF5 mRNA expression [32]. There seems a potentially important role of the IRF5-associated immune response in the pathogenesis of secondary HLH.

In many cases of HLH, viral infections trigger both primary and secondary HLH [18, 33]. Also, IRF5 has a key role in the induction of the antiviral and inflammatory response and controls the production of pro-inflammatory cytokines [22]. Therefore, the association between gene polymorphisms of *IRF5* and susceptibility to HLH is plausible. In order to assess whether there is an influence of *IRF5* gene polymorphisms on IHLH, we analyzed the association between *IRF5* gene polymorphisms and EBV-HLH. The *IRF5* gene polymorphisms tended to be associated with EBV-HLH, but without statistical significance, presumably because of the small number of patients in this study. Ineffective activation of histiocytes, NK cells, and CTL following viral infections is considered important in the pathogenesis of HLH [5–7]. Recently, several research outcomes were reported about the influence of IRF5 on the function of these immune cells [34–37]. For instance, M1 macrophages, which produce pro-inflammatory cytokines and mediate resistance to pathogens, were characterized by large amounts of IRF5 compared with

Table IV Comparison of IRF5 haplotypes in patients with secondary HLH

The order of SNPs in haplotype is rs729302-rs2004640-rs2280714

Haplotype	Haplotype frequencies in control subjects	Haplotype frequencies in secondary HLH patients	*p* value	OR	95% CI
A-G-C	0.405	0.302	0.02	1.0	–
C-G-T	0.208	0.174	0.37	1.19	0.70–2.04
A-T-T	0.333	0.461	<0.001	1.92	1.21–3.04

M2 macrophages, which produce anti-inflammatory cytokines and promote tissue repair [36]. In addition, IRF5 controls the induction of chemokines, such as IL-8, that mediate recruitment of T lymphocytes [34]. Therefore, IRF5 presumably serves as one of the key factors for the pathogenesis of HLH via influencing the function of these immune cells.

The present study still has some limitations. The first issue is the definition of secondary HLH. The patients with the following criteria were excluded from the study: positive defects of known genes (*PRF1*, *UNC13D*, *STX11*, *STXBP2*, and *SAP*), <1 year old at onset, and low or deficient CTL/NK activity. In male patients who had recurrent HLH episodes or were refractory to treatment, mutations in the *SH2D1A* genes were ruled out [38]. With using these criteria, almost all of the patients can be diagnosed with secondary HLH.

The second issue is that we could not perform a validation study. Although a genetic association study should be validated, the incidence of HLH is too low to validate this association in a single institution and even in a nationwide study. Therefore, it is important that the association between the *IRF5* genotype/haplotype and HLH susceptibility is confirmed by other groups.

We found a close relationship between polymorphisms in the *IRF5* gene and susceptibility to secondary HLH in general and its subtypes (MAS and non-MAS HLH), respectively. This finding suggests a potentially important role of the IRF5-associated immune response in the pathogenesis of HLH.

Acknowledgments This work was supported by a grant from Yokohama Foundation for Advancement of Medical Science. We thank the physicians who participated in the HLH-94 and HLH-2004 studies in Japan and all members of the HLH Study Committee of the Japan Leukemia/Lymphoma Study Group. We also thank M Maesato for her secretarial assistance.

References

1. Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: clinical features, pathogenesis and therapy. Expert Rev Clin Immunol. 2010;6(1):137–54.
2. Janka GE. Hemophagocytic syndromes. Blood Rev. 2007;21(5):245–53.
3. Henter JI, Elinder G, Soder O, Ost A. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. Acta Paediatr Scand. 1991;80(4):428–35.
4. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124–31.
5. Jordan MB, Hildeman D, Kappler J, Marrack P. An animal model of hemophagocytic lymphohistiocytosis (HLH): $CD8^+$ T cells and interferon gamma are essential for the disorder. Blood. 2004;104 (3):735–43.
6. Marcenaro S, Gallo F, Martini S, Santoro A, Griffiths GM, Arico M, et al. Analysis of natural killer-cell function in familial hemophagocytic lymphohistiocytosis (FHL): defective CD107a surface expression heralds Munc13-4 defect and discriminates between genetic subtypes of the disease. Blood. 2006;108(7):2316–23.
7. Schneider EM, Lorenz I, Muller-Rosenberger M, Steinbach G, Kron M, Janka-Schaub GE. Hemophagocytic lymphohistiocytosis is associated with deficiencies of cellular cytolysis but normal expression of transcripts relevant to killer-cell-induced apoptosis. Blood. 2002;100(8):2891–8.
8. Akashi K, Hayashi S, Gondo H, Mizuno S, Harada M, Tamura K, et al. Involvement of interferon-gamma and macrophage colony-stimulating factor in pathogenesis of haemophagocytic lymphohistiocytosis in adults. Br J Haematol. 1994;87(2):243–50.
9. Imashuku S, Hibi S, Sako M, Ishii T, Kohdera U, Kitazawa K, et al. Heterogeneity of immune markers in hemophagocytic lymphohistiocytosis: comparative study of 9 familial and 14 familial inheritance-unproved cases. J Pediatr Hematol Oncol. 1998;20(3):207–14.
10. Ohga S, Matsuzaki A, Nishizaki M, Nagashima T, Kai T, Suda M, et al. Inflammatory cytokines in virus-associated hemophagocytic syndrome. Interferon-gamma as a sensitive indicator of disease activity. Am J Pediatr Hematol Oncol. 1993;15(3):291–8.
11. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr. 2007;166(2):95–109.
12. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, et al. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. 2003;115(4):461–73.
13. Ishii E, Ohga S, Imashuku S, Yasukawa M, Tsuda H, Miura I, et al. Nationwide survey of hemophagocytic lymphohistiocytosis in Japan. Int J Hematol. 2007;86(1):58–65.
14. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. 1999;286(5446):1957–9.
15. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet. 2005;14(6):827–34.
16. zur Stadt U, Rohr J, Seifert W, Koch F, Grieve S, Pagel J, et al. Familial hemophagocytic lymphohistiocytosis type 5 (FHL-5) is caused by mutations in Munc18-2 and impaired binding to syntaxin 11. Am J Hum Genet. 2009;85(4):482–92.
17. Fisman DN. Hemophagocytic syndromes and infection. Emerg Infect Dis. 2000;6(6):601–8.
18. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007;7(12):814–22.
19. Grom AA, Mellins ED. Macrophage activation syndrome: advances towards understanding pathogenesis. Curr Opin Rheumatol. 2010;22(5):561–6.
20. Kelly A, Ramanan AV. Recognition and management of macrophage activation syndrome in juvenile arthritis. Curr Opin Rheumatol. 2007;19(5):477–81.
21. Yanagimachi M, Naruto T, Miyamae T, Hara T, Kikuchi M, Hara R, et al. Association of IRF5 polymorphisms with susceptibility to macrophage activation syndrome in patients with juvenile idiopathic arthritis. J Rheumatol. 2011;38:769–74.
22. Takaoka A, Yanai H, Kondo S, Duncan G, Negishi H, Mizutani T, et al. Integral role of IRF-5 in the gene induction programme activated by Toll-like receptors. Nature. 2005;434 (7030):243–9.
23. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol. 2001;19:623–55.
24. Henter JI, Arico M, Egeler RM, Elinder G, Favara BE, Filipovich AH, et al. HLH-94: a treatment protocol for hemophagocytic lymphohistiocytosis. HLH Study Group of the Histiocyte Society. Med Pediatr Oncol. 1997;28(5):342–7.
25. Arico M, Janka G, Fischer A, Henter JI, Blanche S, Elinder G, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children

from the International Registry. FHL Study Group of the Histiocyte Society. Leukemia. 1996;10(2):197–203.
26. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004;31(2):390–2.
27. Ravelli A, Magni-Manzoni S, Pistorio A, Besana C, Foti T, Ruperto N, et al. Preliminary diagnostic guidelines for macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. J Pediatr. 2005;146(5):598–604.
28. Matsuda K, Nakazawa Y, Yanagisawa R, Honda T, Ishii E, Koike K. Detection of T-cell receptor gene rearrangement in children with Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis using the BIOMED-2 multiplex polymerase chain reaction combined with GeneScan analysis. Clin Chim Acta. 2011;412:1554–8.
29. Gonzalez JR, Armengol L, Sole X, Guino E, Mercader JM, Estivill X, et al. SNPassoc: an R package to perform whole genome association studies. Bioinformatics. 2007;23(5):644–5.
30. Lake SL, Lyon H, Tantisira K, Silverman EK, Weiss ST, Laird NM, et al. Estimation and tests of haplotype–environment interaction when linkage phase is ambiguous. Hum Hered. 2003;55(1):56–65.
31. Shimane K, Kochi Y, Yamada R, Okada Y, Suzuki A, Miyatake A, et al. A single nucleotide polymorphism in the IRF5 promoter region is associated with susceptibility to rheumatoid arthritis in the Japanese population. Ann Rheum Dis. 2009;68(3):377–83.
32. Graham RR, Kozyrev SV, Baechler EC, Reddy MV, Plenge RM, Bauer JW, et al. A common haplotype of interferon regulatory factor 5 (IRF5) regulates splicing and expression and is associated with increased risk of systemic lupus erythematosus. Nat Genet. 2006;38(5):550–5.
33. Henter JI, Ehrnst A, Andersson J, Elinder G. Familial hemophagocytic lymphohistiocytosis and viral infections. Acta Paediatr. 1993;82(4):369–72.
34. Barnes BJ, Kellum MJ, Field AE, Pitha PM. Multiple regulatory domains of IRF-5 control activation, cellular localization, and induction of chemokines that mediate recruitment of T lymphocytes. Mol Cell Biol. 2002;22(16):5721–40.
35. Hao S, Li P, Zhao J, Hu Y, Hou Y. 17beta-estradiol suppresses cytotoxicity and proliferative capacity of murine splenic NK1.1+ cells. Cell Mol Immunol. 2008;5(5):357–64.
36. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, et al. IRF5 promotes inflammatory macrophage polarization and T (H)1–T(H)17 responses. Nat Immunol. 2011;12(3):231–8.
37. Krausgruber T, Saliba D, Ryzhakov G, Lanfrancotti A, Blazek K, Udalova IA. IRF5 is required for late-phase TNF secretion by human dendritic cells. Blood. 2010;115(22):4421–30.
38. Sumazaki R, Kanegane H, Osaki M, Fukushima T, Tsuchida M, Matsukura H, et al. SH2D1A mutations in Japanese males with severe Epstein–Barr virus-associated illnesses. Blood. 2001;98(4):1268–70.

Rheumatol Int
DOI 10.1007/s00296-011-2225-z

ORIGINAL ARTICLE

Novel mutations of MVK gene in Japanese family members affected with hyperimmunoglobulinemia D and periodic fever syndrome

Takahisa Mizuno · Hidemasa Sakai · Ryuta Nishikomori · Koichi Oshima · Osamu Ohara · Ikue Hata · Yosuke Shigematsu · Takashi Ishige · Kazushi Tamura · Hirokazu Arakawa

Received: 12 July 2011 / Accepted: 22 October 2011

Abstract Hyperimmunoglobulinemia D with periodic fever syndrome (HIDS) is a recessively inherited recurrent fever syndrome. We describe a family of eldest son and monozygotic twin younger sisters with characteristic syndrome of HIDS, but normal level of IgD. Mevalonate kinase (MK) activity was deficient in all of them, and analysis of the MVK gene revealed compound heterozygosity for 2 new mutations, one of which was the disease-causing splicing mutation and the other was a novel missense mutation. All the patients had the same compound heterozygous mutations c.227-1 G > A and c.833 T > C, which resulted in exon 4 skipping and p.Val278Ala. This is the first case in which exon skipping mutation of the MVK gene has been certainly identified at the genomic DNA level. In each case, in which HIDS is clinically suspected, despite normal IgD level, analysis of MK activity and the MVK gene should be performed.

Keywords HIDS · MVK gene · Novel mutation · Compound heterozygous mutation · Splicing mutation · Inherited recurrent fever syndrome

T. Mizuno · T. Ishige · K. Tamura · H. Arakawa (✉)
Department of Pediatrics and Developmental Medicine, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan
e-mail: harakawa@gunma-u.ac.jp

H. Sakai · R. Nishikomori
Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

K. Oshima · O. Ohara
Laboratory for Immunogenomics, RIKEN Research Center for Allergy and Immunology, Kanagawa, Japan

I. Hata
Department of Pediatrics, Faculty of Medical Sciences, University of Fukui, Fukui, Japan

Y. Shigematsu
Department of Health Science, Faculty of Medical Sciences, University of Fukui, Fukui, Japan

Introduction

Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) is a rare autosomal recessive auto-inflammatory disorder characterized by recurrent febrile attacks with lymphadenopathy, abdominal distress, skin eruptions, and joint involvement [1–3]. Febrile attacks usually last for 3–7 days and are interrupted by asymptomatic intervals of several weeks' duration [4–6]. Symptoms appear in early infancy and may persist throughout life with gradual increases in serum IgD [7, 8]. The diagnostic hallmark of HIDS is a constitutively elevated level of serum IgD, although parts of the patients have been reported to have normal amount of serum IgD levels.

The HIDS is caused by mutations on mevalonate kinase gene (MVK), which encodes an enzyme involved in cholesterol and non-sterol isoprenoid biosynthesis. We present herein a Japanese family, eldest son and monozygotic twin younger sisters, with HIDS that had compound heterozygous mutations on MVK gene, one of which was the disease-causing splicing mutation and the other was a novel missense mutation. Serum concentrations of IgD were repeatedly within the normal range. These cases demonstrate that detail analysis with more specific diagnostic tests such as urinary excretion of mevalonic acid and MVK genetic analysis should be performed not to miss the correct diagnosis in patients, especially younger children with HIDS.

Published online: 11 December 2011

Case reports

Patients are the eldest son and monozygotic twin younger sisters of parents of Japanese origin. The eldest son (patient 1) had presented with recurrent fever from 5 months of age. The twin younger sisters (patient 2 and 3) presented with fever from 1 month of age. Vomiting and diarrhea were presented in the younger sister (patient 3). Febrile episodes appeared every 4–8 weeks and lasted for 3–5 days on all the three patients. During febrile episodes, peripheral blood leukocytosis and CRP elevations (more than 10 mg/dl) were observed. In intermittent period between fever episodes, serum CRP levels decreased, but did not always become negative. Their parents had no history of recurrent fever. Sepsis work-up did not show any foci and any pathogens causing the febrile episodes. The repeated bacterial cultures resulted in negative, and administration of the antimicrobial agents did not change the clinical courses of the febrile episodes, indicating that the fever was not induced by pathogen. In addition, immunological analysis such as serum IgA, IgM, IgG, and IgD, lymphocytes counts including T, B, NK cells, and mitogen proliferation assays of peripheral blood mononuclear cells (PBMCs) were normal.

Due to the recurrent high fevers caused most unlikely by pathogen and the heavy family history of the periodic fevers, we suspected hereditary periodic fever syndromes and performed genetic study. After written informed consents approved by institutional review board of the Kyoto University Hospital were obtained, peripheral blood samples were collected from the patients and their parents for isolating genomic DNA and total RNA.

First, we performed genomic DNA sequencing for MEFV gene for familial Mediterranean fever, MVK gene for HIDS, NLRP3 for cryopyrin-associated periodic syndrome, and TNFRSF1A for TNF receptor-associated periodic syndrome. Genomic DNA sequencing analysis of the MVK gene revealed the presence of heterozygous mutations of c.227-1 G > A at the exon/intron border of exon 4 and c.833T > C (p.Val278Ala). Subsequent amplification of the cDNA by RT-PCR showed that the former mutation caused deletion of exon 4 (Fig. 1a). Genomic DNA sequence analysis on their parents revealed that the parents inherited c.227-1 G > A from their father and c.833T > C from their mother, indicating that the three patients were compound heterozygous for MVK gene (Fig. 1b). The patients had markedly elevated excretion of mevalonic acid in urine, especially in febrile periods, and their mevalonate kinase enzyme activities were very low, which confirmed that all the three patients suffered from HIDS (Table 1).

While the patients did not have any mutations on TNFRSF1A and NLRP3, we identified MEFV non-synonymous nucleotide alterations on the elder brother, who was a heterozygote for L110P, E148Q, and R202Q, and the younger twin, who was a heterozygote for R202Q in addition to MVK gene mutations. These MEFV gene nucleotide alterations were regarded as SNPs, and the clinical diagnosis of FMF was not compatible with the patients, although the complex MEFV gene alterations of L110P/E148Q/R202Q have been reported on the clinically-diagnosed FMF patients.

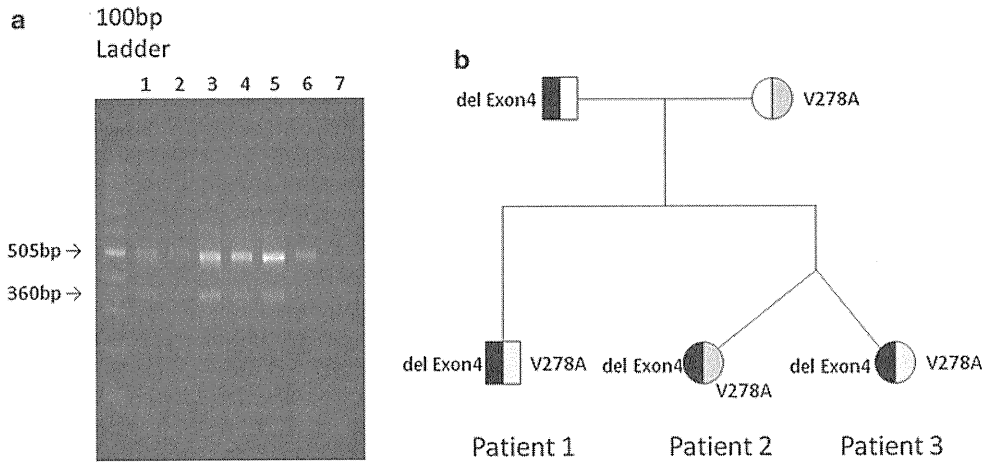

Fig. 1 Molecular genetic findings in the study patients. **a** Agarose gel electrophoresis of RT-PCR products for exon 2 to exon 5 of MVK shows the normal 505-bp alleles in samples from normal healthy control (*lane 6*) and mother (*lane 2*), as well as both the normal allele and the mutant 362-bp allele in the sample from father (*lane 1*), patient 1 (*lane 3*), patient 2 (*lane 4*), and patient 5 (*lane 5*). Subsequent cDNA sequencing confirmed that this 144-bp deletion in cDNA corresponds to codon 303–407 (exon 4). The molecular size marker was a 100-bp ladder. *Lane 7* represents PCR with distilled water added but not with DNA, indicating that there was no background amplification. **b** Pedigree of the affected family. The three patients are heterozygous for del exon 4 and V278A

Table 1 Urinary mevalonic acid and mevalonate kinase levels in the study patients

Patient no.	Mevalonic acid in urine (μg/mgCr)		Mevalonate kinase (pmol/minute/mg)
	Febrile period	Intermittent period	
1	67.9	11.3	3
2	55.6	17.7	2
3	58.8	18.5	2
Control		0.078 ± 0.012[a]	214 ± 62[a]

Control data are given as mean ± SD

[a] Values form healthy subjects were used to obtain a control range for urinary mevalonic acid levels (mean ± SD) and mevalonate kinase levels (mean ± SD)

Discussion

We present herein a sibling of HIDS that demonstrated compound heterozygous for two novel mutations of MVK gene. All the patients had the same compound heterozygous mutations c.227-1 G > A and c.833T > C, which resulted in exon 4 skipping and p.Val278Ala. The mutations are novel, especially the splicing mutation of MVK gene was identified at the genomic DNA level.

Cuisset et al. [9] reported that HIDS mutations were evenly distributed along the coding region of the MVK gene, in contrast to mutations causing MA, which clustered between 243 and 334. The sequence variations seen in MA are missense mutations that are in the same region as the variants described in HIDS. Further studies will be needed to clarify the association of phenotypical differences with MVK gene mutations. Over 80% of patients with HIDS were reported to have compound heterozygous mutation in the MVK gene. To our knowledge, both the skipping of exon 4 and V278A mutation have not been reported previously in HIDS. Moreover, this is the first case in which exon skipping mutation of the MVK gene has been certainly identified at the genomic DNA level. Only few groups reported HIDS patients with the skipping of exon in the cDNA of the MVK gene [10, 11]. They suggested that these exon skipping was probably due to the presence of a potential splice site mutation, but could not identify mutations responsible for these altered splicing through the sequence analysis at the genomic level. Most MVK mutations in patients with HIDS and MA have only been determined at the cDNA level; however, analysis of cDNA sometimes appeared troublesome, probably due to instability of the MVK mRNA. More detailed studies through the sequence analysis at the genomic level lead us to elucidate the role of MVK mutations in HIDS and MA, and expression studies in *E. coli* will be necessary to evaluate the effect of each mutation.

HIDS is classically defined as a high concentration of mevalonic acid in the urine and is characterized by a high serum IgD concentration during each febrile episode, but some reports from the Netherlands stated that high levels of serum IgD levels were not seen and affirmed that other diseases also showed high serum IgD levels [12]. In our cases, the analysis of enzymes and molecular genetics of MVK gene yielded the correct diagnosis, although serum concentrations of IgD were within the normal range. Thus, it should be now common practice to examine the MVK gene in order to diagnose this disease.

In conclusion, we present a Japanese family with HIDS that appeared to have novel mutations of MVK gene. Most of the HIDS cases were reported from European, especially Dutch, whereas only one HIDS case of Japanese patient was reported by Naruto et al. [13], which is only one report of Asian patient. Cases of HIDS may so far have been overlooked or misdiagnosed as infectious diseases or autoimmune disorders in Japan, besides there may be difference in race. It is necessary that accumulation of case in hereditary mutation and in other race leads to solve a detailed cause of HIDS.

Acknowledgments The authors thank Dr. Georg F. Hoffmann for measurement regarding the mevalonic kinase activity.

Conflict of interest There is no financial or other potential conflict of interest for each author.

References

1. van der Meer JW, Vossen JM, Radl J, Van Nieuwkoop JA, Meyer CJ, Lobatto S et al (1984) Hyperimmunoglobulinaemia D and periodic fever: a new syndrome. Lancet 1:1087–1090
2. Church LD, Churchman SM, Hawkins PN, McDermott MF (2006) Hereditary auto-inflammatory disorders and biologics. Semin Immunopathol 27:494–508
3. Simon A, van der Meer JW (2007) Pathogenesis of familial periodic fever syndromes or hereditary autoinflammatory syndromes. Am J Physiol Regul Integr Comp Physiol 292:R86–R98
4. Fenkel J, Houten SM, Waterham HR, Wanders RJ, Rijkers GT, Duran M et al (2001) Clinical and molecular variability in childhood periodic fever with hyperimmunoglobuninaemia D. Rheumatology (Oxford) 40:579–584
5. Stojanov S, Lohse P, Lohse P, Hoffmann F, Renner ED, Zellerer S et al. (2004) Molecular analysis of the MVK and TNFRSF1A genes in patients with a clinical presentation typical of the hyperimmunoglobulinemia D with periodic fever syndrome: a low-penetrance TNFRSF1A variant in a heterozygous MVK carrier possibly influences the phenotype of hyperimmunoglobulinemia D with periodic fever syndrome or vice versa. Arthritis Rheum 50:1951–1958
6. Touitous I, Lesage S, McDermott M, Cuisset L, Hoffman H, Dode C et al (2004) Infevers: an evolving mutation database for autoinflammatory syndromes. Hum Mutat 24:194–198
7. Pouchot J, Sampalis JS, Beaudet F, Carette S, Decary F, Salusinsky-Sternbach M et al (1991) Adult Still's disease: manifestations, disease course, and outcome in 62 patients. Medicine (Baltimore) 70:118–136

8. Haas D, Hoffmann GF (2006) Mevalonate kinase deficiencies: from mevalonic aciduria to hyperimmunoglobulinemia D syndrome. Orphanet J Rare Dis 1:13
9. Cuisset L, Drenth JP, Simon A, Vincent MF, Van der Velde Visser S, Van der Meer JW (2001) Molecular analysis of MVK mutations and enzymatic activity in hyper-IgD and periodic fever syndrome. Eur J Hum Genet 9(4):260–266
10. Drenth JP, Cuisset L, Grateau G, Vasseur C, van de Velde-Visser SD, de Jong JG et al (1999) Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. International Hyper-IgD Study Group. Nat Genet 22:178–181
11. Takada K, Aksentijevich I, Mahadevan V, Dean JA, Kelley RI, Kastner DL (2003) Favorable preliminary experience with etanercept in two patients with the hyperimmunoglobulinemia D and periodic fever syndrome. Arthritis Rheum 48:2645–2651
12. Drenth JP, Haagsma CJ, van der Meer JW (1994) Hyperimmunoglobulinemia D and periodic fever syndrome. The clinical spectrum in a series of 50 patients. International Hyper-IgD Study Group. Medicine (Bartimore) 73:133–144
13. Naruto T, Nakagishi Y, Mori M, Miyamae T, Imagawa T, Yokota S (2009) Hyper-IgD syndrome with novel mutation in Japanese girl. Mod Rheumatol 19(1):96–99