RESEARCH ARTICLE

Identification of Disease-Promoting HLA Class I and Protective Class II Modifiers in Japanese Patients with Familial Mediterranean Fever

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Abstract

Objectives

The genotype-phenotype correlation of MEFV remains unclear for the familial Mediterranean fever (FMF) patients, especially without canonical MEFV mutations in exon 10. The risk of FMF appeared to be under the influence of other factors in this case. The contribution of HLA polymorphisms to the risk of FMF was examined as strong candidates of modifier genes.

Methods

Genotypes of HLA-B and -DRB1 loci were determined for 258 mutually unrelated Japanese FMF patients, who satisfied modified Tel-Hashomer criteria, and 299 healthy controls. The effects of carrier status were evaluated for the risk of FMF by odds ratio (OR). The HLA effects were also assessed for clinical forms of FMF, subsets of FMF with certain MEFV genotypes and responsiveness to colchicine treatment.

Results

The carriers of B*39:01 were increased in the patients (OR = 3.25, p = 0.0012), whereas those of DRB1*15:02 were decreased (OR = 0.45, p = 0.00050), satisfying Bonferroni’s
correction for multiple statistical tests (n = 28, p < 0.00179). The protective effect of DRB1*15:02 was completely disappeared in the co-existence of B*40:01. The HLA effects were generally augmented in the patients without a canonical MEFV variant allele M694I, in accordance with the notion that the lower penetrance of the mutations is owing to the larger contribution of modifier genes in the pathogenesis, with a few exceptions. Further, 42.9% of 14 colchicine-resistant patients and 13.5% of 156 colchicine-responders possessed B*35:01 allele, giving OR of 4.82 (p = 0.0041).

Conclusions
The differential effects of HLA class I and class II polymorphisms were identified for Japanese FMF even in those with high-penetrance MEFV mutations.

Introduction
Familial Mediterranean fever (FMF) has been considered to be an autosomal recessive trait which is characterized by self-limiting recurrent fever and serositis (OMIM #249100),[1] and categorized to an autoinflammatory disease.[2] MEFV, which was identified as the responsible gene for FMF, encodes cytosolic protein pyrin (also known as marenostrin) which regulates the activity of NLRP3 inflammasome.[3] Two hundred and ninety six sequence variants in MEFV gene have been registered to “Infevers” database (http://fmf.igh.cnrs.fr/ISSAID/infevers/) as of September 1st, 2014, including hot spots for pathogenic amino acid substitutions in the C-terminus region of the protein. Recently, dominant form (OMIM #134610) of the disease was reported,[4] and overlapping and continuum to other autoinflammatory diseases were proposed.[5] We performed a nation-wide surveillance of FMF in Japan and found that only M694I is commonly identified among hot spot mutations in the exon 10, homozygotes of which comprise as many as 10% of Japanese FMF.[6] We also noticed that more than one third of the patients did not carry two copies of pathological mutations in their MEFV gene.[6] It is possible that the indistinct genotype-phenotype correlation observed in Japanese FMF is owing to the effect of modifier genes. Genes in the HLA region are candidates for the modifier genes because they are involved in various inflammatory diseases such as ankylosing spondylitis,[7] rheumatoid arthritis,[8] and Behçet’s disease.[9]

In the present study, we examined the effect of carrier status of HLA-B and -DRB1 alleles on FMF by the comparison of the frequencies between patients and non-disease control individuals by employing the probands of FMF pedigree and sporadic FMF cases who were captured by our previous national surveillance study.[6] HLA effects on clinical forms of FMF, subsets of FMF with certain MEFV genotypes and poor responders to colchicine treatment were also evaluated.

Methods
Study population
Blood samples were donated by the probands of FMF pedigree or sporadic cases of FMF, who satisfied the modified Tel-Hashomer criteria for FMF with either “typical” or “incomplete” attacks,[10] after the acquisition of written informed consent from each patient. Information about clinical feature was reported by health care providers participated to national surveillance study in Japan as reported previously;[6] in brief, epidemiological data (including gender,
consanguinity of parents, familial history and age of onset of inflammation signs) and major clinical data (including fever, thoracic, abdominal, articular, cutaneous signs, duration and frequency of episodes, presence of amyloidosis, and response to colchicine) were provided by a standard form. Differential diagnosis between typical and incomplete attacks was made according to the following criteria: a typical FMF attack is defined as 3 or more episodes of generalized peritonitis, or monoarthritis of the hip, knee or ankle lasting 12 hours to 3 days with a fever of 38°C or more, while an incomplete attack is defined as a fever less than 38°C of ill-defined duration (from 6 hours to 1 week), no sign of peritonitis or localized abdominal sign during abdominal attack and atypical distribution of arthritis. Genotype of MEFV was determined during a diagnostic procedure; E84K, L110P, E148Q, R202Q, E225K, G304R, R354Q, P369S, R408Q, R410H, S503C, M694I and P751L were identified. All the study procedures were approved by institutional review boards of National Hospital Organization Nagasaki Medical Center and Nagasaki University.

### HLA-B and -DRB1 genotyping

DNA was extracted from the blood sample and subjected to HLA-B and -DRB1 genotype determination by WAKFlow HLA typing kit (Wakunaga Pharmaceutical, Osaka, Japan) based on the reverse sequence-specific oligonucleotide probes method coupled with xMAP technology designed for use with the Luminex system (Luminex Japan, Tokyo, Japan).

### Statistics

The risk/protective effects of carrier status of HLA alleles were evaluated by odds ratio (OR) obtained by the comparison of frequencies between patients and controls. HLA genotype data of 299 healthy volunteers, who were staff of National Hospitals, were used as controls in the present study. Independency or interaction between HLA factors was assessed by subgroup analysis after the stratification by carrier status of HLA alleles of interest. Further, the HLA effect on the responsiveness to colchicine treatment was evaluated by the comparison of HLA carrier status between poor responders and responders. All the statistical analyses were performed using STATA12 (StataCorp, College Station, TX, USA).

### Results

#### Clinical feature of patients with FMF

Two hundred fifty eight mutually unrelated patients with FMF were enrolled in the present study. Among them, 149 met the criteria for “typical” FMF (FMF with typical attacks) and the other 109 were classified as “incomplete” FMF patients (patients with incomplete FMF attacks). MEFV M694I was carried by 85 FMF patients (32.9%) and 159 patients (61.6%) possessed two copies of MEFV sequences with detectable pathological mutations (Table 1). In accordance with our previous report, [6] almost all patients carrying MEFV M694I (98.8%) exhibited clinical features of “typical” FMF (Table 1).

#### Effect of HLA-B and -DRB1 on the risk of FMF

Fourteen HLA-B alleles (B*07:02, B*15:01, B*35:01, B*39:01, B*40:01, B*40:02, B*40:06, B*44:03, B*46:01, B*48:01, B*51:01, B*52:01, B*54:01 and B*55:02) and 13 HLA-DRB1 alleles (DRB1*01:01, DRB1*15:01, DRB1*15:02, DRB1*04:03, DRB1*04:05, DRB1*04:06, DRB1*04:10, DRB1*12:01, DRB1*13:02, DRB1*14:54, DRB1*08:02, DRB1*08:03 and DRB1*09:01) were possessed by 5% or more of the patients or controls. Carriers of B*15:18 occupied more than 5% of typical FMF. These alleles were examined whether the carrier frequencies were different
between patients and controls. Consequently, 4 HLA-B (B*39:01, B*52:01, B*40:01 and B*44:03) and 3 HLA-DRB1 (DRB1*15:02, DRB1*04:03 and DRB1*08:02) alleles were significantly different (Table 2 and S1 Table). The carriers of B*39:01 were increased in the patients (OR = 3.25, p = 0.0012), whereas those of DRB1*15:02 were decreased (OR = 0.45, p = 0.00050), satisfying Bonferroni's correction for multiple statistical test (n = 28, p < 0.00179) among them.

**HLA effects on the clinical forms of FMF and MEFV genotype**

Next, the HLA effects were examined for two clinical forms of FMF, typical and incomplete FMF, as well as patients with certain MEFV genotypes (Table 2). The precipitating effect of B*39:01 was observed in incomplete FMF and patients without high-penetrance MEFV allele M694I with higher OR and smaller p value than the comparison between FMF and controls (OR = 4.30 and 3.83, p = 0.00028 and 0.00037, respectively), but not in typical FMF or patients carrying M694I. In contrast, the protective effect of DRB1*15:02 was evident in both typical and incomplete FMF (OR = 0.55 and 0.32, p = 0.025 and 0.0010) and in both carriers and non-carriers of M694I (OR = 0.47 and 0.42, p = 0.028 and 0.0013), while the effect was slightly stronger in incomplete FMF and patients without M694I. The major HLA effects were augmented in the patients without canonical MEFV variant allele M694I, in accordance with the notion that the lower penetrance in the subset of the disease is owing to the larger contribution of modifiers in the pathogenesis. Additional precipitating alleles such as B*40:01, B*15:18, B*15:01, DRB1*04:03 and DRB1*08:02 were significant in typical FMF, whereas B*35:01 and DRB1*04:10 were increased only in incomplete FMF.

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**Table 1. Basic characteristics of the patients with FMF.**

|                              | All patients | Clinical form | MEFV genotype† | M694I mutation | M694I genotype | Clinical form | MEFV mutation |
|------------------------------|--------------|---------------|----------------|----------------|----------------|---------------|---------------|
|                              | Number       | Typical FMF   | Incomplete FMF | M694I-positive | M694I-negative | homo or compound het | homo or compound het |
| Number                       | n = 258      | n = 149       | n = 109        | n = 85         | n = 172        | n = 159       | n = 99        |
| Age of onset (years, mean±SD)| 28.5±18.9    | 23.6±16.1     | 35.8±20.6      | 19.7±12.7      | 32.9±20.1      | 26.9±18.2    | 30.9±20.0    |
| Family history of FMF        | 53/245 (21.6%) | 38/147 (25.9%) | 15/98 (15.3%)  | 25/83 (30.1%)  | 28/161 (17.4%) | 35/152 (23.0%) | 18/93 (19.4%) |

**Clinical form**

- Typical FMF: 149/225 (66.2%), - Incomplete FMF: 76/225 (33.8%)

**M694I mutation**

- M694I-positive: 85/257 (33.1%), 84/148 (56.8%), 1/109 (0.9%), -
- M694I-negative: 172/257 (66.9%), 64/148 (43.2%), 108/109 (99.1%), -

**Mutation homo or compound het**

- mutation homo or compound het: 99/258 (38.4%), 45/149 (30.2%), 54/109 (49.5%), 16/85 (18.8%), 83/172 (48.3%)
- mutation hemi or no mutation: 159/258 (61.6%), 104/149 (69.8%), 55/109 (50.5%), 69/85 (81.2%), 89/172 (51.7%)

† Patients with FMF were stratified in two ways according to MEFV genotype; (i) presence/absence of a canonical mutation M694I and (ii) homozygosity (homo), compound heterozygosity (compound het) or hemizygosity (hemi) in terms of detectable pathological mutations.

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Interaction between HLA alleles

Independence/interaction of HLA factors was evaluated by the comparison of carrier frequencies between subpopulations of patients and controls after stratification by carrier status of another HLA allele of interest. Mantel-Haenszel weighed mean was adopted to estimate an OR for each of 4 HLA-B and 3 HLA-DRB1 significant alleles adjusted for carrier status of the other 6 HLA alleles (Table 3). Among 42 estimations, only OR of B*52:01 adjusted for DRB1*15:02 and that of DRB1*15:02 adjusted for B*52:01 were profoundly attenuated by adjustment, because B*52:01 and DRB1*15:02 were associated non-randomly as one of ancestral HLA haplotypes in the Japanese population.[11] Although it did not reach statistical significance, a point estimation of OR of DRB1*15:02 remained less than one regardless of the presence or absence of B*52:01, but not in vice versa (Table 4), suggesting the primary role of DRB1*15:02 in the protective effect conferred by the B*52:01-DRB1*15:02 haplotype. Adjusted ORs were similar to un-adjusted ORs irrespectively of the carrier status of the second HLA allele for the other combinations, indicating independence of these HLA factors as the modifiers of the disease risk except for B*40:01. Further, the unexpected interaction between B*40:01 and DRB1*15:02 was also demonstrated by stratification analysis, in which the protective effect of DRB1*15:02 was completely disabled in the presence of B*40:01 (Table 4).

Table 2. HLA carrier status in the patients with FMF.

| HLA allele | All patients | Clinical form | MFFV genotype | mutation homo or compound het | mutation hemic or no mutation |
|------------|--------------|---------------|----------------|-------------------------------|-------------------------------|
| HLA-B      | n = 257      | n = 149       | n = 108        | n = 85                        | n = 171                       | n = 159                       | n = 98                        |
| B*39:01    | 3.25 (1.53–6.94) | 2.53 (1.06–6.04) | 4.30 (1.82–10.2) p = 0.00028 | ns                        | 3.83 (1.73–8.48) p = 0.00037 | 3.01 (1.31–6.92) p = 0.0064 | 3.65 (1.48–9.00) p = 0.0025 |
| B*52:01    | 0.52 (0.34–0.81) p = 0.0030 | ns                        | 0.36 (0.19–0.71) p = 0.0020 | ns                        | 0.43 (0.26–0.73) p = 0.0013 | 0.42 (0.25–0.73) p = 0.0013 | ns                        |
| B*40:01    | 2.25 (1.24–4.07) p = 0.0060 | 2.42 (1.25–4.68) p = 0.0069 | ns                        | 2.91 (1.38–6.13) p = 0.0034 | ns                        | 2.62 (1.38–4.94) p = 0.0023 | ns                        |
| B*44:03    | 0.63 (0.39–1.00) p = 0.047 | 0.51 (0.28–0.93) p = 0.025 | ns                        | ns                        | ns                        | ns                        | ns                        |
| B*15:18    | 4.69 (1.18–13.9) ns | 3.68 (1.03–13.1) p = 0.032 | ns                        | ns                        | ns                        | ns                        | ns                        |
| B*15:01    | 1.86 (1.03–3.35) ns | ns                        | ns                        | ns                        | ns                        | ns                        | ns                        |
| B*35:01    | ns            | 1.92 (1.07–3.42) p = 0.025 | ns                        | ns                        | ns                        | ns                        | ns                        |
| HLA-DRB1   | n = 256      | n = 148       | n = 108        | n = 85                        | n = 172                       | n = 158                       | n = 98                        |
| DRB1*15:02 | 0.45 (0.28–0.71) p = 0.0050 | 0.55 (0.32–0.93) p = 0.025 | 0.32 (0.16–0.66) p = 0.0010 | 0.47 (0.23–0.94) p = 0.028 | 0.42 (0.24–0.72) p = 0.0013 | 0.36 (0.20–0.64) p = 0.00033 | ns                        |
| DRB1*04:03 | 2.97 (1.20–7.32) p = 0.013 | 3.35 (1.26–8.90) p = 0.010 | ns                        | ns                        | ns                        | ns                        | 3.43 (1.31–8.97) p = 0.0075 | ns                        |
| DRB1*08:02 | 2.17 (1.07–4.40) p = 0.027 | 2.48 (1.14–5.39) p = 0.018 | ns                        | ns                        | 2.44 (1.15–5.19) p = 0.016 | 3.19 (1.53–6.66) p = 0.0011 | ns                        |
| DRB1*04:10 | ns            | 2.75 (1.20–6.28) p = 0.012 | ns                        | ns                        | ns                        | 2.78 (1.19–6.48) p = 0.0013 | ns                        |

Odds ratio and its 95% confidence intervals (in parenthesis) for the carriers of HLA-B and DRB1 alleles were listed when the comparison with the controls gave p<0.05. The results which remained significant after the Bonferroni's procedure are highlighted in bold with their p values: 15 HLA-B and 13 DRB1 alleles, in total 28 HLA allele carriers were tested because their frequencies in patients and/or controls were 5% or more; p<0.05/28=0.00179. ns: not significant, (p ≥ 0.05).

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Table 3. Odds ratio adjusted for carrier status of second HLA allele.

| HLA allele | un-adjusted | adjusted for B*39:01 | B*52:01 | B*40:01 | B*44:03 | DRB1*15:02 | DRB1*04:03 | DRB1*08:02 |
|------------|-------------|----------------------|---------|---------|---------|------------|------------|------------|
| HLA-B      |             |                      |         |         |         |            |            |            |
| B*39:01    | 3.25 (1.53–6.94) | 2.88 (1.35–6.18)    | 3.32 (1.58–6.98) | 3.25 (1.52–6.97) | 2.85 (1.33–6.10) | 3.29 (1.53–7.10) | 3.18 (1.47–6.88) |
|            | p = 0.0012  | p = 0.0044           | p = 0.00081  | p = 0.0013 | p = 0.0048 | p = 0.0013 | p = 0.0020  |
| B*52:01    | 0.52 (0.34–0.81) | 0.57 (0.37–0.88)    | 0.54 (0.35–0.83) | 0.51 (0.33–0.80) | 1.00 (0.45–2.24) ns† | 0.54 (0.35–0.83) | 0.54 (0.35–0.83) |
|            | p = 0.0030  | p = 0.010            | p = 0.0047   | p = 0.0024 | p = 0.0043 | p = 0.0043 | p = 0.0042  |
| B*40:01    | 2.25 (1.24–4.07) | 2.31 (1.29–3.82)    | 2.13 (1.18–3.82) | na         | 2.10 (1.15–3.82) | 2.19 (1.23–4.26) | 2.34 (1.28–4.26) |
|            | p = 0.0060  | p = 0.0097           | p = 0.0013   |           | p = 0.0062 | p = 0.0041 | p = 0.0043  |
| B*44:03    | 0.63 (0.39–1.00) | 0.63 (0.39–0.97)    | 0.68 (0.42–1.09) ns | na         | 0.60 (0.37–0.97) ns | 0.63 (0.39–1.01) ns | 0.65 (0.41–1.05) ns |
|            | p = 0.047   | p = 0.037            | p = 0.037    |           | p = 0.035  | p = 0.035  | p = 0.035   |

HLA-DRB1

| DRB1*15:02 | 0.45 (0.28–0.71) | 0.44 (0.19–1.05) ns† | 0.45 (0.28–0.71) | 0.43 (0.28–0.70) | na         | 0.47 (0.30–0.74) | 0.45 (0.29–0.72) |
|            | p = 0.00050 | p = 0.0019           | p = 0.00053  | p = 0.00037 | p = 0.0010 | p = 0.00062 | p = 0.0014  |
| DRB1*04:03 | 2.97 (1.20–7.32) | 3.00 (1.19–7.56) | 2.83 (1.15–6.97) | 3.15 (1.26–7.86) | 2.85 (1.18–6.88) | 2.72 (1.08–6.86) | na         |
|            | p = 0.013   | p = 0.014            | p = 0.018    | p = 0.0093 | p = 0.015 | p = 0.027  | 2.87 (1.19–6.95) |
| DRB1*08:02 | 2.17 (1.07–4.40) | 2.06 (1.00–4.29) | 2.08 (1.03–4.22) | 2.28 (1.11–4.68) | 2.08 (1.02–4.22) | 2.10 (1.04–4.23) | 2.12 (1.06–4.26) |
|            | p = 0.027   | p = 0.047            | p = 0.037    | p = 0.020  | p = 0.039 | p = 0.035  | p = 0.029   |

Odds ratio (and its 95% confidence intervals) for the carriers of HLA-B and DRB1 alleles were adjusted for carrier status of those alleles. The results which remained significant after the Bonferroni procedure are highlighted in bold with their p values; p < 0.05/28 = 0.00179. na: not applicable. ns: not significant, (p ≥ 0.05).

† Because B*52:01 and DRB1*15:02 are in strong linkage disequilibrium in the Japanese population, statistical significance of these tests was severely attenuated.

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Response to colchicine treatment

Although the good response to the treatment with oral colchicine administration is one of diagnostic criteria for FMF,[10] a relatively small but not negligible part of the patients are resistant to colchicine-treatment and require alternative anti-inflammatory treatment. Among 170 patients whose clinical data for the response to colchicine were available, 14 patients (8.2%) were clinically classified as non-responders to colchicine-treatment. When the HLA alleles significantly associated with FMF, clinical forms of FMF or subgroups of the patients with characteristic MEFV genotypes identified above were analyzed (Table 5), B*35:01 carriers were more likely resistant to colchicine-treatment; 42.9% of 14 treatment-resistant patients and 13.5% of 156 colchicine-responders possessed B*35:01 allele (OR = 4.82, 95% confidence interval of OR: 1.47–15.8, p = 0.0041), satisfying Bonferroni’s collection for multiple statistical tests (n = 11, p < 0.0045).

Discussion

Similarity to other inflammatory diseases with respect to HLA-disease association and differential effects of HLA classes

As an attempt to identify a modifier gene of FMF, we analyze polymorphisms in the HLA-B and DRB1 loci because of their possible roles in the determination of antigen specificity in immune response as well as known association with various health conditions including...
Table 4. Interaction between HLA alleles.

| HLA allele / Subpopulation† | odds ratio   | p     | test for homogeneity‡ |
|-----------------------------|--------------|-------|-----------------------|
| B*52:01                     |              |       |                       |
| DRB1*15:02-negative         | 0.91 (0.36–2.28) | ns (p = 0.84) |                       |
| DRB1*15:02-positive         | 1.36 (0.26–7.22)  | ns (p = 0.71)  | ns (p = 0.67)         |
| Mantel-Haenszel estimate controlling for DRB1*15:02 | 1.00 (0.45–2.24)  | ns (p = 0.99)  |                       |
| B*40:01-negative            | 0.50 (0.32–0.79)  | p = 0.0024 |                       |
| B*40:01-positive            | 1.47 (0.25–8.57)  | ns (p = 0.67)  | ns (p = 0.23)         |
| Mantel-Haenszel estimate controlling for B*40:01 | 0.54 (0.35–0.83)  | p = 0.0047 |                       |
| B*40:01                     |              |       |                       |
| B*52:01-negative            | 1.86 (1.00–3.50)  | ns (p = 0.051) |                       |
| B*52:01-positive            | 5.44 (0.96–30.8)  | ns (p = 0.24)  |                       |
| Mantel-Haenszel estimate controlling for B*52:01 | 2.12 (1.18–3.82)  | ns (p = 0.0097) |                       |
| DRB1*15:02-negative         | 1.69 (0.89–3.21)  | ns (p = 0.10)  |                       |
| DRB1*15:02-positive         | 9.80 (1.73–55.4)  | p = 0.0015 | p = 0.043               |
| Mantel-Haenszel estimate controlling for DRB1*15:02 | 2.19 (1.23–3.88)  | p = 0.0062 |                       |
| DRB1*15:02                  |              |       |                       |
| B*52:01-negative            | 0.34 (0.07–1.69)  | ns (p = 0.17)  |                       |
| B*52:01-positive            | 0.51 (0.18–1.39)  | ns (p = 0.67)  |                       |
| Mantel-Haenszel estimate controlling for B*52:01 | 0.44 (0.19–1.05)  | ns (p = 0.056) |                       |
| B*40:01-negative            | 0.38 (0.22–0.63)  | p = 9.7×10^{-5} |                       |
| B*40:01-positive            | 2.20 (0.40–12.2)  | ns (p = 0.35)  | p = 0.034               |
| Mantel-Haenszel estimate controlling for B*40:01 | 0.45 (0.28–0.71)  | p = 0.00053 |                       |

† The effect of carrier status of HLA-B and-DRB1 alleles were separately calculated in two subpopulations for different carrier status of the second HLA allele. Odds ratio and its 95% confidence interval (in parenthesis) are given. Mantel-Haenszel weighed mean was given (as shown as adjusted odds ratio in Table 3). ns: not significant, ( p ≥ 0.05).

‡ Test for homogeneity of the effects in two subpopulations. The tests showing differential effects are highlighted in bold.

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Table 5. HLA carrier status in the poor-responders to colchicine treatment.

| HLA alleles | Poor responders | Responders | OR      | p       |
|-------------|----------------|------------|---------|---------|
| HLA-B       | n = 14         | n = 156    |         |         |
| B*39:01     | 4 (28.6%)      | 17 (10.9%) | 3.27 (0.91–11.8) | ns (0.055) |
| B*52:01     | 1 (7.1%)       | 26 (16.7%) | 0.38 (0.05–3.11) | ns (0.87)  |
| B*40:01     | 1 (7.1%)       | 23 (14.7%) | 0.44 (0.05–3.60) | ns (0.61)  |
| B*44:03     | 2 (14.3%)      | 21 (13.5%) | 1.07 (0.22–5.15) | ns (0.92)  |
| B*15:18     | 0 (0.0%)       | 7 (4.5%)   | 0.69 (0.04–12.7) | ns (0.65)  |
| B*15:01     | 0 (0.0%)       | 23 (14.7%) | 0.20 (0.01–3.40) | ns (0.12)  |
| B*35:01     | 6 (42.9%)      | 19 (13.5%) | 4.82 (1.47–15.8) | 0.0041     |
| HLA-DRB1    | n = 14         | n = 155    |         |         |
| DRB1*15:02  | 0 (0.0%)       | 19 (12.3%) | 0.24 (0.01–4.21) | ns (0.17)  |
| DRB1*04:03  | 0 (0.0%)       | 11 (7.1%)  | 0.43 (0.02–7.74) | ns (0.30)  |
| DRB1*08:02  | 4 (26.7%)      | 17 (10.9%) | 0.92 (0.11–7.67) | ns (0.92)  |
| DRB1*04:10  | 2 (14.3%)      | 6 (3.9%)   | 4.14 (0.74–23.2) | ns (0.078) |

Frequency of which was significantly deviated from that of the controls is highlighted in bold.

† OR was obtained by Haldane’s modifications of Woolf’s formula.

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inflammatory diseases, which share certain clinical features with FMF. Consequently, several HLA-B and DRB1 alleles were demonstrated to be associated with FMF; some of them were reported as risk/protective factor for other diseases. B*39:01 was associated with severe and sustained osteoarticular manifestations complicated with Brucella infection,[12] and B*27-negative ankylosing spondylitis,[13] suggesting its proinflammatory nature. Indeed, there are accumulating evidences for special ligand binding characteristics with HLA-B*39.[14,15] B*40:01 which encodes HLA-B60 antigenic specificity was the second most significantly associated HLA-B allele with FMF in the present study and has been identified an additional risk factor for ankylosing spondylitis also.[16–18] In contrast, apart from their primary function of antigen presentation to CD4-positive T helper cells in immune response, certain HLA class II alleles play a role in the protection from autoimmunity as in the case of DRB1*13:02 in systemic lupus erythematosus, rheumatoid arthritis and autoimmune thyroid disease,[19–21] and DRB1*15:02 in rheumatoid arthritis without anti-citrullinated peptide/protein antibodies (ACPA-negative RA).[22] in Japanese for example, although the underlying biological mechanisms remain to be uncovered. Similarly, the biological implication of DRB1*15:02 in the protection from FMF is not clear, but non-competitive interaction between B*40:01 and DRB1*15:02 (Table 4) was distinctive and can provide a potential clue to the understanding of the contribution of protective HLA in the pathogenesis of FMF.

Common genetic predisposition to FMF and other inflammatory diseases

It has been reported that the patients with FMF are predisposed to other inflammatory diseases such as ankylosing spondylitis and Behçet disease.[23,24] MEFV mutations play a role to increase the risk of these diseases even among the patients without the complication of FMF.[25–28] Although it still remains unclear how the symptoms of periodical fevers of FMF are triggered, the MEFV product interacts with cellular components of inflammasome whose activity is augmented by the pathogenic mutations.[29] Therefore, it is reasonable to consider that FMF-associated MEFV mutations enhance the cellular responses in the inflammatory diseases which are also associated with certain HLA polymorphisms. Uncovering of the common underlying mechanism in the pathogenesis of these inflammatory diseases in the context of HLA-MEFV interaction would be helpful to find an effective target of novel therapeutic or preventive measures.

Predictors of colchicine response

Colchicine is the first choice of the medical treatment of FMF. The response to colchicine is one of remarkable characteristics of FMF and has been a very effective criterion for the differential diagnosis FMF from other inflammatory diseases. In the present study, 14 of 170 patients (8.2%) whose response to colchicine treatment was available were reported as poor responders by care-providing physicians. It has been widely recognized that 5–15% of the patients with FMF do not respond to colchicine.[30] We did not examine ABCB1 polymorphisms which are associated with colchicine non-responders,[31] in the present study, but six HLA-B*35:01-carriers were identified among poor responders (42.9%) giving a significant increase in the carrier frequency in comparison to colchicine responders. The positive predictive value of HLA-B*35:01-carriage remained as much as 0.24, but it was a moderately useful predictor of prognosis as assessed by positive likelihood ratio of 4.35.[32] Despite nation-wide collection of FMF in Japan, the evaluation of predictors of colchicine response was made with relatively small number of poor responders only whose clinical documents were available. Therefore the result of the present study should be confirmed by more colchicine poor responders. None the
less for this limitation, combination with other prognostic markers such as ABCB1 SNPs may improve the accuracy of prediction to meet practical demands.

**Conclusions**

The differential effects of HLA class I and class II alleles on FMF were identified for Japanese population. Modifier genes including HLA explain low penetrance of non-canonical mutations at least in part. Further, HLA-B*35:01 can be a useful predictive marker for the failure of colchicine treatment.

**Supporting Information**

**S1 Table. Frequency of HLA carriers in the patients with FMF.** Frequency of which was significantly deviated from that of the controls is highlighted in bold. † Patients with FMF were stratified in two ways according to MEFV genotype; (i) presence/absence of a canonical mutation M694I and (ii) homozygosity (homo), compound heterozygosity (compound het) or hemizygosity (hemi) in terms of detectable pathological mutations.

(DOCX)

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**Author Contributions**

Conceived and designed the experiments: M. Yasunami KM. Performed the experiments: M. Yasunami HN. Analyzed the data: M. Yasunami HN. Contributed reagents/materials/analysis tools: M. Yasunami KA AN M. Yazaki DK AY TT JM HI AK KE HF TN MN KM. Wrote the paper: M. Yasunami.

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