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<td>Migita, Kiyoshi; Agematsu, Kazunaga; Masumoto, Junya; Ida, Hiroaki; Honda, Seiyo; Jiuchi, Yuka; Izumi, Yasumori; Maeda, Yumi; Uehara, Ritei; Nakamura, Yoshikazu; Koga, Tomohiro; Kawakami, Atsushi; Nakashima, Munetoshi; Fujieda, Yuichiro; Nonaka, Fumiaki; Eguchi, Katsumi; Furukawa, Hiroshi; Nakamura, Tadashi; Nakamura, Minoru; Yasunami, Michio</td>
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The Contribution of SAA1 Polymorphisms to Familial Mediterranean Fever Susceptibility in the Japanese Population

Kiyoshi Migita1*, Kazunaga Agematsu2, Junya Masumoto3, Hiroaki Ida4, Seiyo Honda4, Yuka Jiuchi1, Yasumori Izumi1, Yumi Maeda1, Ritei Uehara5, Yoshikazu Nakamura5, Tomohiro Koga6, Atsushi Kawakami6, Munetoshi Nakashima7, Yuichiro Fujieda8, Fumiaki Nonaka9, Katsumi Eguchi9, Hiroshi Furukawa10, Tadashi Nakamura11, Minoru Nakamura1, Michio Yasunami12

1 Clinical Research Center, Department of General Internal Medicine and NHG Nagasaki Medical Center, Kubara, Omura, Japan, 2 Department of Infection and Host Defense Graduate School of Medicine, Shinshu University, Asahi, Matsumoto, Japan, 3 Department of Pathogenomics, Ehime University Graduate School of Medicine, Toone City, Ehime, Japan, 4 Department of Rheumatology, Kurume University School of Medicine, Kurume, Japan, 5 Department of Public Health, Jichi Medical University, Tochigi, Japan, 6 First Department of Internal Medicine, Nagasaki University School of Medicine, Sakamoto, Nagasaki, Japan, 7 Department of Rheumatology, Red Cross Nagasaki Atomic bomb Hospital, Mori, Nagasaki, Japan, 8 Department of Medicine II, Hokkaido University Graduate School of Medicine, Sapporo, Japan, 9 Department of Rheumatology, Sasebo City General Hospital, Hirase, Sasebo, Japan, 10 Department of Rheumatology, NHO Sagamihara Hospital, Sakuradai, Sagamihara, Japan, 11 Department of Rheumatology, NTT WEST JAPAN Kyushu Hospital, Shinyashiki, Kumamoto, Japan, 12 Center for International Collaborative Research (CICORN) and Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

Abstract

Background/Aims: Familial Mediterranean Fever (FMF) has traditionally been considered to be an autosomal-recessive disease, however, it has been observed that substantial numbers of patients with FMF possess only 1 demonstrable MEFV mutation. The clinical profile of familial Mediterranean fever (FMF) may be influenced by MEFV allelic heterogeneity and other genetic and/or environmental factors.

Methodology/Principal Findings: In view of the inflammatory nature of FMF, we investigated whether serum amyloid A (SAA) and interleukin-1 beta (IL-1β) gene polymorphisms may affect the susceptibility of Japanese patients with FMF. The genotypes of the -13C/T SNP in the 5′-flanking region of the SAA1 gene and the two SNPs within exon 3 of SAA1 (2995C/T and 3010C/T polymorphisms) were determined in 83 Japanese patients with FMF and 200 healthy controls. The same samples were genotyped for IL-1β-511 (C/T) and IL-1 receptor antagonist (IL-1Ra) variable number of tandem repeat (VNTR) polymorphisms. There were no significant differences between FMF patients and healthy subjects in the genotypic distribution of IL-1β -511 (C/T), IL-1Ra VNTR and SAA2 polymorphisms. The frequencies of SAA1.1 allele were significantly lower (21.7% versus 34.0%), and inversely the frequencies of SAA1.3 allele were higher (48.8% versus 37.5%) in FMF patients compared with healthy subjects. The frequency of -13T alleles, associated with the SAA1.3 allele in the Japanese population, was significantly higher (56.0% versus 41.0%, p = 0.001) in FMF patients compared with healthy subjects.

Conclusions/Significance: Our data indicate that SAA1 gene polymorphisms, consisting of -13T/C SNP in the 5′-flanking region and SNPs within exon 3 (2995C/T and 3010C/T polymorphisms) of SAA1 gene, are associated with susceptibility to FMF in the Japanese population.

Introduction

FMF is an inherited autoinflammatory disease characterized by recurrent self-limited fever, and serositis [1]. These episodes of inflammation are mainly mediated by a massive influx of neutrophils into serous cavities and are accompanied by an elevation of acute phase reactants [2]. The disease is associated with mutations in the MEFV gene that encodes pyrin, and is transmitted in an autosomal-recessive manner [3]. Therefore, heterozygotes are expected to be carriers or lack the clinical phenotype of FMF. However, mutations in the second MEFV allele have not been observed in 20–30% of patients with typical FMF [4]. Recent studies suggest that subjects with a single MEFV mutation may cross a threshold for the development of an FMF phenotype if they also express a combination of gene polymorphisms that favor increased inflammation [5]. These polymorph-
phisms are thought to belong to genes of the interleukin-1β/innate immune system pathways [5]. The IL-1 family of cytokines is critical to the host’s response to infection, and induction of innate immunity and acute phase inflammation [6]. The overproduction of IL-1β is responsible for a variety of autoinflammatory syndromes including FMF [7]. IL-1β requires cleavage via caspase-1 for proper secretion, which is facilitated by inflamma-
some activation [8]. The NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome has emerged as a critical cytosolic sensor for a number of endogenous mediators, including amyloid protein [9].

Recent studies have shown that serum amyloid A (SAA) induced the expression of pro-IL-1β and activated the NLRP3 inflamma-
some in a cathepsin B and P2X4-dependent manner resulting in secretion of mature IL-1β [10]. SAA is an acute-phase protein, which increases in the serum during inflammation and is susceptible to proteolytic cleavage to amyloid A (AA) protein, the major fibrillar protein in secondary amyloidosis [11]. An allelic variant of SAA1.3, was found to be associated with AA amyloidosis in Japanese rheumatoid arthritis (RA) patients [12]. In view of the recent genetic studies in FMF, other modifying genetic factors may contribute to the susceptibility or clinical expression of FMF in addition to MEFV mutations. Therefore, we attempted to determine the effect of gene polymorphisms on the susceptibility to FMF in the Japanese population.

Materials and Methods

Patients

In early 2007, a laboratory network collecting the genetic diagnosis of periodic fever was established at the Japan Autoinflammation Association (JAA), and MEFV gene analysis was carried out at the Clinical Research Center of National Hospital Organization (NHO) Nagasaki Medical Center. Up to October 2012, 481 consecutive unrelated patients with periodic fever were referred and underwent molecular diagnosis at the NHO Nagasaki Medical Center. All patients, who were originating from Japan, (East Japan n = 96, West Japan n = 47) were asked to complete a questionnaire that included demographics (sex, age of onset), family history (consanguinity of parents, family history of recurrent fever), and the presence of recurrent febrile attacks typical of FMF, including peritonitis, pleuritis, arthritis, and transient inflammato-
ry responses. The genetic analysis of MEFV gene was approved by the Ethics Committee of Nagasaki Medical Center, and written informed consent was obtained from each individual. On the basis of Tel-Hashomer criteria [13], we divided the FMF patients in two groups: Group 1, typical FMF exhibiting the presence of 1 or more major criteria independent to the presence of minor criteria; Group 2, incomplete FMF exhibiting the absence of major criteria and 2 or more minor criteria. It is important to stress that response to colchicine was confirmed in almost all patients. As controls, 200 healthy Japanese individuals without pre-existing medical diseases (90 men and 110 women 14 to 64 years, with a mean age of 38.6 ± 13.9 years) from East Japan (n = 96) and West Japan (n = 114) were enrolled in the study after obtaining informed consent.

MEFV gene Mutation analysis

All patients were undergone genetic analysis of MEFV gene exons 1, 2, 3 and 10 by direct sequencing. 2 milliliters of blood samples were collected from all subjects. Genomic DNA was extracted from whole blood by means of the Promega Wizard® Genomic DNA Purification Kit (Promega, USA). Mutation analysis was performed by genomic sequencing as described previously [14].

Genotyping

SAA1 gene. The genotype of the SAA1 -13C/T in the 5'-region of exon 1 (rs11024595) was determined by the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method [15]. The primers used for the PCR reaction were 5'-ACATCT TGTTCCTCTG AGGTTG-3' (sense) and 5'-GCTGTA GGAGCTGCTGCGG-3' (antisense). The 229-bp PCR products were digested with restriction enzyme AciI (BioLabs, Beverly, MA, USA) and electrophoresed on a 12.5% polyacrylamide gel [15].

The SAA1.1, 1.3, and 1.5 alleles, corresponding to the T-C, C- T, and C-C haplotypes of the C2995T (rs1136743) and C3010T (rs1136747) polymorphisms were also determined by the PCR-RFLP [15]. The primers used for the PCR reaction were 5'-GCC AATTGACGCCCTCAG-3' (sense) and 5'-TGGCGCA AAAATCTCTGAT-3' (antisense).

The 518-bp PCR products were digested with restriction enzyme BclI (Promega, San Luis Obispo, CA, USA) and electrophoresed on a 2.5% agarose gel [15].

The genotype of the SAA2 (rs2468844) was determined by the polymerase chain reaction restriction fragment length polymorphism (PCR–RFLP) method. The primers used for the PCR reaction were 5'-AGAGAATATCCCCAGACTCCAGGCG-3' (sense) and 5'-CAGGCGCAAGAGTAGCAGG-3' (antisense). The 115 bp PCR products were digested with the restriction enzyme Neo I. The digested products were separated by 3% agarose gels by ethidium bromide staining [15].

IL-1Ra. For the IL-1Ra VNTR polymorphism, the region including variable numbers of identical 86-bp tandem repeats was amplified by PCR using the following primers: 5'-CTCAGGCACAGACTCTAT-3' (sense) and 5'-TCGCTTGCTGCTGAGC-3' (antisense). PCR products of 240 (allele 2, two repeats), 325 (allele 3, three repeats), 410 (allele 4, four repeats), and 500 bp (allele 5, five repeats) were distinguished by agarose gel electrophoresis [16].

IL-1B-511. A fragment containing the Aal polymorphic site at promoter region -511 of the IL-1B gene was amplified by PCR. PCR was carried out with primers, forward primer 5'-GCCCTGAAACGCTGATACGT-3' (sense). 5'-GCCAA-TAGGCCTTGTCT-3' (antisense). Fragments were separated by electrophoresis on 3% agarose with ethidium bromide staining using appropriate commercially available size markers for comparison. The C allele was designated if two bands of 92 and 63 bp were obtained, and the T allele was designated if a single band of the undigested 155 bp was obtained. Genotypes were designated as follows: C/C, two bands of 92 and 63 bp: C/T, three bands of 155, 92, and 63 bp; and T/T, a single band of 155 bp [16].

Statistical Analysis

Results are expressed as mean ±SD. Statistical analysis was performed with SPSS18 for windows (SPSS Statistics, Illinois). The statistical significance of differences between groups was calculated by either the chi-square test for categorical data and Mann-Whitney’s U-test for quantitative data. Deviation from Hardy-Weinberg equilibrium was assessed using the SNPAllye software ver. 7.0 (Dynacom, Yokohama, Japan). A p value of <0.05 was considered significant.
Results

Demographic data and MEFV genotypes

We diagnosed 83 subjects, all of Japanese origins, as FMF. Among these patients, 44 were diagnosed as typical FMF and 37 were diagnosed as incomplete FMF. The demographic data of the newly-diagnosed FMF patients are summarized in Table 1. The overall male: female ratio in patients with FMF was 0.8 (37:46). In incomplete FMF patients, the more affected sex is female in contrast to typical FMF (Table 1). The mean age at diagnosis was 37.9 ± 18.8 years. Age at diagnosis of patients with typical FMF was similar to those with incomplete FMF (36.2 ± 18.2 and 39.9 ± 19.6 years, respectively; \( p = 0.419 \); Table 1). By mutation analysis, the MEFV gene mutation could not be identified in 3 of 83 patients (3.6%). The distribution of the MEFV genotype was heterogenous. The most frequent genotype was M694I/E148Q, followed by M694I/normal and M694I/E148Q. AA amyloidosis was histologically confirmed in 4 patients with FMF, whose genotypes were M694I/M694I SAA1.5/1.5, M694I/E148Q/L110P SAA1.1/1.1, M694I/E148Q/L110P SAA1.3/1.5 and E148Q/R202Q/P369S/R408Q SAA1.3/1.5.

IL-1β and IL-1Ra gene polymorphism

The genotype frequencies of IL-1β-511 (C/T), and IL-1Ra VNTR polymorphisms in FMF patients and healthy subjects are summarized in Table 2. There were no significant difference in the frequencies of these polymorphisms between FMF patients and healthy subjects.

Association between SAA2 gene polymorphism and FMF

There was no significant difference in the frequencies of the SAA2 genotype between FMF patients and healthy subjects (Table 2).

Association between SAA1 gene polymorphisms and FMF

A segment of the genomic SAA1 gene with polymorphic sites was subjected to PCR/restriction fragment length polymorphism (PCR-RFLP) analysis. Table 3 shows the frequencies of individuals with various genotypes and alleles at the SAA1 locus in either FMF patients (n = 83) or Japanese healthy subjects (n = 200). The allele frequency of SAA1.1 was significantly lower in FMF patients compared with healthy subjects (21.7% versus 34.0%). Conversely,
Table 2. Frequencies of the genotypes at the IL-1β -511, IL-1Ra and SAA2 loci in patients with FMF and healthy subjects.

<table>
<thead>
<tr>
<th>Genotype at IL-1β -511 locus</th>
<th>FMF patients</th>
<th>Healthy subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>27(32.5)</td>
<td>59(29.5)</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>43(51.8)</td>
<td>100(50.0)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>13(15.7)</td>
<td>41(20.5)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype at IL-1Ra locus</th>
<th>FMF patients</th>
<th>Healthy subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1</td>
<td>73(88.0)</td>
<td>167(83.5)</td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>5(6.0)</td>
<td>20(10.0)</td>
<td></td>
</tr>
<tr>
<td>1/3</td>
<td>0(0.5)</td>
<td>2(1.0)</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>4(4.8)</td>
<td>7(3.5)</td>
<td></td>
</tr>
<tr>
<td>2/2</td>
<td>2(2.4)</td>
<td>3(1.5)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotype at SAA2 locus</th>
<th>FMF patients</th>
<th>Healthy subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/A</td>
<td>62(74.7)</td>
<td>163(81.5)</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>19(22.9)</td>
<td>35(17.5)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>2(2.4)</td>
<td>1(1.0)</td>
<td></td>
</tr>
</tbody>
</table>

The allele frequency of SAA1.3 was higher in FMF patients compared with healthy subjects (48.8% versus 37.5%). The -13C/T polymorphism, in the 5′-flanking region of the SAA1 gene is associated with the SAA1.3 allele and susceptibility to amyloidosis in Japanese RA patients [17]. We analyzed the frequency of -13C/T polymorphisms in FMF patients and Japanese healthy subjects. Allele frequencies of -13C/T were significantly increased in FMF patients compared with healthy subjects (56.0% versus 41.0%, p = 0.001). These data suggest that the -13T allele is associated with susceptibility to FMF in the Japanese population. Allele frequencies of -13 C/T polymorphisms were also analyzed in typical or incomplete FMF patients. There was no significant difference in the frequencies between typical and incomplete FMF patients (Table 5).

Discussion

FMF is considered to be an autosomal recessive disease [18]. The gene causing FMF is MEFV, which encodes pyrin, expressed in the cytoplasm of myeloid cells [2]. Pyrin is postulated to act as a negative regulator of IL-1-mediated inflammation [19]. However, approximately 30% of FMF patients exhibit a single MEFV mutation, despite sequencing of the entire MEFV genomic region and other autoinflammatory genes [20]. More recently it was demonstrated that pyrin truncation in mice did not show an overt phenotype of FMF, however, pyrin-deficient and MEFV-associated B30.2 mutations “knock in” mice showed severe spontaneous inflammatory phenotype, suggesting that FMF may be caused by a gain of function by disease-associated missense changes in pyrin.

Table 3. Frequencies of the genotypes and alleles at the SAA1 locus of Japanese patients with FMF and healthy subjects.

<table>
<thead>
<tr>
<th>Genotype at SAA1 locus</th>
<th>FMF patients</th>
<th>Healthy subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1/1.1</td>
<td>4(4.8)</td>
<td>24(12.0)</td>
<td></td>
</tr>
<tr>
<td>1.1/1.3</td>
<td>22(25.6)</td>
<td>49(24.5)</td>
<td></td>
</tr>
<tr>
<td>1.1/1.5</td>
<td>6(7.2)</td>
<td>39(19.5)</td>
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</tr>
<tr>
<td>1.3/1.3</td>
<td>15(18.1)</td>
<td>27(13.5)</td>
<td></td>
</tr>
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<td>1.3/1.5</td>
<td>29(34.9)</td>
<td>47(23.5)</td>
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</tr>
<tr>
<td>1.5/1.5</td>
<td>7(8.4)</td>
<td>14(7.0)</td>
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SAA1: Serum amyloid A1. Chi-square test was used to examine differences of genotype and allele frequencies between FMF patients and healthy subjects. doi:10.1371/journal.pone.0055227.t003

Table 4. Frequencies of the genotypes and alleles at -13C/T SAA1 locus of Japanese patients with FMF and healthy subjects.

<table>
<thead>
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<th>FMF patients</th>
<th>Healthy subjects</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>13(15.7)</td>
<td>67(33.5)</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>47(56.6)</td>
<td>102(51.0)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>23(27.7)</td>
<td>31(15.5)</td>
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</table>

SAA1: Serum amyloid A1. Chi-square test was used to examine differences of genotype and allele frequencies between FMF patients and healthy subjects. doi:10.1371/journal.pone.0055227.t004

Hardy-Weinberg equilibrium test

Finally, Hardy-Weinberg equilibrium was estimated by chi-square test with Yates’ correction. There was no significant difference between observed and experienced frequencies of each genotype (SAA1 -13C/T, SAA2, IL-1β -511) in the both FMF patients (Table 7) and healthy subjects (Table 7). These results indicated that these populations had a relatively stable genetic background and were stable for genetic statistical analysis.

The allele frequency of SAA1.3 was higher in FMF patients compared with healthy subjects (48.8% versus 37.5%). The -13C/T polymorphism, in the 5′-flanking region of the SAA1 gene is associated with the SAA1.3 allele and susceptibility to amyloidosis in Japanese RA patients [17]. We analyzed the frequency of -13C/T polymorphisms in FMF patients and Japanese healthy subjects. Allele frequencies of -13C/T were significantly increased in FMF patients compared with healthy subjects (56.0% versus 41.0%, p = 0.001). These data suggest that the -13T allele is associated with susceptibility to FMF in the Japanese population. Allele frequencies of -13 C/T polymorphisms were also analyzed in typical or incomplete FMF patients. There was no significant difference in the frequencies between typical and incomplete FMF patients (Table 5). Among 83 patients with FMF, 30 patients had 0 to 1 MEFV mutation (no mutation 3; heterozygous 27) and 53 patients at least 2 mutations (homoygous or compound heterozygous). There was no significant difference in SAA1 gene polymorphisms between FMF patients with different numbers of MEFV mutations (Table 6).

Discussion

FMF is considered to be an autosomal recessive disease [18]. The gene causing FMF is MEFV, which encodes pyrin, expressed in the cytoplasm of myeloid cells [2]. Pyrin is postulated to act as a negative regulator of IL-1-mediated inflammation [19]. However, approximately 30% of FMF patients exhibit a single MEFV mutation, despite sequencing of the entire MEFV genomic region and other autoinflammatory genes [20]. More recently it was demonstrated that pyrin truncation in mice did not show an overt phenotype of FMF, however, pyrin-deficient and MEFV-associated B30.2 mutations “knock in” mice showed severe spontaneous inflammatory phenotype, suggesting that FMF may be caused by a gain of function by disease-associated missense changes in pyrin.

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<tr>
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<td>102(51.0)</td>
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<td>T/T</td>
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and that FMF may not be a pure autosomal recessive disease due to
the loss of protein function [21]. One explanation is that subjects
having a single MEFV mutation may develop an FMF phenotype
in the presence of other inflammasome-related genes or
in the presence of other environmental factors [22]. Therefore, the
role of potential modifier genes and polymorphisms within these
gene families should be assessed in conjunction with genotype-
phenotype association studies. Polymorphisms in genes associated
with the inflammasome pathway can affect the development of
FMF [5]. For example, TLR2 polymorphisms may be an
important factor in the susceptibility of FMF [23,24].

In this study, we investigated the SAA1 and IL-1β gene
polymorphisms in Japanese patients with FMF. There was no
different significance in IL-1β-511 (C/T) or IL-1Ra VNTR
polymorphisms between FMF patients and healthy subjects in
accord to the previous report [25]. However, we demonstrated that
SAA1 gene polymorphisms, which are attributed to AA
amyloidosis, might be also responsible for susceptibility to FMF. It
is clear that genotypes at the SAA1 locus are associated with an
increased susceptibility to AA amyloidosis [26]. However, the
contribution of these genotypes to the occurrence of non-amyloid,
inflammatory disease has not been elucidated. In this study, we
investigated the allele frequencies of SAA1.1, SAA1.3, and SAA1.5
polymorphisms of the SAA1 promoter region in Japanese patients
with FMF. Our data demonstrated that the -13T allele polymor-
phism was a major risk factor and that the SAA1.1 allele was
protective for the occurrence of FMF in Japanese case-control
studies.

The presence of 2 single-nucleotide polymorphisms (SNPs)
within exon 3 of the SAA1 gene, 2995 G/T and 3010 C/T, defined
3 haplotypes that corresponded to the SAA1.1, SAA1.3, and SAA1.5
isofoms [26]. In Japanese patients with RA, homozygote
expression of the SAA1.3 allele was a proven risk factor, whereas
SAA1.1 appeared to be protective for AA amyloidosis [27]. In
contrast, a strong positive association with SAA1.1 has been
established in Caucasian patients with amyloidosis secondary to
juvenile idiopathic arthritis and FMF [28–30]. Moriguchi et al.
identified another SAA1 SNP, the -13T/C SNP in the 5’-flanking
region of the SAA1 gene [17]. They observed the -13T allele was
associated with AA amyloidosis, and associated with the SAA1.3
allele in Japanese RA patients [17]. Interestingly, a polymorphism
in the SAA1 promoter -13T allele was found to be significantly
associated with increased AA amyloidosis risk in both populations
and to be in linkage disequilibrium with SAA1.1 and SAA1.3 in
Caucasian and Japanese patients, thus apparently explaining the
previous discrepancy [31–35]. Functional studies have demon-
strated that the -13T allele is responsible for a higher transcription-
al rate [36]. However, this did not result in higher serum levels of
SAA, possibly due to increased proteolytic processing rates of
SAA1.1 and SAA1.3 compared to SAA1.3 [37]. The mechanisms by
which the -13T allele predisposes to FMF remains to be unraveled
and many possibilities have been suggested.

The overproduction of IL-1β, induced by NLRP3 inflamma-
some activation, is responsible for a variety of autoinflammatory
syndrome including FMF. The NLRP3 inflammasome has
emerged as a critical cytosolic sensor for a number of endogenous
mediators, including amyloid proteins [6]. Recent studies indic-
ate that SAA activates the NLRP3 inflammasome in a cathepsin B
and P2X7–dependent manner, resulting in the secretion of mature
IL-1β [10]. The accumulation of newly formed AA amyloid fibrils
and aberrant processing of SAA is relevant to AA amyloidosis
[30]. Therefore, in subjects with AA amyloidogenic genetic
factors, such as -13T allele, the presence of SAA-derived AA
amyloid fibrils may implicate the NLRP3 inflammasome activa-
tion pathway, which is thought to be relevant to the pathogenesis
of FMF. Jeru et al. demonstrated that the SAA1 genotype
influenced the severity of FMF and disease susceptibility through
a negative selection process, providing new insights into the role of
SAA1 in the pathophysiology of FMF [39]. Assuming that SAA1
gene polymorphisms induce the formation of AA amyloid fibrils,
this suggests that the polymorphisms may be associated with the
NLRP3 inflammasome activation process and susceptibility to
FMF. These findings may provide insights into modifier factors,
other than MEFV, in the development of FMF.

The gender discrepancy (female dominant in incomplete FMF)
seen in the present study may result from hormonal or associated
environmental factors, which generate a disease of atypical or
milder severity in females. For example, the risk for developing
amyloidosis was shown to be higher in male patients with
FMF [40,41]. These findings suggest that clinical variability
observed in FMF may be partly attributed to the influence of
environmental factors including gender. The main limitations of
the study are its localization to a certain country, and a limited
number of patients.

**Table 5.** Allele frequencies of SAA1 gene polymorphisms in
typical and incomplete FMF patients.

<table>
<thead>
<tr>
<th>Allele at SAA1 locus</th>
<th>Typical</th>
<th>Incomplete</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>16(17.4)</td>
<td>20(27.0)</td>
<td>3.733</td>
<td>0.155</td>
</tr>
<tr>
<td>1.3</td>
<td>44(47.8)</td>
<td>37(50.0)</td>
<td>0.029</td>
<td>0.865</td>
</tr>
<tr>
<td>1.5</td>
<td>32(34.8)</td>
<td>17(23.0)</td>
<td>3.686</td>
<td>0.055</td>
</tr>
</tbody>
</table>

**Table 6.** Number of MEFV gene mutations and SAA1 gene
polymorphisms in FMF patients.

<table>
<thead>
<tr>
<th>Allele at SAA1 locus</th>
<th>0–1 mutations</th>
<th>≥ 2 mutations</th>
<th>χ²</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>11(18.3)</td>
<td>23(23.6)</td>
<td>0.955</td>
<td>0.620</td>
</tr>
<tr>
<td>1.3</td>
<td>29(48.3)</td>
<td>52(49.1)</td>
<td>0.040</td>
<td>0.841</td>
</tr>
<tr>
<td>1.5</td>
<td>20(33.3)</td>
<td>29(27.4)</td>
<td>0.029</td>
<td>0.865</td>
</tr>
</tbody>
</table>

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In occlusion, this study shows a significant prevalence of the -13T allele in Japanese patients with FMF. This comparative case-control study demonstrated that the SAA1 gene polymorphisms might affect susceptibility to FMF, which is presumed to be a monogenic disease. Further studies are required to determine the impact of SAA1 gene polymorphisms and the occurrence of FMF in large studies in different geographic areas.

**Ethics approval**
This study was conducted with the approval of the ethical committees of Nagasaki Medical Center.

**Author Contributions**
Conceived and designed the experiments: KM KA JM HI AK RU YN. Performed the experiments: YJ YM MY. Analyzed the data: KM MN. Contributed reagents/materials/analysis tools: SH YI TK MNF KE HF TN. Wrote the paper: KM MN.

**Table 7. Frequencies of SAA1 -13C/T, SAA2, IL1β -511 genotypes in Japanese patients with FMF and frequencies of SAA1 -13C/T, SAA2, IL1β -511 genotypes in healthy subjects.**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genotype</th>
<th>Observed number(%)</th>
<th>Expected number*</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA1 -13C/T</td>
<td>C/C</td>
<td>13(15.7)</td>
<td>16.1</td>
<td>χ²=1.292  p=0.256</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>47(56.6)</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>23(27.7)</td>
<td>26.1</td>
<td></td>
</tr>
<tr>
<td>SAA2</td>
<td>A/A</td>
<td>62(74.7)</td>
<td>61.6</td>
<td>χ²=0.007  p=0.932</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>19(22.9)</td>
<td>19.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>2(2.4)</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>IL1β -511</td>
<td>C/C</td>
<td>27(32.5)</td>
<td>28.3</td>
<td>χ²=0.144  p=0.704</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>43(51.8)</td>
<td>40.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>13(15.7)</td>
<td>14.3</td>
<td></td>
</tr>
</tbody>
</table>

*Expected genotype frequencies based on observed allele frequencies and assuming Hardy-Weinberg equilibrium.

doi:10.1371/journal.pone.0055227.t007

**References**


