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ORIGINAL RESEARCH

Inflammatory biomarker analysis confirms reduced disease severity in heterozygous patients with familial Mediterranean fever

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ABSTRACT

Introduction Familial Mediterranean fever (FMF) is a genetic disease leading to recurrent episodes of inflammation. Two pathogenic variants are required for classical disease, but the disease can occur in heterozygous patients. Patients are treated continuously with colchicine to prevent amyloid A (AA) amyloidosis, including heterozygous patients who display a moderate form of FMF and rarely develop AA amyloidosis. The need for lifelong colchicine treatment in heterozygous FMF is therefore controversial. We aimed to characterise genotype-specific levels of inflammatory biomarkers, and to focus on heterozygous patients who discontinued colchicine.

Methods All patients with FMF from the European databases AIDnet and JIRcohort who received colchicine during follow-up were included. Demographics, C reactive protein (CRP), serum amyloid A (SAA), S100A8/A9 and S100A12 levels, leucocyte and neutrophil counts were extracted. Visits were classified as active, subclinical or inactive according to symptoms, CRP and SAA levels.

Results Data from 747 patients were extracted (233 homozygous, 201 compound heterozygous, 224 heterozygous patients, 49 heterozygous with one class III variant and 40 compound heterozygous with two class III variants). During active visits, all biomarker levels were higher compared with inactive visits ($p<0.001$). Heterozygous patients showed lower levels of CRP, SAA, S100A8/A9 and S100A12 during inactive and subclinical visits than patients with two class IV-V variants. Colchicine was discontinued in 52 heterozygous patients and reintroduced in 23 of them (44%).

Conclusion S100A8/A9 and S100A12 proteins are biomarkers that can be used to assess disease activity. Heterozygous patients have lower levels of inflammatory biomarkers and some of them can sustainably discontinue colchicine treatment.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Familial Mediterranean fever is an autoinflammatory disease that classically requires two pathogenic variants to manifest. Subjects with only one variant may develop a moderate form of the disease, but are usually treated with lifelong colchicine. Classical inflammatory markers (C reactive protein and serum amyloid A) are not specific for the disease.

WHAT THIS STUDY ADDS

⇒ S100A8/A9 and S100A12 levels are elevated in familial Mediterranean fever following a gene-dose effect and reflect disease activity. Colchicine was sustainably discontinued in 44% of patients in whom it was attempted.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Colchicine could be discontinued in some patients with heterozygous familial Mediterranean fever. S100A8/A9 and S100A12 could be used to monitor disease activity in familial Mediterranean fever.

INTRODUCTION

Familial Mediterranean fever (FMF) is a long-known genetic disease primarily affecting patients from the Mediterranean region.[1] Patients display periodic fever beginning in childhood, associated with systemic inflammatory symptoms, especially aseptic serositis, joint inflammation and pseudoerysipelas.[1] Colchicine is the mainstay of treatment used in FMF,[2] preventing inflammatory attacks and complications. Indeed, if uncontrolled, FMF can lead to AA amyloidosis, which is caused by chronic

and/or recurrent systemic inflammation.[1] FMF is classically seen as recessive disease, meaning that two pathogenic variants in *MEFV* must be present for the disease to develop. However, the possibility of diagnosing FMF in patients with a heterozygous mutation of *MEFV* is accepted. The current EUROFEVER/PRINTO (Paediatric Rheumatology INternational Trials Organisation) diagnostic criteria consider the clinical diagnosis of FMF in patients with only one pathogenic variant (non-confirmatory genotype) and compatible clinical manifestations.[3] In FMF, disease severity and presentation are strongly influenced by genotype.[4 5] Most (>98%) individuals carrying a single mutation—even with a highly pathogenic one—are clinically healthy,[6] but some may have increased subclinical inflammation.[7] Less than 2% of heterozygous carriers develop an FMF-like disease with a relative risk of 6.3–8.1 in comparison to healthy controls.[7 8] These FMF-like patients tend to have a different clinical presentation: first, the disease seems less severe, with less-intense organ involvements.[9–12] Second, a subset of heterozygous patients may experience remission of FMF symptoms after childhood and perhaps some of these patients could be more accurately classified as having Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Adenitis (PFAPA) syndrome.[13] Finally, heterozygous FMF-like patients scarcely, if ever, develop AA amyloidosis.[14] Therefore, the relevance of lifelong colchicine use in heterozygous patients is questionable, as shown in several cohort studies.[15–18]

The main objectives of colchicine treatment are to reduce inflammatory episodes and normalise inflammatory parameters in order to prevent amyloidosis.[2] According to European recommendations, C reactive protein (CRP) and serum amyloid A (SAA) should be used to determine disease activity and to adapt treatments.[2] However, both parameters increase during inflammatory processes, regardless of whether they might be infectious or related to a flare of FMF.[19] Moreover, in most cases, both CRP and SAA return to normal levels between attacks and cannot be used as a means of monitoring overall disease control. The search for biomarkers specific to FMF has been ongoing for two decades. Among the candidate biomarkers, proteins from the S100 family have been of interest. The Damage-Associated Molecular Pattern (DAMP) proteins S100A12 and S100A8/A9 (the later complex also called calprotectin) are suggested to be specifically elevated in patients with autoinflammatory diseases. Both biomarkers have been shown to be elevated in patients with FMF compared with healthy subjects, even when out of flare.[20–23]

The objectives of our study were to investigate inflammatory biomarkers, including the phagocyte-specific S100-proteins, in patients with FMF in and out of flares and according to genotype, and to analyse the influence of gene dosage on biomarkers in heterozygous patients for which colchicine was sustainably stopped.

METHODS

Patients

Patients were identified from the JIR (Juvenile Inflammatory Rheumatism) cohort, an ongoing international multicentre data that started in 2013 (http://www.fondationres.org/fr/jircohorte—NTC02377245), and AID-net, a German retrospective registry that was in place from 2008 to 2018, with patient recruitment to the registry and patient material deposition to biomaterial banks.[24 25] These registries collect clinical and biological data at each medical visit throughout the follow-up period. We considered for inclusion all patients with a registered diagnosis of FMF with genetic status provided, who had been included in the registries before the age of 18 years old, and who needed long-term medication with colchicine due to clinical FMF. The export took place in March 2023 and included all eligible visits since the start of both databases. Clinical data including sex, mutational status, disease history and medication history were extracted.

Ethical approval for AID-Net was obtained from University Muenster (Ref.: 2009-031 f-S) and from University Duisburg-Essen (08-3866). The JIRcohort protocol was approved by the French Ethics Committee (CCTIRS) on 21 April 2015 (decision number 14.302). The electronic form of the JIRcohort was approved by the National Commission of data processing and liberties (CNIL) on 27 March 2015 (decision number DR-2015-218).

Genetic classifications

Variant pathogenicity was defined according to the Infevers registry ranging from class I (benign) to class V (pathogenic) (https://infevers.umai-montpellier.fr/web/search.php?n=1). Homozygous and compound heterozygous FMF was defined by the presence of two class IV-V variants (likely pathogenic and pathogenic variants) in exon 10 of *MEFV*, and heterozygous patients were defined by the presence of one class IV/V variant in exon 10 of *MEFV*. Patients with one class III variant and one class IV-V variant were also classified as heterozygous.[26] Patients with variants described as causative of dominant FMF (exon 8) as well as patients with no variant ≥III were excluded. Habits of genetic testing vary according to the year in which the diagnosis was made, but also according to local habits. Since 2013, according to the national recommendations for diagnosis and care (PNDS) in France (https://www.has-sante.fr/upload/docs/application/pdf/2013-02/pnds_-_fievre_mediterraneenne_familiale.pdf), Exon 2 variant E148Q is not reported to physicians as it is considered a benign variant, although it is a class III variant in the Infevers database.[27] Furthermore, since 2020, the international recommendations for the genetic diagnosis of autoinflammatory diseases stipulate that these class III variants should no longer be reported in laboratory reports even if they used to be.[28] To harmonise patient data, we have based our

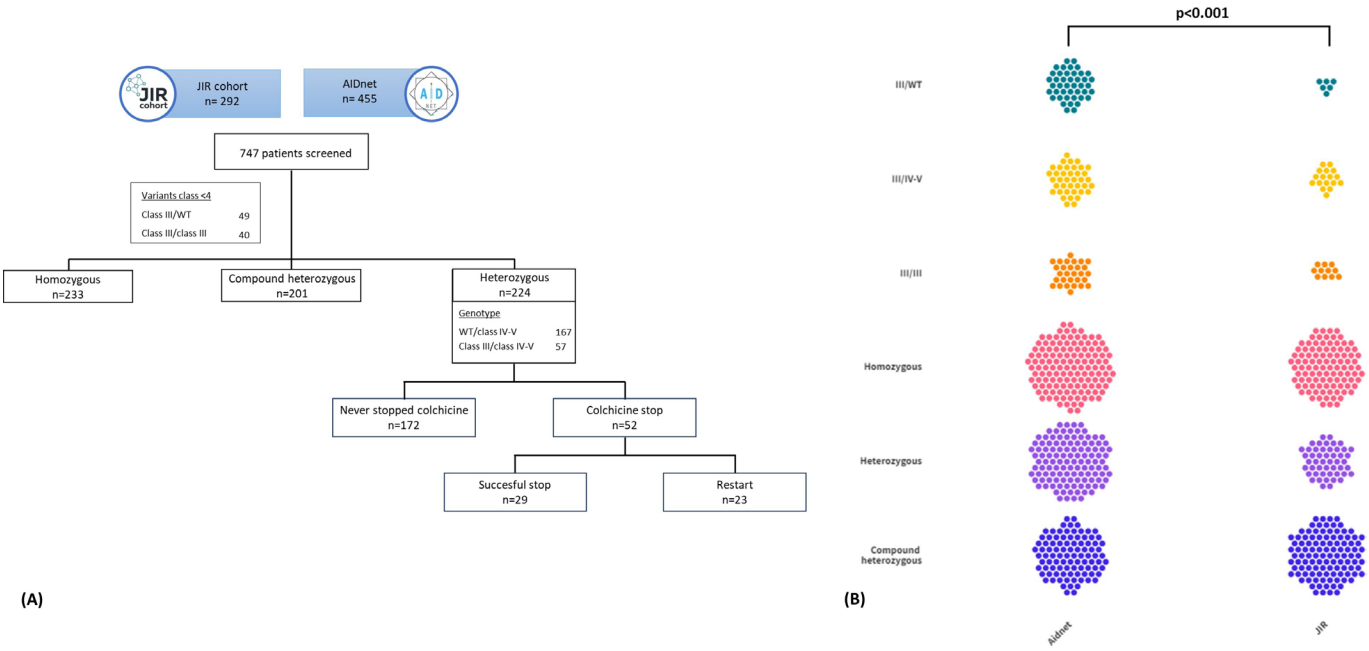

Figure 1 Characteristics of included patients according to database. (A) Flow chart of included patients and corresponding mutation status. (B) Dot plot of included patients according to genotype and database (one patient is represented by one dot). WT, wild type; III, class III variant.

definitions on these more recent restrictive recommendations.

Clinical visits

Visits were taken into account if they included the result of at least one CRP or SAA value. For each patient, each visit was classified into three categories according to disease activity: 'active disease visits' (ongoing inflammatory attacks, fever, serositis or pseudoerysipelas during the visit), 'subclinical disease visits' (raised CRP and/or SAA levels without clinical signs related to inflammatory attacks) or 'inactive disease visits' (normal CRP and SAA levels, outside an inflammatory flare). Categorisation of patients was similar for our AID-Net analysis of patients with FMF from May 2014, that included a minor group of patients (n=128), that is now part of the whole study group.[29] Raised inflammatory markers were defined as CRP >5 mg/L and SAA >7 mg/L.

Table 1 Patients' characteristics according to database

	AIDnet	JIRcohort	P value
n	455	292	
Females	215 (47)	145 (50)	0.59
Mutational status			
Homozygous	132 (34)	101 (37)	**<0.001**
Compound heterozygous	94 (25)	107 (39)	
Heterozygous	157 (41)	67 (24)	
Class III	72 (29)	17 (6)	
Colchicine stop ever in heterozygous patients	36 (23)	16 (24)	1
Median number of visits	6 (3–10)	3 (2–5)	**<0.001**
Age at colchicine stop in heterozygous patients (years)	8.08 (5.25–17.17)	10.41 (9.6–16.71)	0.08
Total time under colchicine in heterozygous patients (years)	1.17 (0.9–5)	6.54 (4.58–12.5)	**0.006**
Number of patients with ≥1 measurements			
S100A8/A9	137 (60)	20 (7)	**<0.001**
S100A12	170 (37)	2 (1)	**<0.001**

Values are number (%) for categorical variables or median (IQR) for continuous variables.
Bold values indicate p-value<0.05.

Table 2 Inflammatory biomarkers according to disease activity in all included patients

	Inactive visits		Subclinical visits		Active visits		Global p value	Inactive versus subclinical	Subclinical versus active
	n	Value	n	Value	n	Value			
CRP (mg/L)	589	1 (1–2.51)	508	7.95 (5.18–14.15)	108	48 (18.4–84)	**<0.001**	**<0.001**	**<0.001**
SAA (mg/L)	443	2.56 (1.08–4)	423	16.65 (9–44)	55	147 (26.4–422)	**<0.001**	**<0.001**	**<0.001**
ESR (mm/hour)	473	8.5 (6–12.5)	414	18 (11–26)	87	25 (15–44)	**<0.001**	**<0.001**	**0.002**
S100A8/A9 (ng/mL)	157	1074.8 (19451–7740)	171	9266.6 (4017–21 089)	9	55671 (11807–75 891)	**<0.001**	**<0.001**	0.082
S100A12 (ng/mL)	172	97 (47–201)	198	202 (7–575)	13	1160 (590–2860)	**<0.001**	**<0.001**	**0.002**
Leucocytes (10^9/L)	501	7.3 (6.35–8.5)	39	8 (6.74–10.9)	98	8.34 (6.8–11.5)	**<0.001**	**0.021**	0.54
Neutrophils (10^9/L)	190	3.72 (2.86–4.51)	136	4.15 (3.05–5.02)	42	4.85 (3.43–16.05)	**<0.001**	**0.012**	**0.013**

Values are number (%) for categorical variables or median (IQR) for continuous variables.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; SAA, serum amyloid A.

Determination of inflammatory markers

Inflammatory markers (erythrocyte sedimentation rate (ESR), CRP, SAA, leucocyte count, neutrophils count, Proteins S100A8/A9 and S100A12) were obtained from registry visits as inserted by the participating centres. S100A8/A9 and S100A12 levels from AID-Net patients were collected centrally at University of Muenster and measured with Bühlmann MRP8/14 Calprotectin ELISA (Bühlmann Laboratories AG) according to manufacturer's instructions, or for S100A12 using an inhouse ELISA (normal level <150 ng/mL) as previously reported.[23] S100A8/A9 and S100A12 concentrations in the JIRcohort were assessed at individual participating centres and obtained as part of routine visits.

For each patient, the median values of biomarkers were calculated for each category of visits (active, subclinical and inactive). This means that each patient has a maximum of three values per biomarker that are included in the analysis. Only if patients presented in all three disease activity categories could three values (median for multiple presentations) be analysed, otherwise fewer.

Statistics

Numeric variables were expressed as median (IQR) and discrete outcomes as absolute and relative (%) frequencies. Normality and heteroskedasticity of continuous data were assessed with Shapiro-Wilk and Levene's test, respectively. Continuous outcomes were compared with Anova, Welch Anova or Kruskal-Wallis tests according to data distribution. Discrete outcomes were compared with χ^2 or Fisher's exact test accordingly. The alpha risk was set to 5% and two-tailed tests were used. Statistical analysis was performed with EasyMedStat (V.3.28; www.easymedstat.com).

RESULTS

FMF genotypes in the cohorts

A total of 747 individuals diagnosed with FMF and treated with colchicine were identified from registries, including 455 patients from AIDnet and 292 from JIRcohort (figure 1A). No gender disparity was observed between the groups. Nevertheless, genotype distribution was significantly different between the two databases (p<0.001), with more heterozygous and patients with class III variants in AIDnet, while there were more compound heterozygous patients in JIRcohort (figure 1B). The distribution of homozygous (n=233), compound heterozygous (n=201) and heterozygous patients (n=224) differed between the registries, with heterozygous patients being more prevalent in AIDnet and compound heterozygous patients being more prevalent in JIRcohort (p<0.001).

Heterozygous patients with FMF

The percentage of heterozygous patients who discontinued colchicine, either temporarily or sustainably, was evenly distributed between the two databases (table 1). In heterozygous patients who discontinued colchicine, the median total duration of colchicine use was 5 years longer in the JIR cohort compared with AIDnet (p=0.006). However, the age at which colchicine was discontinued did not exhibit a significant difference, suggesting that patients from JIRcohort initiated treatment at an earlier age.

Differences between the registers in terms of data collection

The content of both databases was also heterogenous. For instance, while 37%–60% (n=170–137) of patients from AIDnet had at least one measurement of S100A8/A9 and S100A12, because of the included biorepository,

Table 3 Mutational status in *MEFV* gene of included patients

Heterozygous (class IV-V/WT and class IV-V/III)					Homozygous (only class IV/V variant)	Compound heterozygous (only class IV/V variant)	Class III		
Class IV-V variant	n	Additional class III variant	n	Class IV-V variant	n	n	Cass III variant	III/WT n	III/III n
Total	224	Total	57	Total	233	402 (201 patients)	Total	49	80 (40 patients)
M694V	152	E148Q	34	M694V	196	154	A744S	6	
M694I	14	P369S	5	M694I	7	30	E148Q	28	42
M680I	13	A744S	6	M680I	14	73	K695R	8	1
V726A	35	K695R	8	V726A	12	104	I591T	3	2
R761H	4	E230K	1	R761H	3	26	R202Q	2	0
M680L	1	I641F	1	M680L	0	1	R653H	1	0
Q97K	1	R354W	1	F479L	0	8	G196W	1	0
S42R	1	I59T	1	E167D	0	4	L110P	0	4
T267I	1			M694L	1	0	P369S	0	20
A89T	1			I692del	0	1	R408Q	0	9
M694L	1			Other	0	2	Other	0	2

WT, wild type.

it only concerned 1%–7% (n=2–20) of patients from JIRcohort. Likewise, SAA and leucocytes count were more frequently entered into Aidnet than JIR cohort. Overall, not all biomarkers were entered at all visits for both registries.

All biomarkers differentiate between active attacks and inactive disease states

All tested biomarkers were higher when measured during an active visit compared with subclinical and inactive visits and increased gradually. While CRP, SAA, ESR, Neutrophil count and S100A12 were significantly different for all activity groups, S100A8/A9 and leucocyte count were not significantly different between subclinical and active visits (table 2).

Biological differences between FMF genotypes in patients with class IV-V variants

Mutational status is described in table 3. During follow-up, homozygous patients were more likely to register active visits or subclinical visits (p<0.001, respectively). Conversely, heterozygous patients were more frequently classified as inactive than homozygous and compound heterozygous patients (p<0.001, table 4). In inactive visits, median SAA, ESR, S100A8/A9 and S100A12 levels were higher in homozygous patients. Pairwise analyses further suggested that values of SAA, S100A8/A9 and S100A12 levels were specifically lower in heterozygous patients than in compound heterozygous patients during inactive visits (table 4, figure 2A,B). Moreover, in subclinical visits, ESR, S100A8/A9 and S100A12 levels were higher in homozygous than in heterozygous patients. Leucocyte and neutrophil counts were not different between genotypes in neither inactive nor subclinical visits. We could not perform statistical tests for active visits because there were too few datapoints reported.

In a substantial proportion of heterozygous patients with FMF colchicine could be stopped

Colchicine was stopped during follow-up in 52 patients (23%). Among these, discontinuation was sustained up to last visit in 29 patients, and colchicine was restarted in 23 patients (figure 1A). Reasons for discontinuation in the patients were not reported in both registries. Median age at colchicine stop was 9.7 years old (2.5–17.2). Median follow-up time after colchicine stop was 12 months (0–79) and was >2 years in 13 patients (45%). In patients who restarted colchicine, it was restarted after 1.92 years (0.23–3.86). There was no difference in genotype between patients who had sustainably stopped and those who had restarted colchicine (p=0.3). The most frequent variant was M694V (69%), followed by V726A (23%), R761H (4%), M680I (2%) and M694I (2%).

S100 proteins are key biomarkers to differentiate patients with class IV-V variants from those with class III variants

As the clinical significance of class III variants (such as E148Q) is controversial, and as described in the Methods section, we excluded these patients from our overall analysis and from the colchicine discontinuation analysis. Nevertheless, we were interested in an additional comparison of these patients, as shown in table 4, in which we compared 89 patients with only class III variants with the other genotypes. We found that S100A12 levels were specifically lower in patients with only class III variants compared with heterozygous patients at both inactive and subclinical visits (table 4). In addition, S100A8/A9 levels were significantly lower in inactive disease. No other biomarker was different between the two groups.

Table 4 Visits characteristics and inflammatory biomarkers according to genotype

	Homozygous	Compound heterozygous	Heterozygous	Class III variants	a	b	c	d	e
n	233	201	224	89					
Females	125 (54)	93 (42)	97 (48)	45 (49)	NS	ND	ND	**ND**	ND
Median number of visits	7 (2–13)	3 (1–9)	5 (2–8)	4 (2–11)	***	*	***	*	NS
Inactive/total ratio	0.33 (0.08–0.6)	0.56 (0–1)	0.6 (0.3–0.9)	0.6 (0–0.9)	***	NS	***	***	NS
Subclinical/total	0.54 (0.2–0.8)	0.2 (0–0.5)	0.25 (0–0.57)	0.27 (0–0.5)	***	NS	***	***	NS
Inactive visits	2 (1–5)	1 (0–5)	3 (1–5)	2 (1–7)	**	**	NS	*	**NS**
CRP (mg/L)	1.2 (1–3)	1 (0.6–2.6)	1 (1–2.2)	1 (1–2.1)	NS	ND	ND	ND	ND
SAA (mg/L)	2.8 (1.19–4.96)	3 (1.59–3.82)	2.2 (0.8–3.68)	2.4 (1.4–3.3)	*	*	NS	**	NS
ESR (mm/hour)	10 (7.62–15.75)	8 (6–11)	8 (5–11)	8 (6–11)	***	NS	***	***	NS
S100A8/A9 (ng/mL)	7095.2 (3427–14729)	5788.4 (3176.6–11 446.7)	2689.7 (1274.86)	1137 (700–2567)	***	***	NS	***	*
S100A12 (ng/mL)	135 (81–230)	170 (91–280)	70 (37–106.11)	41 (25–110)	***	***	NS	***	*
Leucocytes (10^9/L)	7.2 (6.18–8.28)	7.2 (6.25–8.4)	7.4 (6.54–8.71)	7.2 (6.4–8.1)	NS	ND	ND	ND	ND
Neutrophils (10^9/L)	3.72 (2.82–4.59)	3.92 (2.98–4.41)	3.81 (2.87–8.42)	3.5 (2.7–7.4)	NS	ND	ND	ND	ND
Subclinical visits	3 (1–7)	1 (0–3)	1 (0–3)	1 (0–3)	***	NS	***	***	NS
CRP (mg/L)	9.85 (6–16.8)	7.5 (5–11.65)	6.5 (3.9–11.35)	6.6 (3.1–11.3)	***	NS	**	***	NS
SAA (mg/L)	21 (11.48)	17 (10.12–39.5)	13.75 (7.5–37.9)	12.2 (7–38)	*	NS	NS	NS	NS
ESR (mm/hour)	20 (13–28.38)	17 (11–23.5)	15 (9.12–24)	18 (10.5–30)	**	NS	*	***	NS
S100A8/A9 (ng/mL)	14 118 (5561–50 850)	9319 (1870–16 637)	6100 (3081–13 111)	2416 (1462–6338.6)	***	NS	NS	***	NS
S100A12 (ng/mL)	303 (130–1590)	219 (111–510)	140 (57–335)	58 (34.3–133.1)	***	NS	NS	*	**
Leucocytes (10^9/L)	8 (6.759.2)	7.12 (6–8.78)	10 (8.97–12.26)	NA	ND	ND	ND	ND	ND
Neutrophils (10^9/L)	4.4 (3.3–5.23)	4.05 (3–4.63)	4.05 (2.9–5.15)	3.7 (3–5.2)	NS	ND	ND	ND	ND

Values are number (%) for categorical variables or median (IQR) for continuous variables.
a: Global p value; b: heterozygous versus compound heterozygous; c: homozygous versus compound heterozygous; d: heterozygous versus homozygous; e: class III variants versus heterozygous.
*P<0.05. **p<0.01. ***p<0.001. Bold values indicate p-value<0.05.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; NA, not available; ND, not done; NS, not significant; SAA, serum amyloid A.

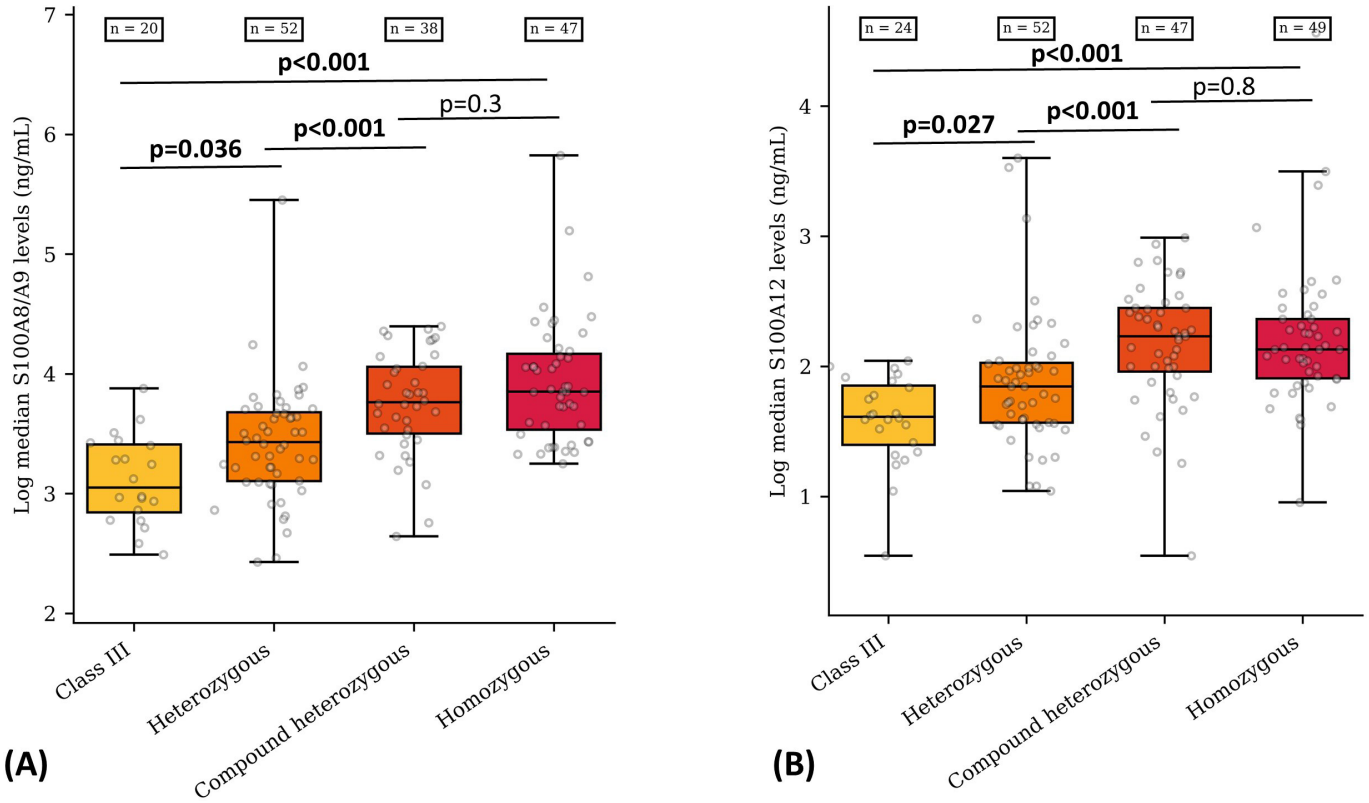

Figure 2 S100 proteins levels measured during inactive visits levels according to genotype. (A) Decimal logarithm conversion (10^n) of median S100A8/A9 levels in patients with class III variants, heterozygous, compound heterozygous and homozygous patients. (B) Decimal logarithm conversion (10^n) of median S100A12 levels in patients with class III variants, heterozygous, compound heterozygous and homozygous patients.

Furthermore, they are significantly elevated in homozygous and compound heterozygous patients when compared with heterozygous patients in inactive disease.

DISCUSSION

In this study, we (1) successfully pooled data from two European databases of patients with well-characterised FMF disease during their childhood follow-up. We (2) showed that the levels of inflammatory biomarkers in FMF are genotype dependent, reflect gene dosage and correlate with the clinically described severity of the variants. Patients with heterozygous variants had lower levels of both classical (CRP, SAA and ESR) and novel (S100A8/A9, S100A12) biomarkers than compound heterozygous and homozygous patients. In addition, we (3) showed that colchicine could be discontinued in a significant number of heterozygous patients with a treatment-free follow-up of one to 2 years, adding to the growing body of knowledge about disease outcomes in heterozygous FMF. Finally, (4) we found that patients with only class III variants could be clearly distinguished from class IV/V variants based on S100 protein analysis.

The first step of our study—combining two different databases—revealed different practices in the management of FMF. As the diagnosis of FMF is a combination of phenotype and genotype, a definitive diagnosis may be difficult in heterozygous patients. [6 7] Differences in genotype ordering and interpretation may account for the higher proportion of patients with class III variants in AIDnet than in JIRcohort. This was also influenced by the national genotype reporting practices as described in the Methods section. Genotype reporting could also differ according to the genetic analysis performed (next-generation sequencing panel, exon 10 Sanger or whole gene Sequencing). Overall, the merging of two databases required additional revisions with the exclusion of patients with class III variants for the global analysis and a careful interpretation.

The role of *MEFV* genotype for the FMF phenotype is very well known.[4] While M694V variants are associated with a more severe disease with a dose-dependent effect, they additionally result in higher frequency of attacks, higher biomarkers and higher rates of AA amyloidosis.[4 30] A previous report had already shown higher CRP levels in patients with exon 10 homozygosity than in those with compound heterozygosity and heterozygosity.[31] In addition, healthy carriers have elevated levels of inflammatory biomarkers compared with non-carriers.[32] Conversely, patients with two M694V variants have higher levels than those heterozygous for M694V.[32]

S100A8/A9 and S100A12 have been candidate biomarkers to sensitively assess inflammation in FMF before.[22 29 33 34] In this study, we confirm that median S100A8/A9 and S100A12 levels are higher during flares

than during subclinical visits and inactive visits.[29] We also found that heterozygous patients displayed lower inflammatory biomarkers in both inactive and subclinical state than patients with FMF with two class IV/V variants. Taken together, S100-protein evaluation according to disease activity status in patients with FMF clearly reflects gene dosage of MEFV. We had recently identified pyroptosis as a key event inherent of FMF pathophysiology, to be responsible for S100A12 secretion, especially in inactive patients with FMF.[29 30 33 35] This is the first study, that also identifies S100A8/A9 as mirror of MEFV-gene dosage in a real-world setting, not surprisingly, as all three proteins are biochemically closely related with intracellular and extracellular functions according to their DAMP activity.[36] The exact place of these two biomarkers in the management of FMF still needs to be determined, particularly in the therapeutic management and in classifying patients in terms of risk of colchicine resistance and/or disease severity. However, a single cut-off for all patients with FMF may not be appropriate as genotype affect their levels. In addition, the added value of these biomarkers in predicting the risk of AA amyloidosis remains to be determined.

Lifelong colchicine medication is the rule in FMF because of the risk of the life-threatening complication AA amyloidosis in homozygous patients.[2 27] As previously mentioned, this risk is minimal in heterozygous patients.[37] In this study, we found that colchicine stop was sustained until last visit in 56% of heterozygous patients for which colchicine stop was tried. Colchicine discontinuation in FMF has been up to now investigated in four studies,[15–18] which included patients with class III variants and without *MEFV* variants depending on the study. This approach differs from ours, which excluded patients with FMF without genetic confirmation and separated those with a class III variant. High-dose colchicine medication prior to withdrawal, high attack severity, compound heterozygosity and M694V variants were associated with higher relapse rates in previous studies.[16] Although the median follow-up of 2 years in our study may be short, compared with a lifetime perspective, the relevance of continuous colchicine treatment in patients who can go several months to years without flares should be questioned. Heterozygous patients may experience successive periods of active and inactive disease influenced by genetic modifiers and/or environmental factors. Therefore, the need to restart colchicine may indicate the re-emergence of a transient active phase of the disease rather than a definitive need for treatment. In previous studies, colchicine was discontinued for 6 months to 3 years in patients with inactive disease.[15–18] Further studies will need to be conducted to specifically determine the duration without colchicine restart in these patients. Definite treatment discontinuation may however apply to a subgroup of heterozygous patients whose characteristics remain to be determined. In addition, it is controversial whether these patients, especially during infancy, should be reclassified as having PFAPA or syndrome of undifferentiated recurrent fever rather than classical FMF.

International recommendations do not consider genotype when determining treatment strategy. However, our results suggest that the management of these patients should probably be different from that of patients with two pathogenic variants. Factors that may be considered in the decision to discontinue colchicine in heterozygous FMF include the exact genotype (M694V variant), family history of AA amyloidosis, predictable phase of active disease (attacks triggered by life events, pregnancy…), and other biomarkers that still need to be determined.

Our study has several limitations intrinsic due to its retrospective nature. Differences in genetic testing protocols and variant classification, such as the reporting of class III variants may have introduced heterogeneity in the diagnosis and classification of patients. However, we have used the latest recommendations for variant classification to reduce this bias. In addition, some analyses could not be carried out due to missing data, especially regarding novel biomarkers and active visits. Patients were included if they had had at least one measurement of CRP and SAA. Therefore, patients with a more severe disease could be over-represented. Moreover, the reasons for colchicine discontinuation were not specified, but could include physician's decision or non-compliance. Finally, the decision to restart colchicine was not described and may vary between physicians, centres and countries. Further prospective studies should investigate heterozygous patients with controlled colchicine discontinuation and evaluate their biochemical inflammatory activity including S100-proteins at the time of discontinuation and according to disease status.

Interestingly, classical biomarkers (SAA, CRP and ESR) were not different between patients with only class III variants and those with a class IV-V variant. S100 proteins were the only biomarker that differed specifically between the two groups. The influence of class III variants on phenotype is still debated: A Turkish study found that patients with both class III/IV-V had more severe symptoms than those with just heterozygous class IV-V, but biomarker levels were not discussed,[38] whereas an Israelian study showed the contrary.[26] The low levels of S10012A measured in patients with class III variants may be similar to those found in healthy individuals or those with non-inflammasome-related diseases.[39] Therefore, these results suggest that the inflammatory activity in these patients is less than in classical FMF and therefore even more supportive of a trial of colchicine withdrawal in these patients.

CONCLUSION

In conclusion, we could show that S100A8/A9 and S100A12 levels are elevated in FMF following a gene-dose effect and reflect disease activity. For patients with heterozygous FMF, we see a specific less-severe phenotype of FMF, that may require specific management. Possibly this

includes discontinuation of colchicine, which could be monitored with the aid of new biomarker levels, that is, S100A8/A9 and S100A12. Their uniquely lower levels of inflammatory biomarkers should prompt clinicians to consider genotype when interpreting laboratory results in patients with FMF.

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