EXTENDED REPORT

Severe disease in patients with rheumatoid arthritis carrying a mutation in the Mediterranean fever gene

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Ann Rheum Dis 2005;64:1009–1014. doi: 10.1136/ard.2004.029447

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Accepted
28 December 2004

Background: Pylrin is a newly recognised intracellular regulator of inflammation, and mutations in *MEFV*, the gene encoding pyrin, are the cause of familial Mediterranean fever.

Objective: To determine if known mutations of *MEFV* are associated with rheumatoid arthritis (RA) morbidity or can modify RA severity.

Methods: The frequency of the three most common *MEFV* mutations: M694V, V726A, and E148Q, was determined in 98 Israeli patients with RA (74 women, 24 men) and compared with that in 100 healthy subjects matched for origin. RA severity was determined using a new clinical score of 126 grades. The median severity score of mutation carrier and non-carrier groups was compared after confounding measures were eliminated by logistic regression.

Results: 17/98 (17%) patients with RA (all women) were heterozygous for common *MEFV* mutations, predominantly E148Q (12 patients), and one patient was homozygous for the V726A mutation. The overall mutation rate was comparable between patients with RA and healthy subjects. Patients carrying a mutation had a higher median severity score than the non-carrier group (42 v 29, $p=0.0005$). The logistic regression model assigned a 15-fold odds ratio for severe RA in carriers, after adjusting for sex, presence of rheumatoid factor, age at onset, and disease duration ($n=97$, $p=0.01$, 95% CI 1.74 to 128).

Conclusion: *MEFV*, and particularly the E148Q mutation, is an independent modifier of the clinical manifestations of RA. This is the second Th1-type autoimmune disease in which *MEFV* mutations have been shown to aggravate the clinical status.

Rheumatoid arthritis (RA (MIM180300)) is a systemic autoimmune disease primarily affecting the joints, and present in about 0.8% of the world population. The risk of developing RA is ascribed in part to genetic factors, which, besides the important HLA class II DRB1 gene, encoding the consensus, high risk amino acid sequence QKRAA or related sequences,^{1–3} include less well characterised susceptibility intervals on chromosomes 1, 6, 12, 16, 17, 18, and 21.^{4–6} Moreover, additional immune system genes may affect RA manifestations. For instance, the variable extent of joint erosions among patients^{7–8} has been related to the DR antigens,⁹ rheumatoid factor (RF),^{10–11} and to several gene variants of the innate immune system.^{12–14} RA development and expression may therefore be associated with heterogeneous predisposing gene combinations, and with additional, as yet unidentified, loci.

Pyrin and several other proteins containing the pyrin domain are intracellular modules of inflammatory signalling that are associated with autoinflammatory disorders.¹⁵ Mutations in *MEFV*, the gene encoding for pyrin, cause familial Mediterranean fever (FMF), a recessive recurrent polyserositis^{16–17} and are associated to a lesser extent with several other autoinflammatory conditions.^{18–22} When combined with a Th1 related autoimmune disease, carrying an *MEFV* mutation may aggravate the disease course, as shown for carriers among patients with multiple sclerosis of non-Ashkenazi origin.²³

A connection between *MEFV* mutations and arthritic diseases was suggested by Booth *et al*, who found that the E148Q mutation was overrepresented in white and Indian patients with inflammatory arthritis and amyloidosis.²⁴ A recent report extended this association and showed overrepresentation of the M694V mutation in a cohort with juvenile RA.²⁵ Interestingly, arthritis is a common manifestation in FMF, especially in M694V homozygotes.^{26–30} Moreover,

monocytes and synovial fibroblasts have been reported to express *MEFV* in culture.^{31–32}

Because *MEFV* mutations are carried by 10–20% of the general population amongst Semite people (see, for example, Stoffman *et al*³³) it is important to investigate the impact of this genotype on RA.

We adopted a case-control design, to compare the *MEFV* mutation frequency between patients with RA and healthy subjects, and to compare disease severity between mutation carriers and non-carriers, as this design is considered the most sensitive method of identifying disease risk factors in a small sample.³⁴

PATIENTS AND METHODS

Patients and healthy subjects

The study included 98 patients with RA (74 women, 24 men), recruited consecutively and followed up at the rheumatology clinic of Sheba Medical Centre, Tel Hashomer, Israel. All patients fulfilled the American College of Rheumatology criteria for diagnosis of RA.³⁵ The median duration of RA in the entire cohort was 11 years, and the median onset was at 47 years of age. One hundred healthy blood donors (67 men, 33 women), serving as controls, were recruited consecutively at the National Blood Services donation centre, at Tel-Hashomer, Israel. An attempt was made to match the ethnic composition of the healthy group to that of the patients with RA. There were two exceptions to this, Iraqi-Jewish patients were overrepresented in the RA group (14 v 3, $p=0.03$) and Yemenite-Jewish patients were overrepresented in the healthy subject group (3 v 1, $p=0.05$). In addition, the healthy blood donors completed a questionnaire inquiring about FMF, RA, or other autoinflammatory or autoimmune

Abbreviations: CI, confidence interval; FMF, familial Mediterranean fever; IL, interleukin; RA, rheumatoid arthritis; RF, rheumatoid factor

Table 1 Score system for RA severity

Parameter	Method of scoring	Maximal score*
Tender or swollen joints	1 for each inflamed joint	Upper limbs 10 Lower limbs 10
Deformed joints	1 for each joint	Upper limbs 10 Lower limbs 10
Joint disability affecting daily tasks†	5 when affecting complex movement in an independent subject 10 when subject needs help in eating and dressing 15 when subject totally depends on assistance 5 when walking with a cane 10 when using a walker 15 when using a wheelchair or bed-ridden	Upper limbs 15 Lower limbs 15
Extra-articular, RA related disease‡		Lower limbs
Neurological	2.5 for each affected limb	10
Respiratory	2 for shortage of breath during effort 4 for shortage of breath at rest 6 for need of oxygen	6
Heart insufficiency	2.5 for each level according to AHA	10
Amyloidosis/vasculitis	2 for each affected organ/limb	10
Drug load	1 for each RA intended drug	10
Unresponsiveness to treatment	1 for each month of current year with acute symptoms	10
Total for all measures		126

*Score grades are allocated to each RA affected organ or system according to severity, dysfunction, and suffering, to ensure that one affected system does not outweigh the total score; †the scoring rheumatologist determined the relationship of a given manifestation to RA by excluding other diagnostic possibilities, to an extent he/she considered appropriate.

diseases, and reported none. All participants, patients and healthy subjects, consented to the study in writing and donated 2 ml of blood, collected in an EDTA tube.

Detection of common *MEFV* mutations

Genomic DNA was prepared from 200 µl of whole blood using a commercial kit (high template polymerase chain reaction preparation kit, Roche IL, USA). Three common *MEFV* mutations—M694V, V726A, and E148Q—identify 70% of the FMF chromosomes in Israeli-Jewish patients with FMF. These mutations were detected on polymerase chain reaction amplified fragments of exon 10 and exon 2 of *MEFV* by enzymatic restriction analysis as previously described.³⁶

RA severity score: content, applicability, and construct validation

All patients were examined, interviewed, and then scored once during the year 2000. Table 1 describes the new scoring system. It grades RA manifestations according to the extent of disease, organ dysfunction, disability, and suffering. It is simple and does not require an expert opinion other than that of a rheumatologist, it only minimally depends on the patient's or physician's perception of the severity of the disease, and there is no prerequisite for laboratory investigation. It was developed in the absence of a composite scoring system for RA, and the growing use of a combination of complementary scoring systems, each evaluating one aspect

of disease activity (for example, Sharp or Larsen scores to evaluate joint destruction, RA Disease Activity Index (RADAI) or Disease Activity Score (DAS) scores to assess disease activity, and Health Assessment Questionnaire (HAQ) score to measure health status). Validation of the present scoring system was obtained by correlating its outcome with the presence of the RF, a known, external marker of RA severity.

Rheumatoid factor

Patients whose plasma contained high titre (1/20 or higher) IgM antibodies capable of aggregating IgG coated latex beads were considered RF positive.

Statistical analyses

The rate of *MEFV* mutations, $n/2N$, is the number of mutations (n) in the *MEFV* genes of N screened subjects. This rate was compared between RA and healthy cohorts by Fisher's exact test. The RA severity score is non-parametric (disability is not a continuous variable) and therefore the medians of the total score and of the disability score were compared between groups using the Mann-Whitney test. The statistical significance of all other score differences was assessed by Student's t test. A multiple logistic regression model was used to determine the independent effect of five variables (carrier status, female sex, RF, age at onset, and disease duration) on the risk of scoring above median severity. Differences were considered significant when the probability that they occurred by chance was <0.05 . These analyses were performed using SAS software (SAS Institute, Cary, North Carolina).

RESULTS

MEFV mutation frequency in patients with RA

A screen of 98 patients with RA for common mutations identified 17 heterozygous and one homozygous patient with no FMF manifestations (V726A/V726A), and an overall mutation rate of 0.095. A comparable mutation rate (0.06) was found in the healthy cohort of 100 blood donors of matched ethnic composition recruited for this study

Table 2 Rate of *MEFV* mutations in 98 Israeli patients with RA and 100 healthy subjects

Mutation	Patients with RA No (frequency)	Healthy subjects No (frequency)	p Value for χ^2
E148Q	12 (0.06)	6 (0.03)	0.13
M694V	4 (0.02)	3 (0.015)	
V726A	3 (0.015)	3 (0.015)	
Total rate	19 (0.095)	12 (0.06)	0.17

One patient with RA was homozygous for the V726A mutation and one healthy subject was compound heterozygous for the V726A and E148Q mutations. Neither had FMF.

Table 3 Clinical and demographic description of the carrier and non-carrier patients with RA

	Carriers	95% CI	Non-carriers	95% CI	p Value
Number of patients (n)	18		80		
Mean age at onset of RA (years)	54.3*	48.9 to 59.7	45.9	41.8 to 49.9	0.07
Mean age at diagnosis (years)	56.8	50.4 to 63.1	46.9	42.7 to 51.0	0.07
RA duration (years)	10.9*	7.9 to 13.8	15.0	12.7 to 17.3	0.1
Women, No (%)	18 (100)		56 (70)		0.01
Familial RA, No (%)	2 (11)		10 (13)		1
Ashkenazi patients, No (%)	8 (44)		37 (46)		0.9
Non-Ashkenazi-Jewish, No (%)	9 (50)		37 (46)		1.0
Others†	1 (6)		6 (8)		
RF positive patients, No (%)	17 (94)		42 (53)		0.001

95% CI, confidence interval for the mean when $\alpha=0.05$.

*The age at onset of RA was unavailable for one carrier patient, $n=17$.

†The "Others" group had one Arab carrier patient, two non-carrier patients of mixed (Ashkenazi and non-Ashkenazi) origin and four non-carrier patients of undetermined Jewish ethnicity. For the entire ethnic distribution of carriers and non-carriers, $p=0.9$.

($p=0.17$, table 2); Omitting the Iraqi-Jewish and Yemenite-Jewish patients, whose frequency was unequally distributed between the patients and control groups (as specified in the "Patients and methods" section) had little effect on the overall mutation rate in either cohort ($0.085 \nu 0.07$, $p=0.4$). All mutation carriers in the RA cohort were women (17/74 female carriers ν 0/24 male carriers, $p=0.009$), whereas mutation carriers in the healthy cohort were distributed among men and women (5/33 female carriers ν 6/67 male carriers, $p=0.3$).

Table 3 presents the clinical and demographic characteristics of the carrier patients. Carriers had a later mean onset of RA of about 8 years ($p=0.07$) and later diagnosis of RA of about 10 years ($p=0.07$) than non-carriers, and correspondingly, a shorter mean disease duration (10.9 years in carriers ν 15.0 years in non-carriers, $p=0.1$). The ethnic composition and rate of familial RA were comparable in both subgroups.

Severity score

The severity score of patients with RA ranged between 6 and 70 out of a maximum score of 126, and was distributed with a median of 34 (fig 1A). The severity score outcome was validated against the presence of RF, an accepted severity risk factor. Forty of the 59 RF positive subjects had a higher than median severity score (sensitivity = 68%, 95% confidence interval (CI) 0.55 to 0.78), whereas 30/39 RF negative patients scored below or equal to the median severity score (specificity = 77%, 95% CI 0.62 to 0.87). A small increase in sensitivity (71%, 95% CI 0.53 to 0.85) was obtained when only quartiles 1 and 4 of the severity chart were considered. The positive predicted value of the score (the fraction of RF positive patients among those with a score above the median) was 0.82 (95% CI 0.7 to 0.9, diagnostic odds ratio (OR) = 7.0, 95% CI 2.7 to 17.7, $p<0.0001$).

Severity score in MEFV mutation carriers

The median score of patients who carried a common MEFV mutation was higher than that of non-carriers by 13 grades: 42 ν 29 respectively ($p=0.0005$, fig 1B). Carriers of the E148Q mutation alone ($n=12$) had a significantly higher median score than non-carriers (46 ν 29, $p=0.0015$, data not shown graphically). The measures that scored significantly higher in carriers were count of deformed joints, disability, extra-articular manifestations, drug load, and the duration of active disease despite treatment in the past year (table 4). Notably, vasculitis was as prevalent in carriers as in non-carriers (3/18 ν 13/80, $p=1$), only one patient (a non-carrier) had amyloidosis, and yet severe heart insufficiency and

respiratory complications were overrepresented among the carrier patients (3/18 ν 1/80, respectively, $p=0.02$).

Because all carrier patients were women, sex was analysed as a confounding factor for increased severity. Indeed the median score of women of the entire RA cohort was higher than that of the men (36 ν 24 respectively, $p=0.002$, fig 1C). Nevertheless, female carriers ($n=18$) had a higher median score than female non-carriers ($n=56$) (42 ν 33, respectively, $p=0.004$, fig 1D).

As expected RF positive patients ($n=59$) had a higher median severity score than RF negative patients with RA ($n=39$) by 10 grades ($p=0.01$, fig 1E), and a higher score in each score variable ($p<0.05$). Because almost all MEFV mutation carrier patients (17/18), but only 42/80 (53%) non-carriers tested positive for RF, the effect of MEFV mutations was also analysed separately in RF positive patients. In this cohort the median score of mutation carriers still remained higher than that of non-carriers by 11 grades (45 ν 34, respectively, $p=0.006$, fig 1F).

Finally, in comparison with female non-carriers who were also RF positive ($n=32$) the corresponding carriers ($n=17$) still had a higher median severity score by 9 grades (45 ν 37, $p=0.03$, not shown graphically).

The effect of MEFV mutations on RA severity was also determined using a multiple logistic regression model with five variables and a dichotomous outcome (score above the median = 1, score equal to or below the median = 0). When the age at onset, disease duration, sex, and RF were adjusted between the carrier and non-carrier groups the presence of a mutation predicted a 15-fold odds ratio to score above median severity ($n=97$, 95% CI 1.7 to 128, $p=0.01$). Similarly, after adjustment of the age at onset, disease duration, sex, and mutation status between the RF negative and positive groups the presence of the RF predicted a 3.0-fold odds ratio to score above the median (95% CI 1.1 to 7.9, $p=0.02$). The age at onset and sex of the subject had no significant effect on severity ($p=0.9$ and $p=0.5$, respectively). Disease duration had a negligible effect on the severity outcome in the model (OR = 0.99, 95% CI 0.9 to 1, $p=0.07$). All in all, there was a good fit between the dichotomous outcome predicted by the five variables combined and the observed severity score of the patient ($c=0.80$, likelihood ratio = 30.2, $df=5$, $p=0.0001$).

DISCUSSION

This study suggests a strong association between RA severity and the presence of mutations in the MEFV gene in patients with RA. The association was found using a new, integrative, and comprehensive clinical scoring system assessing disease

Figure 1 The severity score of patients with RA. The score range was divided into grade intervals of 10 and the score of each patient distributed to the appropriate interval. (A) Score distribution of all patients. (B) Patients with or without *MEFV* mutations. (C) Female or male patients. (D) Female patients with or without *MEFV* mutations. (E) RF positive or negative patients. (F) RF positive patients with or without *MEFV* mutations.

activity, suffering, extent of joint and organ involvement, and physical disability by using measurable and comparable criteria. This scoring system yielded a wide range of values with a Gaussian distribution for all patients. The scoring system was validated by correlating its outcome with two known predictors of RA severity. Specifically, women had a higher median score than men and RF positive patients had a

higher score than RF negative patients when assessed with this system. Carriers of the *MEFV* mutations maintained a higher median score than non-carriers when these confounding factors were eliminated using a multiple logistic regression model. The adjusted odds ratio for severe RA in carriers was five times higher than that associated with the RF, suggesting that disease-causing alleles in *MEFV* are

Table 4 Mean score in each severity variable in relation to the *MEFV* genotype

Variable	Carriers (n = 18)	Non-carriers (n = 80)	p Value
Tender or swollen joints	8.9 (5.2)	7.5 (5.8)	0.38
Deformed joints	10.1 (6.9)	6.9 (5.9)	0.04
Disability in daily tasks*	10	5	0.06
Extra-articular manifestations	2.4 (5)	0.6 (1.4)	0.006
Drug load	5.8 (1.9)	4.5 (2.1)	0.01
Active disease in past year	8.5 (2)	5.8 (2)	0.001
Total score*	42	29	0.001

*Median score, because the variable is non-continuous.

independent and major genetic aggravating factors for RA severity. It is noteworthy that preliminary data showed no association between joint disease and the HLA-DRB1 typing in patients with FMF (Shinar *et al*, unpublished data).

Interestingly, *MEFV* maps within a susceptibility region for RA on chromosome 16p13-q12.2 that has emerged with recent meta-analysis of four separate genome screens.⁵⁻³⁷ How mutations in *MEFV* might increase the severity of RA is not yet known, but this may be related to the function of pyrin. This molecule is a cytoplasmatic protein with an N-terminal domain related by secondary structure to the death domain of transducers of apoptotic and inflammatory signals.³⁸ Pyrin regulates the rate of pro-interleukin (IL) 1 β processing to IL1 β by caspase-1 via binding to apoptotic speck protein (ASC), a protein containing a procaspase-1 recruiting domain.³⁹⁻⁴⁰ Germ line disruption of pyrin in mice resulted in increased IL1 β secretion by peritoneal macrophages in culture, in response to lipopolysaccharide.⁴¹ Possibly, a similar increase in IL1 β secretion occurs in response to proinflammatory proteins in patients with RA who express a mutated form of pyrin. This cytokine is pivotal in inducing clinical manifestations of RA,⁴² and the recombinant IL1 receptor antagonist, anakinra (Kineret), is effective in treating patients with RA and reducing joint destruction.⁴³ An increase in IL1 that is driven by mutated pyrin may therefore exacerbate RA. Also of note is the finding that healthy heterozygotes for *MEFV* mutations appear to have higher than normal blood levels of acute phase reactants,⁴⁴ supporting the role of *MEFV* mutation in the induction of inflammation.

FMF is described as an autoinflammatory disease as it is not thought to be driven by defects in the adaptive immune response. It is therefore interesting that this is the second study in which FMF-causing mutations in *MEFV* have been found to be associated with increased severity of an autoimmune disease. In a previous study of a Jewish cohort with multiple sclerosis increasing disability occurred more rapidly in patients carrying the M694V allele of *MEFV*.²³ Interestingly, in the case of RA, an allele of *MEFV* that is thought to cause less severe FMF, the E148Q mutation, was predominantly present in the carrier cohort. Because E148Q is common among Semites, Punjabi Indians, and Chinese,²⁴⁻³³⁻³⁶ and therefore may be carried by millions, this mutation may affect patients with RA world wide.

It is plausible that patients carrying an *MEFV* mutation may benefit from colchicine, the mainstay drug of FMF treatment, yet careful assessment is warranted because the mode of action of colchicine is still not well understood. For instance, despite an apparent increase in IL1 β expression and synthesis,⁴⁵ the anti-inflammatory action of the drug may be related to an up stream inhibition of leucocyte migration to the synovium. Colchicine has been shown to exert a favourable effect on osteoarthritis in Indian patients.⁴⁶ Intervention using this drug in RA is currently rare and is

limited to certain extra-articular manifestations such as amyloidosis,⁴⁷ nodulosis,⁴⁸ or pericarditis.⁴⁹

Two observations reported herein suggest that FMF mutations are not positively associated with a predisposition to develop RA. Firstly, patients with RA have a mutation frequency similar to that of control subjects, suggesting that the mutation carriers would not have an increased risk of developing RA. Secondly, a trend towards late age at onset in the carrier group of this study suggests that the contribution of the *MEFV* mutations toward triggering RA is relatively weak, or even protective. It is also of note that despite autosomal inheritance of the *MEFV* gene, and contrary to former studies,³³⁻³⁶ all mutation carriers in this study were women. The absence of male carriers may be attributed to the small male group (n = 24). Nevertheless, RA may occur more readily in female carriers as the susceptibility of women to RA might reflect sensitivity also to non-sex related genetic factors.⁵⁰ Studies of larger cohorts would be better able to examine this issue of sex bias.

In conclusion, the results reported herein support the hypothesis that patients with RA carrying one *MEFV* mutation seem to be highly susceptible to developing a severe disease. Moreover, these data suggest that *MEFV* mutations may increase the risk of developing RA in women. These and similar data and the proposed mode of action suggest a modifier role for pyrin in RA and in a wide spectrum of autoinflammatory and autoimmune diseases. Mutations in *MEFV* that are known to cause FMF may therefore be prognostic for autoimmune disease outcome.

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This study was approved by the institutional ethical committee at the Sheba Medical Centre.

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