
Prevalence and significance of the familial Mediterranean fever gene mutation encoding pyrin Q148

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Summary

Familial Mediterranean fever (FMF) is caused by more than 25 mutations in the gene *MEFV*, which encodes pyrin (marenostrin), a protein implicated in the regulation of neutrophil activity. Pyrin Q148, is one of the five most common variants in populations in which FMF typically occurs. Our identification of the pyrin Q148 allele in several patients from ethnic groups in which FMF is not classically recognized who had longstanding fevers or AA amyloidosis prompted us to study the prevalence of pyrin Q148 in healthy British, Indian and Chinese subjects. The gene frequency was also sought in 50 British Caucasian patients with inflammatory arthritis, 25 of whom had AA amyloidosis, five Punjabi Indians with AA amyloidosis complicating inflammatory arthritis, and seven British Caucasian patients with uncharacterized longstanding fever syndromes. The allele frequency

for pyrin Q148 was 21%, 15% and 0%, respectively, among Punjabi Indian, Chinese and Caucasian British controls, and was significantly increased among the patients with AA amyloidosis and the patients with obscure fever syndromes ($p < 0.01$). Pyrin Q148 is a polymorphism and occurs widely in global terms, and, although it may cause FMF when associated with certain other *MEFV* mutations, homozygosity for Q148 alone must usually be insufficient to produce FMF in the populations studied. The association of pyrin Q148 with AA amyloidosis and with obscure chronic inflammatory diseases suggests the variant may augment inflammation non-specifically, which might have been beneficial during evolution, but could potentially exacerbate many chronic inflammatory disorders.

Introduction

Familial Mediterranean fever (FMF) is an inherited inflammatory disease that is principally recognized in Jewish, Armenian, Turkish and Middle Eastern Arab populations. The disorder is characterized by recurrent episodes of fever, peritonitis, pleurisy, rashes and arthritis, and may be complicated by AA amyloidosis.¹ The gene responsible for FMF, *MEFV*,^{2,3} encodes a hitherto-unknown protein called pyrin or marenostrin that is expressed chiefly in neutrophils. About 25 mutations in *MEFV* have

now been associated with FMF, and pairs of *MEFV* mutations can be identified in most FMF patients,^{4–9} consistent with autosomal recessive inheritance. Pyrin Q148 is interesting because it is one of the most common variants found in populations typically affected by FMF, with an allele frequency of up to 10%, but, unlike other common FMF-causing pyrin variants, individuals homozygous for it have not yet been confirmed to suffer from clinical FMF.¹⁰

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During the course of *MEFV* genotyping studies performed in patients attending our amyloidosis and FMF clinic, we identified pyrin Q148 in several Indian, Chinese and non-Jewish Caucasian patients with uncharacterized inflammatory disorders that did not meet the clinical criteria for FMF. This prompted us to investigate the frequency of pyrin Q148 among individuals from these ethnic groups in which FMF is not generally recognized, and we found that it is a common polymorphic variant. Intriguingly, pyrin Q148 was substantially over-represented in British and Indian patients with inflammatory arthritis complicated by AA amyloidosis, suggesting that it might non-specifically upregulate the inflammatory response.

Methods

Patients

We determined the pyrin Q148 allele frequency among the following groups of individuals: anonymized healthy control populations comprising 76 British Caucasians attending an insurance medical examination, 55 Chinese university students (Jiang Su Province), and 51 individuals of Punjabi Indian ethnic origin. In addition, we studied 50 British Caucasian patients with inflammatory arthritis, 25 of whom were confirmed histologically to have AA amyloidosis, and five Punjabi Indian patients with inflammatory arthritis complicated by AA amyloidosis. We also sought the Q148 allele in seven British Caucasian patients who had had uncharacterized recurrent fever syndromes associated with objective evidence of an intermittent acute-phase response for at least 5 years duration. These seven patients represented all such cases referred to our amyloidosis and FMF clinic in whom a diagnosis could not be made. FMF was excluded clinically and by additional *MEFV* genotyping in all patients in this study. Differences

between the proportion of amyloid patients with pyrin Q148 and the proportion with Q148 in ethnically-matched healthy control populations were compared statistically by the two-tailed Fisher's exact test.

Genotyping

A section of exon 2 of the *MEFV* (pyrin) gene was amplified from genomic DNA with the primers ATATTCCACACAAGAAAACGGC (7F) and GAG-GCTTGCCCTGCGCG (17R) using Ready-To-Go tubes (Amersham Pharmacia Biotech) with one cycle at 96 °C, 2 min 30 s; five cycles of 96 °C, 30 s; 64 °C, 15 s; 72 °C, 30 s; then 30 cycles of 96 °C, 30 s; 62 °C, 15 s; 72 °C, 30 s; and a final step of 72 °C for 7 min. The PCR product was digested with *Mva*I (Roche) and the fragments were separated by electrophoresis in a 2% agarose gel. *MEFV* exons 2, 3, 5 and 10 were amplified and sequenced as previously described.¹¹

Results

Of the healthy control populations, the *MEFV* allele encoding pyrin Q148 was not identified in any of the 76 British Caucasians (152 chromosomes), but was present in 16/110 (15%) Chinese, and 21/102 (21%) Indian chromosomes. Whereas pyrin Q148 was not present among the 25 British Caucasian inflammatory arthritis patients without amyloidosis, three of the 25 such cases with amyloid were Q148 heterozygotes ($p < 0.01$ compared with British controls, Table 1). Of the five Punjabi Indian inflammatory arthritis patients with AA amyloidosis, three were homozygous for pyrin Q148 ($p < 0.01$ compared with Punjabi Indian controls) and one was homozygous wild type. Of the seven British Caucasian patients with uncharacterized recurrent fever syndromes (Table 2), one was homozygous for pyrin Q148, four were

Table 1 Frequency of pyrin Q148 in patients with inflammatory arthritis (IA) with and without AA amyloid, and in healthy control populations

Ethnic group	Clinical status	<i>n</i>	Q148 alleles	95%CI
British	Healthy controls	76	0	0–4.7
British	IA without amyloid	25	0	0–13.7
British	IA with amyloid	25	3 (3 heterozygotes)*	2.6–31.2
Indian	Healthy controls	51	21 (3 homozygotes)*	1.2–16.2
Indian	IA with amyloid	5	7 (3 homozygotes)*	14.7–94.7
Chinese	Healthy controls	55	16 (2 homozygotes)*	0.4–12.5

n, number of individuals tested. * $p < 0.01$ compared with ethnically matched patients and controls without amyloid.

Table 2 Pyrin Q148 status of seven British Caucasian patients with uncharacterized recurrent fever syndromes

Patient	Sex	Age (years)	Age of onset	Duration of attacks	Additional features	Pyrin Q148
1	M	21	6	4–5 days	Arthralgia	+/+
2	F	28	17	Months or longer	Rash, arthralgia	+/-
3	M	10	1	Weeks	Arthralgia	+/-
4	F	32	15	Weeks	Vomiting	+/-
5	M	51	46	Months or longer	Pleural effusions	+/-
6	M	37	20	Weeks	Arthralgia, myalgia	-/-
7	M	48	41	Days to weeks	Rash, chest pain	-/-

heterozygous and two were homozygous wild type ($p < 0.001$ compared with British Caucasian controls).

Discussion

Our findings suggest that pyrin Q148 may be a common polymorphic variant in global terms, and raise the possibility that it may augment inflammation non-specifically. Pyrin is an uncharacterized neutrophil protein which was discovered recently when mutations in its gene were found to be responsible for FMF, but the protein and its physiological role are yet to be characterized. The *MEFV* gene is apparently expressed only in cells of mature myeloid lineage, predominantly in neutrophils, and the variant forms of pyrin which cause FMF are thought to lead to neutrophil activation and migration in situations that would not normally produce these effects.⁷ The clinical attacks in FMF are associated with massive influx of neutrophils into serosal linings, consistent with bursts of uncontrolled neutrophil activity. Colchicine, a drug known to inhibit neutrophil chemotaxis, is extremely effective in preventing attacks of FMF. Pyrin presumably has a role in preventing neutrophil activation, or down-regulating established neutrophil activity as part of the process by which inflammation resolves.

Pyrin Q148 evidently occurs in geographically and ethnically diverse populations and, indeed, in all ethnic groups in which it has so far been sought. Although there are not yet any systematic data on its frequency in Black Africans, we have identified pyrin Q148 in one such individual. Studies of Turkish, Jewish and Arabic populations in which FMF typically occurs suggest that pyrin Q148 contributes to the pathogenesis of FMF when it is coupled with other, presumably more disruptive, *MEFV* mutations,^{4,6,8} but that simple homozygosity for Q148 is not associated with clinical FMF.¹⁰ Although the susceptibility to FMF may vary among different ethnic groups, the potential for Chinese and Indian pyrin Q148

homozygotes to develop FMF is probably very low, given that this latter genotype might occur in up to 5% of these large populations.

The increased frequency of pyrin Q148 in patients with AA amyloidosis complicating inflammatory arthritis compared with ethnically-matched healthy and disease controls might have broad clinical implications, and requires further investigation. The possibility that pyrin Q148 may be a risk factor for AA amyloidosis in FMF has also been raised,⁶ but systematic study of this has been confounded by the extremely beneficial ameliorating effect of colchicine in FMF and its widespread use over the past 30 years. Although the gene for pyrin Q148 might be linked with another pro-amyloidogenic gene, it occurs on numerous haplotypes, and none of the genes encoding proteins known to be involved in amyloidosis are located near the *MEFV* locus. The incidence of non-AA amyloid is not increased among populations in which pyrin Q148 is prevalent, indicating that this mutation does not predispose to amyloidosis in general. We recently reported that healthy carriers of the FMF trait had modest but significantly elevated baseline plasma levels of the classical acute-phase reactant, C-reactive protein, suggesting that their general response to inflammatory stimuli may be up-regulated,¹² and have lately extended and confirmed these observations among Turkish carriers of pyrin Q148 compared with wild-type controls.¹³ The acute-phase response to tissue damage is highly conserved among species, and in evolutionary terms is likely to be beneficial, probably by augmenting innate host resistance to infection. On the other hand, an intense prolonged acute-phase response is the sole pre-requisite for developing AA amyloidosis, and we suggest that general upregulation of the inflammatory response in individuals with pyrin Q148 is the most likely explanation for their increased susceptibility to AA amyloidosis.

The presence of pyrin Q148 in five out of the seven British Caucasian patients with uncharacterized chronic relapsing fever syndromes supports the possibility that pyrin Q148 may be

pro-inflammatory, given that this allele was not present among 152 British control chromosomes. These seven cases represent all of the British Caucasian patients seen in our FMF and amyloidosis clinic during the past 10 years with undiagnosed relapsing fever syndromes that have been associated with a substantial intermittent acute-phase response, and in whom symptoms had been present for at least 5 years. A minimum duration of 5 years was chosen in order to exclude patients with covert malignancies, for example lymphomas, which occasionally present with non-specific inflammatory features. Despite extensive investigations and prolonged clinical follow-up for at least 5 years, no specific clinical diagnosis has been made in any of these patients, and we speculate that in these particular individuals pyrin Q148 may confer susceptibility to spontaneous inflammation or augment other uncharacterized chronic inflammatory processes. The latter mechanism is perhaps more likely given the absence of family history of similar disease among these patients. Although the predominant clinical features of fever and malaise associated with a very substantial acute-phase response are superficially reminiscent of FMF, this diagnosis was excluded on clinical criteria and by additional MEFV genotyping in each case, notably including the pyrin Q148 homozygote.

Recognition that pyrin Q148 may occur widely in individuals in different ethnic groups, and our identification of an enhanced inflammatory response as a plausible candidate biological mechanism suggest that the FMF trait may have conferred survival benefit during evolution. The absence of overt disease among potentially very large numbers of Indians and Chinese pyrin Q148 homozygotes supports this hypothesis, although low-grade FMF or other febrile inflammatory disease in these particular populations may conceivably have been overlooked. The role of low-grade inflammation in coronary heart disease has lately been highlighted by robust observations that individuals with a plasma C-reactive protein concentration within the upper end of the normal healthy range have a substantially greater risk of coronary events.^{14–16} The possibility that pyrin Q148 might modulate an individual's baseline CRP concentration is therefore all the more intriguing.

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