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FULL PAPER

Allelic variants in genes associated with hereditary periodic fever syndromes as susceptibility factors for reactive systemic AA amyloidosis

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We investigated the hypothesis that low-penetrance mutations in genes (TNFRSF1A, MEFV and NALP3/CIAS1) associated with hereditary periodic fever syndromes (HPFs) might be risk factors for AA amyloidosis among patients with chronic inflammatory disorders, including rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), Crohn's disease, undiagnosed recurrent fevers and HPFs themselves. Four of 67 patients with RA plus amyloidosis had MEFV variants compared with none of 34 RA patients without amyloid (P value = 0.03). The E148Q variant of MEFV was present in two of the three patients with TNF receptor-associated periodic syndrome (TRAPS) complicated by amyloid in two separate multiplex TRAPS families containing 5 and 16 affected members respectively, and the single patient with Muckle–Wells syndrome who had amyloidosis was homozygous for this variant. The R92Q variant of TNFRSF1A was present in two of 61 JIA patients with amyloidosis, and none of 31 nonamyloidotic JIA patients. No HPF gene mutations were found in 130 healthy control subjects. Although allelic variants in HPFs genes are not major susceptibility factors for AA amyloidosis in chronic inflammatory disease, low-penetrance variants of MEFV and TNFRSF1A may have clinically significant proinflammatory effects.
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Introduction

AA amyloidosis is a progressive and often fatal disorder, which occurs in a proportion of patients with any chronic inflammatory disease that gives rise to a sustained acute phase response.[1] AA amyloid fibrils are derived from the circulating acute phase reactant serum amyloid A (SAA) protein, the most responsive and dynamic marker of the acute phase response. AA amyloid typically presents with proteinuria progressing to nephrotic syndrome and renal failure, and is often fatal within 5–10 years. The most common diseases complicated by AA amyloidosis in the developed world are rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA), in which the lifetime incidence of amyloidosis is 1–5%. AA amyloidosis develops substantially more frequently among patients with some of the hereditary periodic fever syndromes (HPFs), namely TNF receptor-associated periodic syndrome (TRAPS [MIM 142680]), familial Mediterranean fever (FMF [MIM 249100]), Muckle–Wells syndrome (MWS [MIM191900]), and the genetically related syndrome of familial cold urticaria (FCU), also called familial cold autoinflammatory syndrome (FCAS [MIM120100]).[2] These are Mendelian disorders characterized by spontaneously relapsing and remitting multisystem inflammation that is accompanied by a remarkably intense acute phase response, and AA amyloidosis is the most common cause of premature death. The genetic defect underlying all these conditions have been identified;[3] mutations in the *MEFV* gene cause FMF, TRAPS is associated with mutations in *TNFRSF1A* on chromosome 12p13, and mutations in and around the NACHT domain of a novel gene, *CIAS1*, also known as *NALP3*, have been identified as causing both MWS and FCU.

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AA amyloidosis occurs in about 25% of patients with TRAPS and MWS, and prior to the introduction of colchicine therapy occurred in a remarkably high proportion of patients with FMF,[4] particularly in the Sephardic Jewish population.[5] While it is clear that AA amyloidosis occurs only in patients who have sustained or frequent elevation of their plasma SAA concentration, the genetic or environmental factors that determine susceptibility are poorly understood. These include duration of the underlying inflammatory disease,[6] SAA_1 isotype status,[7] a positive family history,[8] and probably male sex status,[9] but the magnitude of risk conferred by these various factors is relatively small. The plasma concentration of SAA increases from healthy values of less than 10 mg/l up to as much as 2000 mg/l in response to inflammatory stimuli,[10] and while cumulative abundance of SAA is a plausible risk factor for AA amyloid deposition, this has not yet been studied in a satisfactory longitudinal manner.

FMF, MWS and TRAPS produce an extremely intense acute phase response. We have previously shown that heterozygous carriers of FMF-causing mutations have increased 'acute phase responsiveness', even if clinically healthy, and the pyrin variant E148Q is over represented among patients with inflammatory arthritis who have developed AA amyloidosis.[11] Other studies have suggested that pyrin E148Q may also be a risk factor for AA amyloidosis in FMF itself in some populations,[12] and have shown that the polymorphism/low-penetrance TRAPS-causing TNF receptor 1 variant R92Q is associated with susceptibility to early arthritis in a North American cohort.[13] We have identified the V200 M polymorphism/low-penetrance MWS/FCU-causing variant of *NALP3/CIAS1* in several patients with uncharacterized intense inflammatory diseases.[14] These findings suggest that mutations in genes associated with HPFs may act as modifier genes by upregulating the host inflammatory response in a nonspecific manner. We investigated the hypothesis that such variants may be susceptibility factors for the development of AA amyloidosis in patients with chronic inflammatory diseases and HPFs themselves.

Results

Two *MEFV* gene variants, encoding pyrin E148Q and V726A, were identified in four out of 67 RA patients with AA amyloidosis, in contrast to none of 34 RA patients without amyloidosis ($P = 0.3$), or any of the 130 healthy controls ($P = 0.013$). Paired exon 10 *MEFV* mutations were present in 14 out of the 18 Turkish FMF patients with AA amyloidosis, and just a single exon 10 mutation was found in three of the remainder. A total of 12 out of 18 (67%) of these FMF patients were homozygous for pyrin M694 V, while two of the others had M694 V coupled with either M680I or V726A. Pyrin E148Q was not identified in the FMF group, but this variant was present in two patients within the TRAPS families (Figure 1); notably one of these was the only patient to develop amyloidosis among five living affected members in a Finnish TRAPS family (C88Y mutation), and the other was one of only two patients with amyloidosis among 16 living affected members of the Irish–Scottish TRAPS family (C33Y mutation). Four out of six patients with TRAPS in the Northern Irish family had developed amyloid but no pyrin variants were found in the four living members of this kindred, or in the Polish–English TRAPS family (a father with amyloid and a child without this complication). Several members of the Indian MWS family (both affected and unaffected) had pyrin E148Q (Figure 1), but only the patient with AA amyloidosis was

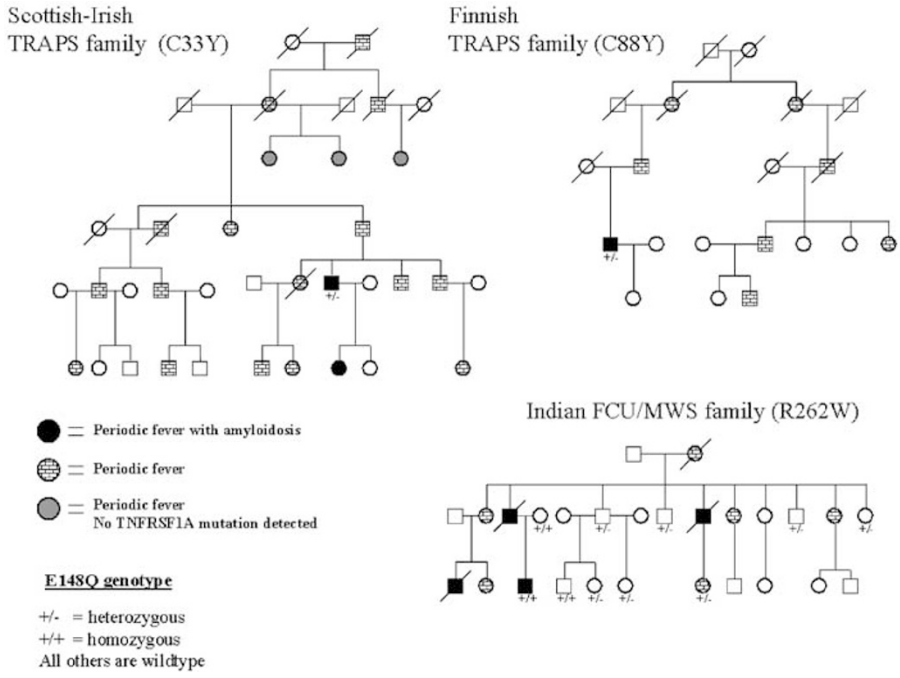

Figure 1 Pedigrees of the Irish-Scottish, Finnish and Indian families showing the presence of AA amyloidosis (black) in affected members. All individuals were negative for the presence of E148Q of *MEFV*, unless otherwise indicated. Three affected individuals in the Irish-Scottish family (grey) were negative for the presence of C33Y, despite having a similar clinical presentation.

homozygous for this variant (two unaffected individuals in the family were also homozygous for E148Q and did not have any symptoms of FMF).

The V200 M variant of *NALP3*/*CIAS1* was identified in one RA patient without amyloidosis and in one amyloidotic FMF patient (Table 1). The *TNFRSF1A* mutation encoding the R92Q variant was found in two of 61 JIA patients with AA amyloidosis, but in none of the 31 JIA patients without AA amyloidosis or 130 normal European controls. A novel C to G transversion in *TNFRSF1A*, producing a histidine to glutamine change at residue 22 (H22Q), was identified in one patient with JIA who did not have amyloidosis. *TNFRSF1A, MEFV* and *NALP3*/*CIAS1* variants were not present in any of the remaining JIA patients (59 with amyloidosis and 31 without amyloidosis), or any of those with amyloidosis associated with Crohn's disease (a small series of seven cases), *de novo* MWS or unclassifiable periodic fever syndromes (Table 1), or any healthy controls.

Discussion

While the data do not suggest that allelic variants in the genes associated with HPFs are major susceptibility factors for the development of AA amyloidosis, our findings are nevertheless consistent with the possibility that low-penetrance variants in *MEFV* and *TNFRSF1A* may upregulate the inflammatory response in some situations, and thus contribute to the likelihood of AA amyloidosis developing in certain patients and families. We were particularly interested in the low-penetrance mutations in *MEFV, TNFRSF1A* and *NALP3* that encode the E148Q, R92Q and V200 M variants, respectively, for two reasons. Firstly, we have previously speculated that pyrin E148Q may nonspecifically augment inflammation,[11] and in our own clinical practice have identified the *TNFRSF1A* and *NALP3*/*CIAS1* variants R92Q and V200 M in several patients with unusually intense chronic inflammatory diseases that do not meet diagnostic criteria for TRAPS or MWS/FCU. Secondly, these particular variants have collectively been described in up to 5% of healthy Caucasian controls, a frequency similar to the overall lifetime incidence of AA amyloidosis in patients with chronic inflammatory diseases. However, it is intriguing that pyrin E148Q was found in three of the RA patients with amyloid and none of those without, and in two out of the seven TRAPS patients with amyloidosis and none of the 19 family members without. Likewise the R92Q variant of *TNFRSF1A* was found in 2/61 of JIA patients with amyloid and none of those without, but the numbers are too small to make any firm conclusions.

There is some evidence that patients with FMF who are homozygous for pyrin M694 V have more severe inflammatory disease, possibly with earlier onset; it is therefore relevant that two-thirds of the Turkish FMF patients with amyloidosis studied here had this particular genotype. However, there is a carrier rate of almost 20% for M694 V in western Turkey,[15] which is the population studied. Similarly, two out of the four TRAPS families in which amyloid occurred had mutations that disrupt cysteine residues, which has been suggested to confer an increased risk of developing AA amyloidosis.[13] Conversely, it is of interest that we identified two patients with TRAPS (T37I and ΔD42) complicated by amyloid in whom we found none of the common *MEFV* mutations, and although it might be supposed that the high frequency of amyloid in the Northern Irish TRAPS family (ΔD42) could be attributed to a severe phenotype associated with marked structural disruption of the TNF receptor by the deletion mutation, the intensity of inflammation even in the amyloidotic members of this family was actually surprisingly mild.

Environmental influences cannot be ignored as potential susceptibility factors for AA amyloidosis, as for example, the incidence of AA amyloidosis complicating rheumatoid arthritis is much lower in similar population in the USA than in Europe with the highest incidence in Finland.[16] Furthermore, even before colchicine therapy was known to suppress FMF, Armenians living in Armenia had a much higher prevalence of amyloidosis than those Armenians living in the USA.[17] It has long

Table 1 Mutations identified in 227 unrelated patients with recurrent fevers

Syndrome	*AA amyloidosis*	*Ethnicity*	*TNFRSF1A*	*MEFV*	*NALP3/CIAS1*
JIA	+ (61)	UK Caucasian	R92Q (2)	—	—
	– (31)	UK Caucasian	H22Q (1)	—	—
RA	+ (67)	Indian (1)	—	E148Q/N	—
		UK Caucasian (66)	—	E148Q/N (1)	—
			—	E148Q/E148Q (1)	—
			—	V726A/N (1)	—
	– (34)	UK Caucasian	—	—	V200 M (1)
FMF	+ (18)	Turkish	—	M694V/ M694 V (1)	V200M
			—	M694V/ M694 V (11)	—
			—	M694V/ M680I (1)	—
			—	M694V/ V726A (1)	—
			—	M694 V /– (3)	—
			—	M680I /– (1)	—
Crohn's	+ (7)	UK Caucasian	—	—	—
MWS	+ (1)	UK Caucasian	—	—	—
Periodic fever	+ (8)	UK Caucasian	—	—	—

Number of cases/categories are within parenthesis.

been known that the induction of murine AA amyloid as a result of experimental inflammation can be reduced from many weeks to just 1 or 2 days when such animals are pretreated with miniscule extracts of *ex vivo* amyloid material by parenteral injection. Disturbingly, this so-called 'amyloid enhancing factor' (AEF) effect has more recently been reproduced following oral administration of an AEF preparation.[18] This raised the possibility that a transmissible agent present in diet may be a contributory factor in AA amyloid fibril formation, analogous to the situation in the prion diseases, where the bovine spongiform encephalopathy has been transmitted from cattle to man as new variant Creutzfeldt–Jakob disease.[19] Hormonal influences may also predispose to AA amyloidosis, it being notable that amyloid developed only in male members of the Indian MWS family, and in none of the six affected female members. In this regard, it is also relevant that the male to female ratio has been equal among 350 patients with AA amyloidosis who have been evaluated in our centre, despite the most common underlying disorder being RA, which is three-fold more common in women.

We identified one new genetic variant in this study, a substitution in *TNFRSF1A* resulting in the replacement of histidine at residue 22 by glutamine. A mutation involving the same residue (H22Y rather than H22Q) has already been described in two Northern European TRAPS families,[13] but even on review the JIA patient who had the H22Q variant was not thought to exhibit any clinical features consistent with TRAPS.

Patients and methods

Unrelated patients and controls

A total of 227 unrelated patients with sporadic chronic inflammatory diseases were studied, all of whom were UK Caucasian (except for 18 Turkish patients with FMF and one Indian RA patient) with sporadic chronic inflammatory diseases, 162 of whom had AA amyloidosis (Table 1). These included 92 children with JIA, 61 of whom had AA amyloidosis and 101 adults with RA, 67 of whom had AA amyloidosis. The remaining 34 cases all had AA amyloidosis, and comprised 18 unrelated patients with FMF, seven with Crohn's disease, one patient with *de novo* MWS, and a further eight patients with unclassifiable recurrent periodic fever syndromes. The control group comprised 130 healthy European subjects.

Familial patients

Studies were also performed in six families with HPFs in whom at least one member had AA amyloidosis. The four TRAPS families were of Finnish (C88Y), Irish–Scottish (C33Y) (Figure 1), Polish-English (T37I) and Northern Irish (ΔD42) ethnic background, respectively. In addition, one North American family had FCU associated with *NALP3*/*CIASI* variant L355P, and an Indian family from Delhi with an overlap FCU/MWS syndrome associated with *NALP3*/*CIASI* variant R262W. Two of four affected members (L355P mutation) of the FCU family had AA amyloidosis[20] (family number 4). All four affected males in the FCU/MWS family, three of whom were deceased, had AA amyloidosis (Figure 1). Samples were not available from the deceased members.[14] It should be noted that the amino-acid sequence of *NALP3*/*CIAS1* is annotated differently by two residues in some reports, since the position of the start site has not been resolved. The diagnosis of amyloidosis was obtained histologically in each case, and AA fibril type was confirmed immunohistochemically with monoclonal antibodies that were specific for SAA.

Mutational analysis

Exons 2–4, where all *TNFRSF1A* mutations identified to date are clustered were amplified as described[21] and analysed in all patients and controls. We also screened for the five most frequent *MEFV* mutations; M680I, M694 V, M694I, and V726A in exon 10, and the E148Q variant in exon 2.[22] The V200 M variant of *NALP3*/*CIAS1* was screened by restriction fragment length polymorphism (RFLP) assay, as described.[14] Two-tailed Fisher's exact test was used to analyse the data.

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Electronic-Database Information

URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for FCU/FCAS [MIM 120100], FHF [MIM 142680], FMF [MIM 249100], HIDS [MIM 260920], and MWS [MIM 191900])

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