British kindred with dominant FMF associated with high incidence of AA amyloidosis caused by novel *MEFV* variant and a review of literature

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Abstract

Objectives: Hereditary systemic autoinflammatory diseases (SAIDs) are rare genetic disorders, which if untreated, can be complicated by AA amyloidosis leading to renal failure and premature death.

Objective: To find a genetic cause in a British family with a dominantly inherited autoinflammatory syndrome complicated by AA amyloidosis.

Methods: The index patient and his sister underwent comprehensive clinical and laboratory assessment including the next generation sequencing (NGS) panel targeting autoinflammatory genes. Subsequently, other relatives underwent clinical evaluation and genetic testing. Screening of the *SAA1* gene was performed in all symptomatic cases.

Results: The index case and his sister presented with proteinuria due to AA amyloidosis. They have been suffering from episodes of fever accompanied by severe abdominal and chest pain, arthritis and erythema from childhood. Their father died aged 52 from complications following a cadaveric renal transplantation. The post-mortem examination demonstrated AA amyloidosis. The index case's grandmother, two paternal cousins and two of their children described similar symptoms. All symptomatic individuals had excellent responses to colchicine.

NGS analysis identified a single *MEFV* p.P373L variant in the index case, his sister and subsequently, in symptomatic family members. Sequencing of the *SAA1* gene revealed all cases were heterozygous for the *SAA1.1* allele.

Conclusion: Typically FMF is an autosomal recessive disorder; nonetheless rare cases of dominantly inherited disease have previously been described. Here we report a novel *MEFV* variant p.P373L causing dominant FMF complicated by AA amyloidosis in four generations of a British family.

Keywords: Familial Mediterranean Fever (FMF), *MEFV* gene, colchicine, AA amyloidosis

Key messages:

- FMF and pyrin associated autoinflammatory diseases can present atypically in non-Mediterranean populations
- Dominantly inherited FMF is rare and may lead to delayed diagnosis
- Treatment with colchicine prevents FMF attacks and protects against development of AA amyloidosis

Introduction

Familial Mediterranean Fever (FMF) is the commonest monogenic systemic autoinflammatory syndrome (SAIDs), originally described in patients from the Eastern Mediterranean region, in particular in Sephardic Jews, Armenians, Turks and Arabs, but is now recognised in many parts of the world. FMF is characterised by recurrent episodes of fever, serositis and elevated inflammatory markers. The effective administration of colchicine for FMF was first published in 1972 [1] and prophylactic therapy both suppresses FMF related inflammation and the development of AA amyloidosis [2]. The causative gene for FMF is *MEFV* (MEditerranean FeVer) identified in 1997 by two different consortia [3, 4], located on chromosome 16, it consists of 10 exons and encodes a 781-amino acid protein called pyrin. Most pathogenic variants are clustered in exon 10 [5]. In patients of Mediterranean ancestry, the p.M694V, p.M680I and p.V726A are the commonest. Homozygosity for the p.M694V has been linked with a higher prevalence of AA amyloidosis [6, 7], which is the most severe complication of FMF, leading to renal failure and death. Previous studies have suggested that homozygosity for the SAA1.1 polymorphism in Caucasian population carries a 7-fold increased risk of AA amyloidosis, compared with heterozygotes and other SAA1 genotypes [8]. FMF is generally considered to be inherited in an autosomal recessive fashion, although both heterozygous disease and dominant transmission are increasingly recognised. Dominant FMF was first suggested in 1954 by Reimann et al. with the description of several families with two or more consecutive affected generations [9]. Subsequently, a variety of pathogenic variants have been implicated in dominant FMF [10-13].

Here we report the clinical features and laboratory investigations, including genetic analysis, performed in a British family with a clear autosomal dominant SAIDs complicated by AA amyloidosis. We have analysed the *SAA1* gene in this family to assess the prevalence of the

SAA1.1 allele. We also provided a comprehensive review of the dominant FMF cases published to date.

Patients and Methods

The index case and his sister (patient 1 and 2 respectively) were referred to the National Amyloidosis Centre (NAC) with a suspicion of AA amyloidosis. They underwent comprehensive clinical assessment and laboratory investigations including serial measurements of the inflammatory markers: serum amyloid A protein (SAA) and C-reactive protein (CRP). Subsequently, other relatives (patients 4 to 7) underwent clinical evaluation and genetic testing at the NAC.

Informed consent was provided by all subjects and the ethical approval for the study was obtained from Royal Free Hospital Research Ethics Committee (REC Reference number 06/Q0501/42) in accordance with the declaration of Helsinki.

Histology and immunohistochemistry

6 μm thick sections of kidney biopsies from the proband and his sister were stained for amyloid with Congo red and viewed under crossed polarised light [14]. Confirmation of AA type amyloid was sought immunohistochemically using monoclonal antibodies specific to SAA (Euro-Diagnostica). Specificity of staining was confirmed by prior absorption of the antiserum with pure antigen in each case and positive and negative controls were included in each run.

Radiolabeled serum amyloid P component (SAP) scintigraphy

The proband and his sister underwent whole body anterior and posterior scintigraphic imaging 24 hr after administration of 123I labelled SAP using a GE Infinia Hawkeye gamma camera, as previously described [15]. The labelled SAP studies results were interpreted by a panel of physicians with experience of over 29000 SAP scans.

Genetic Analysis

The DNA of the proband and his sister was analysed by the Next Generation Sequencing (NGS) method targeting 32 genes associated with SAIDs.

This gene panel was designed using the Agilent EArray online tool (https://earray.chem.agilent.com/suredesign/) (Supplementary Table 1). Llibraries (QXT Target Enrichment System) were sequenced as a multiplex of 16 samples on an Illumina MiSeq genetic analyser. Read alignment, variant calling, and annotation were performed using Agilent Sure Call v3.0 software [16].

After the causative genetic variant in the proband and his sister was identified using the above NGS method, results were confirmed by PCR and Sanger sequencing, also used for genetic analysis in the other family members. The electrophoretic profiles were analysed on the ABI 3130xl Genetic Analyser using Sequencing Analysis Software version 5.4.
Sequencing of the exon 3 of the *SAA1* gene (NCBI Reference Sequence: NC_000011.9) was performed as previously described [8].

Results

Genetic studies

NGS analysis, in the index case and his sister, identified a heterozygous single base substitution (c.1118C>T) in the *MEFV* gene (NCBI Reference Sequence: NG_007871) resulting in the replacement of proline with leucine amino acid at position 373 (p.P373L) in

exon 3. This variant is not reported in the population databases EXAC (http://exac.broadinstitute.org/) and gnomAD (https://gnomad.broadinstitute.org/) and the Sift and PolyPhen in silico predictions imply this amino acid substitution may have a damaging effect on the protein function. We did not identify any other potential pathogenic variant in the SAIDs genes included in this NGS panel.

This p.P373L variant was identified by Sanger sequencing in all affected living family members (Figure 1). The mutant allele was absent in the one asymptomatic individual. All cases were heterozygous for the *SAA1.1* allele.

Patients

The index case was of British ancestry and had been diagnosed with systemic juvenile idiopathic arthritis (sJIA) at the age of seven. In retrospect, he recollects episodes of fever accompanied by severe abdominal and chest pain, small joint arthritis, occasional erythematous rash and night sweats from early childhood. He developed proteinuric renal disease with biopsy proven AA amyloidosis aged 23 and required four years of haemodialysis before cadaveric renal transplantation at the age of 32. His post-transplant course was complicated by a monoarthritis of the right elbow for which he was given a full course of anti-tuberculous therapy at the age of 35. A few months earlier he had been started on low dose of colchicine (0.5mg/day) for presumed post-transplant gout, and generally felt very much better in terms of symptoms so never stopped it. His transplant function was stable without development of proteinuria. Nine years post transplantation he sought a specialist opinion on his amyloidosis following media interest in novel potential treatments for amyloid in Alzheimer's.

At the time of assessment at our centre he had no symptoms suggestive of systemic inflammation. On examination, he had mild splenomegaly and no evidence of joint damage. Investigations showed a large total body amyloid load with deposition on the spleen but not the liver nor renal transplant. His renal graft function was good with an eGFR 37 ml/min and no proteinuria. Serial monitoring of his CRP and SAA showed supressed inflammation with medians of 2 and 4 mg/l respectively (median plasma concentration of CRP and SAA in healthy subjects is <1mg/L and <3 mg/L respectively). The only time the CRP and SAA were elevated (to 84 and 182mg/l), was when he briefly run out of colchicine.

Nearly 20 years after he was diagnosed with AA amyloidosis his sister presented at the age of 47 with nephrotic syndrome. Given her family history amyloidosis was strongly suspected. An SAP scan showed a small amyloid load in spleen and kidney. She too gave a history of recurrent episodes of severe abdominal and chest pain lasting 3 days to a week and recurring up to twice month. She also reported lifelong intermittent swelling of large joints and red eyes but denied fevers or night sweats. At baseline her, the CRP and SAA were 25 and 50 mg/l, but normalised to below <1 and <3 mg/l once she commenced colchicine prophylaxis (1.5 mg/day).

Family History

Their father (patient 3) had died at the age of 52 from complications after cadaveric renal transplant. He had been diagnosed with rheumatoid arthritis and at post-mortem was found to have extensive AA amyloidosis. His mother was said to have had recurrent episodes of abdominal pain and arthritis and died of cancer. The probands two cousins (patients 4 and 7) and three of their children were subsequently investigated at our centre with all but one giving histories of recurrent abdominal and chest pain with onset in childhood or early teens

(patients 5 and 6) (Table 1). All symptomatic individuals were treated with colchicine (1-2mg/day), which resulted in a complete resolution of all inflammatory symptoms.

Histology

Extensive amyloid deposits identified on the renal biopsies obtained from the proband and his sister. These stained with monoclonal anti-SAA antibodies. There was no staining with antibodies against other proteins known to be associated with acquired or hereditary renal amyloidosis.

Discussion

Here, we report a novel MEFV substitution p.P373L causing dominant FMF complicated by AA amyloidosis. This variant has not been reported in population databases and demonstrated clear segregation with disease over four generations in this family.

The *MEFV* gene is very polymorphic with nearly 350 sequence changes reported on the Infever database (fmf.igh.cnrs.fr/ISSAID/infevers/) [5]. A number of syndromes have been associated with mutations in the *MEFV* gene; these recently have been grouped as 'pyrin-associated autoinflammatory diseases' (PAAD) [17]. A recent paper on consensus proposals for the taxonomy and definition of the autoinflammatory diseases implies that classically the term FMF should be used only for patients of Mediterranean origin with two pathogenic variants in *MEFV* exon 10. Other PAADs include periodic fever with autoinflammation and neutrophilic dermatosis (PAAND), chronic nonbacterial osteomyelitis (CNO) like disorder and livedoid ulcerative dermatitis [17]. In the family described here the clinical symptoms and response to colchicine are very much in line with the classical FMF, although the autosomal dominant inheritance, Northern European ethnicity and substitution in exon 3 rather exon 10, fall outside the strictest interpretation of its definition. Nonetheless, there are

four different *MEFV* variants located in exon 3, which are registered in the Infever database with the provisional classification “likely pathogenic”, and detected in patients with PAAD-FMF.

Autosomal dominant FMF like disease has now been reported caused by several pathogenic variants in the *MEFV* gene. Recent work on the molecular consequences of variant pyrin support FMF caused by a single MEFV mutation [18]. We previously identified p.M694del in 21 patients of British origins, whose symptoms were in line with the classical FMF, including good response to colchicine treatment. In three subjects, their disease was complicated by AA amyloidosis [11]. Stoffels M, et al. reported dominant FMF caused by: p.T577N, p.T577S and p.T577A, identified in the British, Turkish and Dutch patients respectively [10]. The clinical phenotype associated with these variants varied from classical FMF to symptoms overlapping with other SAIDs. The British and Turkish families responded to colchicine, there was no treatment data in one Dutch family and the other reported good outcome to anakinra treatment. The p.H478Y substitution was identified in five patients from a three-generation Spanish family with dominant inheritance of severe periodic inflammatory syndrome with long fever episodes, up to 25 days, dominant joint involvement and resistance to colchicine treatment. Two subjects developed AA amyloidosis [13]. Less contentiously dominant FMF can be caused by more frequent MEFV variants inherited as complex alleles in cis, for example p.[M694I;p.E148Q] and p.[L110P;E148Q;M694I] [12, 19].

AA amyloidosis is a rare potential complication of sustained inflammation. Additional risk factors for the development of AA amyloidosis have been identified and the most robust in European and Mediterranean populations is the homozygosity for the SAA1.1 polymorphism. However, none of our family were homozygous for the *SAA1.1* genotype. In our opinion, a

more likely risk factor of AA amyloidosis in these families, is delayed disease recognition due to lack of awareness of FMF type disease in atypical populations. It is well recognised that prompt initiation of long term colchicine is extremely effective in preventing AA amyloidosis. In Turkey, prior to the widespread use of colchicine, up to 60% of patients with FMF developed AA amyloidosis [20] suggesting that dominant *MEFV* variants do not intrinsically carry a higher risk than classical exon 10 substitutions. FMF is a high value diagnosis, as untreated disease has severe effects on quality of life and carries a risk of potentially fatal complications, but colchicine provides highly effective, safe and affordable prophylaxis.

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Table 1. Characteristics of the affected family members assessed at the NAC.

Characteristics	**Proband (Patient 1)**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**
Gender	M	F	M	M	F	M	F
MEFV p.P373L	+	+	ND	+	+	+	+
Age at FMF diagnosis (years)	42	47	ND	61	21	25	58
Age at onset	childhood	childhood	childhood	16	12	15	childhood
Duration of episodes (days)	1-3	1-3	U	1-3	1-2	1-2	1-3
Frequency of attacks	weekly	weekly	U	weekly	weekly	weekly	weekly
Fever	+	+	+	+	+	+	+
Abdominal pain	+	+	+	+	+	+	+
Pleuritis	+	+	+	+	+	+	+
Arthralgia	+	+	+	+	+	+	+
AA Amyloidosis	+	+	+	-	-	-	-
Age at diagnosis with AA amyloidosis (years)	23	47	52 (post-mortem)	-	-	-	-
End stage renal disease with kidney transplantation	+	-	+	-	-	-	-
Colchicine response	+	+	NT	+	+	+	+

Symbols: - / + indicate absence or presence, U, unknown, ND, not done, NT, not treated

Figure 1. Segregation of the *MEFV* p.P373L variant with the disease. The p.P373L variant was found in all family members shown here in solid black and grey shapes with and without AA amyloidosis respectively. Open shapes represent healthy individuals without p.P373L variant. The proband is indicated by an arrow, boxes represent males, circles females. Family members assessed at the NAC are numbered P1-P7.