Autosomal-dominant periodic fever with AA amyloidosis: Novel mutation in tumor necrosis factor receptor 1 gene

**Rapid Communication**

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**Autosomal-dominant periodic fever with AA amyloidosis: Novel mutation in tumor necrosis factor receptor 1 gene.**

**Background.** The recent identification of genes responsible for syndromes of periodic fever with amyloidosis has opened the way to a molecular diagnosis of hereditary AA amyloidosis. Until very recently, hereditary amyloidosis occurring in the context of periodic fever was only known to be transmitted as an autosomal-recessive trait. No mutations were detected in the *MEFV* (Mediterranean fever) and tumor necrosis factor receptor-1 (TNFR1 or TNFRSF1A) genes causing familial Mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), respectively.

**Methods.** A Belgian woman presented for genetic counseling. Three first-degree relatives had a diagnosis of renal amyloidosis with a history of recurrent fever and inflammatory episodes. Medical records and pathological specimens were obtained from all physicians who had been in charge of her three relatives. Immunohistochemical staining was performed on paraffin-embedded material. A mutation search was performed in the *MEFV* and tumor necrosis factor receptor 1 (TNF or TNFRSF1A) genes causing familial Mediterranean fever (FMF) and tumor necrosis factor receptor-associated periodic syndrome (TRAPS), respectively.

**Results.** The family history was consistent with autosomal-dominant transmission of periodic fever with arthralgias, abdominal pain, and eventual AA amyloidosis involving the kidneys, digestive tract, and thyroid. Recurrent amyloidosis in kidney graft was demonstrated in one patient and was suspected in the other. A novel heterozygous mutation (C55S) in TNFRSF1A was identified in the affected patient available for genetic testing but not in the asymptomatic woman requiring counseling. No mutation was detected in *MEFV*.

**Conclusions.** We report a novel mutation (C55S) in TNFRSF1A, resulting in autosomal-dominant periodic fever and AA amyloidosis. This condition, known as TRAPS, should be added to the differential diagnosis of hereditary renal amyloidosis, with obvious implications for management and genetic counseling.

Hereditary amyloidosis encompasses a wide range of entities [1, 2]. The correct diagnosis is based on the ethnic background, the mode of inheritance, a constellation of clinical features, the nature of amyloid fibrils, and ultimately the identification of the mutated gene. Until very recently, hereditary amyloidosis occurring in the context of periodic fever was only known to be due to mutations in the Mediterranean fever (*MEFV*) gene [3, 4]. As familial Mediterranean fever (FMF) is transmitted as an autosomal-recessive trait [3, 4], the autosomal-dominant form of amyloidosis following periodic fever, reported in several families of European origin, remained unexplained [5, 6].

In 1999, McDermott et al described seven families affected by autosomal-dominant periodic fever, with amyloidosis in two of them. Six different missense mutations in *TNFRSF1A*, the gene encoding the tumor necrosis factor (TNF) receptor 1 (also known as TNFR1 or p55) were found, and the authors proposed the name of TRAPS (TNF Receptor-Associated Periodic Syndrome) for such mutations [7].

In a Belgian family with autosomal-dominant AA amyloidosis, we report a novel missense mutation (C55S) in TNFRSF1A. As illustrated in this family, recognition of the entity and identification of the mutation have implications for management of the disease and genetic counseling.

**METHODS**

**Family study**

Individual II-3 was seen at our outpatient nephrology clinic. Her serum creatinine and urinalysis were normal. She nevertheless wondered whether she was at risk of transmitting to her potential lineage the renal amyloidosis that affected her father and two of her three siblings (Fig. 1). Medical records of all affected family members were obtained from the general practitioners, internists, and...
Mutation search in TNFRSF1A (GenBank accession number M75866)

Using standard procedures, genomic DNA was prepared from peripheral blood lymphocytes [8]. Polymerase chain reaction (PCR) amplification of exons 2, 3, and 4 of TNFRSF1A, encoding the two first extracellular cystein-rich domains (CRDs), was performed as previously described [9].

Restriction analysis of the C55S mutation

Exon 3 of TNFRSF1A was PCR amplified in each member of the family. The 220 bp amplified fragment was then digested with the Hinf I restriction enzyme (BioLabs, Beverly, MA, USA) according to the manufacturer’s instructions. Normal alleles displayed fragments of 191 and 29 bp, whereas the C55S allele was characterized by three fragments of 95, 96, and 29 bp (the 95 and 96 bp fragments comigrated on agarose gel electrophoresis).

Mutation search in MEFV (GenBank accession number Y14441)

The region of exon 10 between codons 663 and 771 (including the 4 most frequent mutations, namely, M680I, M694 V, M694I, and V726A) was PCR amplified and sequenced; the E148Q mutation in exon 2 was also searched as described previously [10].

RESULTS

Clinical reports

Patient II-1 was born in 1959. Since age four he has suffered from recurrent episodes of abdominal and/or back pain and/or diarrhea and/or arthralgias with fever (up to 41°C). These episodes lasted for up to four weeks. Still’s disease was tentatively diagnosed, and steroids were given intermittently from 1965, with some improvement. In 1971, severe abdominal pain led to a laparotomy, which failed to reveal any abnormality. In 1972, proteinuria was noticed (1.8 g/L), and in 1973, a full-blown nephrotic syndrome developed. Recurrent febrile episodes and/or arthralgias partially responded to steroids. In 1974, a kidney biopsy showed extensive glomerular (mesangial and arteriolar) amyloid deposits (Fig. 2A). Azathioprine was added to steroids, with a poor tolerance. It was replaced by chlorambucil, with a poor compliance. Steroids were continued and transiently increased during bouts of abdominal pain or arthralgias. In 1976, serum creatinine reached 2.3 mg/dL with a persistent nephrotic syndrome. In February 1978, end-stage renal failure with acute pericarditis required maintenance hemodialysis. On hemodialysis, attempts to withdraw steroids were followed by recurrent episodes of arthralgias, fever, and abdominal pain.

In 1979, a rectal biopsy revealed amyloid deposits. Echocardiography and bone marrow examination were normal. In July 1980, a cadaveric kidney transplantation (TP) was performed. Immunosuppressive regimen included azathioprine and steroids. A single rejection episode was successfully treated by intravenous methylprednisolone. In 1987, gallstones prompted cholecystectomy. Pathological examination of the gallbladder showed amyloid deposits. In 1992, serum bilirubin level rose to 7.2 mg/dL. Liver biopsy showed veno-occlusive disease, but no amyloid. Azathioprine was stopped, and cyclosporine was started, with subsequent normalization of the bilirubin level. In 1993, total thyroidectomy was performed for progressive large goiter. Pathological examination disclosed massive interstitial amyloidosis. In 1999, proteinuria recurred (1.3 g/day). Graft biopsy showed glomerular, arteriolar, and arteriolar amyloid deposits. Treatment with colchicine (1 mg/day) was started.

Patient II-4 was born in 1964. Since childhood, she complained of recurrent episodes of polyarthralgias and/or
fever, lasting approximately 10 days. In 1985, proteinuria developed (1.2 g/day); serum creatinine was normal. Kidney biopsy showed mesangial amyloid deposits. Low-dose colchicine was started. In 1988, the nephrotic syndrome developed, and in 1990, serum creatinine rose from 1.8 mg/dL to end-stage renal failure requiring hemodialysis. At that time, a rectal biopsy failed to reveal amyloid. While on hemodialysis, recurrent episodes of otherwise unexplained fever and arthralgias developed, together with biochemical signs of serum acute phase reaction. In May 1994, a cadaveric kidney TP was performed under cyclosporine, azathioprine, and low-dose steroids. In 1998, a thyroidectomy was performed because of a progressive goiter. Massive interstitial amyloid deposits were found. Currently, serum creatinine is normal, but mild proteinuria (0.41 g/L) has recurred. Gastro-duodenal biopsies show small amyloid deposits.

Patient I-1 was born in 1927. In the late 1960s, he started to develop febrile episodes two to three times yearly, lasting approximately 10 days. These were inconstanty associated with cough and/or rhinorrhea and/or back pain. In 1969, mild proteinuria was detected together with the acute-phase reaction (CRP+). He was temporarily given steroids (10 mg prednisolone/day) with clinical and biochemical improvement. In 1980, he developed arterial hypertension and the nephrotic syndrome. A left pleural rub was detected on physical examination. Chest x-ray showed a right pleural effusion. Serum creatinine was normal. The serum CRP level was markedly elevated (+++). A kidney biopsy showed

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**Fig. 2.** Congo Red-positive amyloid, with apple-green birefringence under polarized light, in mesangium and interlobular artery (A; ×200). Strong staining of amyloid deposits with anti-SAA antibody labeled with a peroxidase is shown (B; ×400).
massive amyloid deposition in glomeruli, tubules, and interstitium, as well as in a few arterioles. A rectal biopsy also showed amyloid in submucosal vessels. Symptomatic treatment was prescribed. He died suddenly in 1985. At that time, serum creatinine was mildly elevated.

**Immunohistochemistry on pathological samples**

In all three patients with amyloidosis (I-1, II-1, II-4), a review of pathological slides (I-1, kidney; II-1, native kidney; II-4, thyroid) after appropriate immunohistochemical staining showed that amyloid deposits stained strongly with anti-SAA antibodies (Fig. 2B) but not with anti-κ, anti-λ, and antitransthyretin antibodies.

**Differential diagnosis**

The unequivocal demonstration of SAA protein in amyloid deposits in this family excluded other forms of autosomal-dominant amyloidosis caused by deposition of different proteins (transthyretin, fibrinogen, lysozyme, apolipoprotein A1, and gelsolin) [1, 2] and restricted the differential diagnosis to three hereditary disorders potentially complicated by AA amyloidosis: TRAPS (not included in a comprehensive recent review of hereditary amyloidosis [2]), FMF (infrequently transmitted as an autosomal-dominant trait [11]), and finally the Muckle–Wells syndrome [12], characterized by recurrent urticarial rash and deafness (both absent in our patients) or the closely related syndrome of autosomal-dominant periodic fever with AA amyloidosis and urticarial lesions without deafness described recently in an Indian family [13].

**Mutation search in TNFRSF1A**

Mutation analysis of exons 2, 3, and 4 of TNFRSF1A coding the two N terminal CRDs was performed as described previously [9]. A heterozygous mutation was identified in exon 3 of patient II-1. This variant is a G→C transversion that substitutes a serine for a cysteine at position 55 (C55S; TGT→TCT; Fig. 3). This mutation C55S creates a Hinf I restriction site (data not shown). Individuals I-2 and II-3 had normal restriction patterns electrophoresis. In order to rule out the possibility that this nucleotide substitution corresponds to a polymorphism, PCR amplification of the TNFRSF1A exon 3 was performed on 100 alleles from unrelated French individuals. The Hinf I restriction site was absent in all of them, thus providing strong genetic evidence that this G→C transversion was responsible for the disease.

**Mutation search in MEFV**

An FMF mutation search was done in patient II-1, consisting of the sequencing of the hot-spot region located in exon 10 of MEFV and the search for the E148Q mutation in exon 2. No mutation was detected.

**DISCUSSION**

The disease affecting this Belgian family associates periodic fever with eventual AA amyloidosis transmitted as an autosomal-dominant trait. Genetic testing disclosed a novel mutation (C55S) in the gene encoding TNFRSF1A. Mutations in TNFRSF1A have very recently been shown to cause autosomal-dominant recurrent fever [7, 14], and the name TRAPS has been proposed to include the genetically characterized clinical syndromes previously described as FMF-like syndrome with amyloidosis, autosomal dominant periodic fever, familial Hibernian fever, or familial periodic fever [15–17].

Several features suggested a diagnosis of TRAPS rather than FMF in this family. First, the autosomal dominant transmission strongly favored TRAPS [9, 14], although pseudodominant or actual dominant transmission of FMF with amyloidosis has been documented infrequently [11]. Second, attacks of fever often lasted weeks in our patients. This is suggestive of TRAPS rather than FMF, in which attacks usually last at most three days [14]. Again, this feature is not fully specific. Third, attacks were apparently improved by steroids in our patients, an observation more compatible with TRAPS than with FMF [14]. Finally, the family was not known to have any Mediterranean ancestors. Mutations in the MEFV have, however, been found in some patients without Mediterranean ancestry [10].

A third diagnostic possibility was the syndrome of autosomal-dominant periodic fever with AA amyloidosis described recently in an Indian family [13]. Interestingly, this syndrome maps, like the Muckle–Wells syndrome [12], to distal chromosome 1q; the gene(s) responsible for both syndromes remains to be identified. Both syndromes share the existence of urticarial lesions,
which were not observed in our patients, making this diagnosis less likely.

Overall, TRAPS appeared to be very likely, but only molecular studies could ascertain the diagnosis. A new mutation (C55S) was detected in TNFRSF1A, while no mutation was identified in MEFV.

In his seminal report on TRAPS, McDermott et al studied seven affected families and detected six different missense mutations in the first two CRDs: CRD1 (C30R, C33Y, T50M, C52F) and CRD2 (C88R, C88Y) of the TNFRSF1A [7]. An additional mutation in CRD1 (C30S) was recently identified in a French family [9]. All identified mutations are at highly conserved positions in the TNF-receptor superfamily, and six out of seven (including our new one) are expected to disrupt intramolecular disulfide bonds. The CRD1 has been shown to be necessary for the binding of TNF-α to TNFR1 [18]. The exact nature of the interaction between TNF-α and its receptors remains to be elucidated. It has been hypothesized that soluble forms of the receptors buffer the inflammatory reaction by antagonizing the binding of TNF-α to its membrane receptors and that a mutation in CRD impairs the cleavage of the receptor to its soluble form [7]. Further progresses in the pathophysiology of TRAPS may ultimately help unravel mechanisms of inflammation.

A remarkable feature in our family is the development of amyloidosis in all three affected subjects. Amyloidosis has indeed been reported in only two out of the seven other families shown at the molecular level to be affected by TRAPS. Admittedly, other families with autosomal dominant recurrent fever with amyloidosis have been reported decades ago [5, 6]; however, whether they are also affected by TRAPS or other unknown genetic diseases remains unknown. Interestingly, Cazeneuve et al have recently demonstrated that a polymorphism in the SAA1 gene coding for the SAA1 protein isoform is an independent risk factor for the development of AA amyloidosis in FMF [19]. Whether this finding applies to TRAPS will require further investigation.

Another striking feature in our family was the severity of amyloidosis. Both patients required hemodialysis followed by renal transplantation, and several other organs were subsequently affected by amyloidosis (thyroid in both cases, gastrointestinal tract in both cases, gallbladder in one), with an eventual recurrence of AA amyloidosis in the kidney graft in the patient with the longest duration of symptomatic disease and suspected recurrence in the other. Although progression of amyloid involvement may reflect a peculiar amyloidogenic potential of this novel mutation, an alternative, more likely explanation is that the extended life expectancy provided From a practical point of view, the clinician facing either periodic fever with unexplained inflammatory symptoms or signs or unexplained AA amyloidosis should consider not only FMF but also TRAPS. The latter diagnosis is strongly suggested by an autosomal dominant transmis-

Interestingly, all hitherto reported TRAPS families originate from Northern Europe (Scottish, Irish/Scottish, Irish, Finish, Irish/English/German, Irish, French/Canadian, French, and now Belgian). As seven different mutations have been identified in these eight families characterized at the molecular level, a founder effect may be excluded. Thus, the complete absence of detection of TRAPS in populations with other ethnic backgrounds may reflect underdiagnosis.

Making a diagnosis of TRAPS has implications for appropriate management. A diagnosis of TRAPS may help avoid invasive diagnostic procedures and ineffective therapies. For instance, in patient II-1, acute abdominal pain prompted an exploratory laparotomy that proved negative, as has been reported in other TRAPS [15, 17] or FMF families [4]. Because colchicine is ineffective in TRAPS [5, 9], patients II-1 and II-4 could be spared this therapy. Of note, the reportedly favorable effect of steroids in TRAPS [14] is challenged by our observation of the relentless course of the disease after renal transplantation with a combination of steroids, cyclosporine and azathioprine, as witnessed by the persistent acute phase reaction, recurrence of amyloidosis in kidney graft, and progression of amyloidosis in other organs. This risk of late-graft amyloidosis, however, should not deter the nephrologist from considering TRAPS patients for renal transplantation. The overall survival rate (and quality of life) is indeed better in patients who have been transplanted than in those put on the waiting list for a TP but still being dialyzed [21].

Making a diagnosis of TRAPS has also implications for genetic counseling. The absence of attacks of fever at age 38 in individual II-3 did not completely exclude the carriage of TRAPS, as attacks of fever started around the age of 40 in patient II-1, her father. In two other TRAPS families, the age at onset of periodic fever varied from less than 1 to 44 years and from 3 to 30 years, respectively [9, 17]. In the latter family, a carrier of TRAPS was asymptomatic in his fourth decade [9]. Genetic testing was therefore mandatory for definite reassurance of individual II-3. As another implication, genetic diagnosis is also advisable when a living-related kidney transplantation is considered in such families.

Understanding the pathophysiology of TRAPS paves the way for innovative treatments. As the disease is apparently mediated by uncontrolled TNF activation, inhibitors of TNF could be of therapeutic value. Preliminary results with the use of etanercept [22], a TNFR:Fc fusion protein, have been encouraging enough to launch a trial with this agent [14].

From a practical point of view, the clinician facing either periodic fever with unexplained inflammatory symptoms or signs or unexplained AA amyloidosis should consider not only FMF but also TRAPS. The latter diagnosis is strongly suggested by an autosomal dominant transmis-
sion and an absence of Mediterranean ancestry. The diagnosis can now be ascertained by genetic testing.

ACKNOWLEDGMENTS

This work was supported by l’Association Francaise contre les Myopathies (AFM) and by le programme hospitalier de recherche clinique (1997). The authors thank M-A. Lefevre, M.D., for referring individual II-3; H. Samain, M.D., for providing medical records of patients II-1 and II-4; and O. Devuyst, M.D., Ph.D., for fruitful discussion.

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