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SHORT REPORT

Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) in a Dutch family: evidence for a *TNFRSF1A* mutation with reduced penetrance

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Mutations of the tumor necrosis factor receptor 1 (*TNFRSF1A*) gene underly susceptibility to a subset of autosomal dominant recurrent fevers (ADRFs). We report on a two-generation six-member Dutch family in which a novel R92P mutation and reduced plasma TNFRSF1A levels were found in all the children, including two who are unaffected. However, only the daughter proband and father exhibited a typical TNF-receptor associated periodic syndrome (TRAPS) phenotype. PCR-RFLP analysis revealed that the mutation was not present in 120 control chromosomes from unaffected Dutch individuals. As this R92P mutation is present in two unaffected carriers it appears to be less penetrant than previously reported *TNFRSF1A* mutations involving cysteine residues in the extracellular domains. *European Journal of Human Genetics* (2001) **9**, 63–66.

Keywords: *TNFRSF1A* mutation; TRAPS; penetrance; plasma TNFRSF1A levels

Introduction

Autosomal dominant and recessive periodic fevers comprise a group of disorders characterised by fevers, peritonitis, pleurisy, arthritis, skin rashes, and/or conjunctivitis. These attacks are separated by symptom-free intervals and some patients develop AA amyloidosis. The discovery of mutations of the tumor necrosis factor receptor 1 (*TNFRSF1A* or tumor necrosis factor receptor superfamily 1A) gene in a subset of autosomal dominant recurrent fevers (ADRF) has simplified disease classification to some extent.[1,2]

The TNF-Receptor Associated Periodic Syndrome (TRAPS) subsumes a number of older diagnostic entities, including a condition known as familial Hibernian fever[3] (FHF; OMIM No 142680). The TRAPS phenotype is distinguished by attacks of fever of longer duration, which are mostly resistant to colchicine prophylaxis but partly responsive to steroid therapy. Five of the six *TNFRSF1A* mutations reported to date disrupt disulfide bonds in extracellular domains.[1]

Familial Mediterranean fever (FMF: OMIM No 249100), and hyperimmunoglobulinaemia D (HIDS; OMIM No 260920) are the most common autosomal recessive periodic fevers with FMF being the most prevalent overall condition.[4] HIDS has been diagnosed in fewer than 150 people and is most commonly found in the Dutch population.

We report on a two-generation Dutch family, where recurrent attacks of fever and abdominal pain appear to segregate as an autosomal dominant trait. We used informative markers from FMF, HIDS and TRAPS loci to study the family. Linkage analysis was unsuitable in this family due to its structure and size, but genotyping data did support a diagnosis of TRAPS and were not consistent with either FMF or HIDS. DNA sequencing of *TNFRSF1A* as a candidate susceptibility gene revealed a novel R92P mutation that segregates with disease, at reduced penetrance, in this family.

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Methods

Families and DNA samples

The family studied includes six living members, two of whom have a clinical phenotype strongly resembling TRAPS (the daughter proband and affected father), and in addition a son aged 27 has mild symptoms suggestive of irritable bowel disease. The Human Experimentation Committee at each participating institution approved the study, and all participants gave informed consent. Clinical details of this family have been described previously.[5] In summary, the 37-year-old proband daughter became affected at age 24 with recurring abdominal pain at two-month intervals with fevers up to 38.5°C but no joint pains or skin rashes. The attacks are of days to weeks duration and are not related to menses. An ultrasound in 1988 showed a swollen right adnex which was surgically removed – there were no abnormal histopathological findings. Her laboratory tests revealed an acute phase response with elevated ESR (37–111 mm/h), leukocytosis and elevated CRP at 22.5 mg/100 ml (N < 0.5 mg/100 ml). Her serum IgD and IgA levels were normal, as were bowel X-rays. Serological tests for ANF and anti-dsDNA were negative and there was no evidence of active viral or bacterial infections. Treatment with colchicine produces less frequent and milder attacks. The 69-year-old father developed similar symptoms at 38 years but the attacks are less frequent than in the proband (2–3 times per year). He also had coronary bypass operations at 46 and 53 years. Investigations include raised ESR during attacks and normal IgD levels. The father's symptoms also respond to colchicine and anti-inflammatories. A 34-year-old son complains of intermittent abdominal pains but does not have any fevers or arthralgia. Blood was taken from all family members of DNA studies and also from 60 unaffected Dutch individuals who were studied as normal controls.

Microsatellite markers

TNFRSF1A locus *(chromosome 12p13)* An informative microsatellite marker (TNFRp55)[6] from the first intron of the *TNFRSF1A* gene in addition to the D12S99 and D12S77 flanking markers was used to genotype all family members.

FMF locus *(chromosome 16p13.3)* Two microsatellite markers which tightly flank the *MEFV* gene, mutated in FMF, were used: D16S3070 (telomeric) and D16S3275 (centromeric) the MEFV locus plus D16S418, about 30 cM centromeric to *MEFV*.

HIDS locus *(chromosome 12q24)* D12S79 and D1S306 flanking markers were used.[7]

Mutation detection by fluorescent sequencing

The proband daughter was screened for *TNFRSF1A* mutations in exons 1–7, as described.[1] The 5' untranslated region was also investigated: the complete 900 bp segment was first amplified using two external primers and the product was then sequenced using overlapping internal primers (Amersham, UK).

Restriction endonuclease assays for *TNFRSF1A* mutations

The restriction endonuclease assay for the R92P mutation involved the introduction of an enzyme recognition site into the reverse primer by nucleotide substitution. The following primers were used: forward primer 5' TTACAGAGACACACACTTAGG 3' and reverse primer 5' CCTGCAGCCACACACGGTGCCC 3'. The 'C' nucleotide in the reverse primer is substituted for the 'T' nucleotide at position 842 in the published sequence (reference M75865 GenBank) so as to create the *Sma*I restriction enzyme (New England, Biolabs, Herts, UK) recognition sequence. An R92P mutation within the amplified product thus generates a restriction site. 50–100 ng of genomic DNA was amplified in a volume of 15 μl in the presence of 1.5 mmol $MgCl_2$ PCR buffer, 0.06 units of Taq Polymerase (Promega, Southampton, UK), and 5 pmol of each of the appropriate PCR primers. PCR was at 95°C for 10 min, with 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and the final extension at 72°C for 10 min. Amplified products were digested for at least 2 hours with 10 units of *Sma*I and samples loaded on a 4–20% polyacrylamide gel (Flowgen, Leics, UK). The gel was stained with ethidium bromide and controls included.

Measurement of Soluble TNF Receptors

Soluble plasma levels of TNFRSF1A (p55) and TNFR2 (p75) were measured by solid-phase ELISA (R&D Systems, Abingdon, UK) as described.[1]

Results

Microsatellite markers

All the children inherited allele 6 of the TNFRp55 marker from the affected father so there was complete segregation of this allele with the low soluble TNFRSF1A levels (Figure 1). None of the children were haploidentical for either the FMF or HIDS markers as might be expected for an autosomal recessive disease – in fact the proband daughter and her mildly affected brother do not share haplotypes at either loci (Figure 2).

Mutation detection

Sequencing of the *TNFRSF1A* gene in the daughter proband revealed a novel G to C transversion in exon 4 producing an arginine to proline mutation at residue 92 (R92P). The *Sma*I RFLP assay revealed that this mutation was present in all family members except the mother (Figure 1) and that it was not found in any of the 60 controls.

Soluble TNFR levels

Reduced plasma TNFRSFIA levels who found in all the children, including two who are unaffecated (Figure 1). The lowest soluble TNFRSF1A levels were observed in the affected proband daughter (444 pg/ml) and the son with mild symptoms (517 pg/ml) but low levels were also found in the two unaffected carriers (547, 602 pg/ml respectively), whereas the

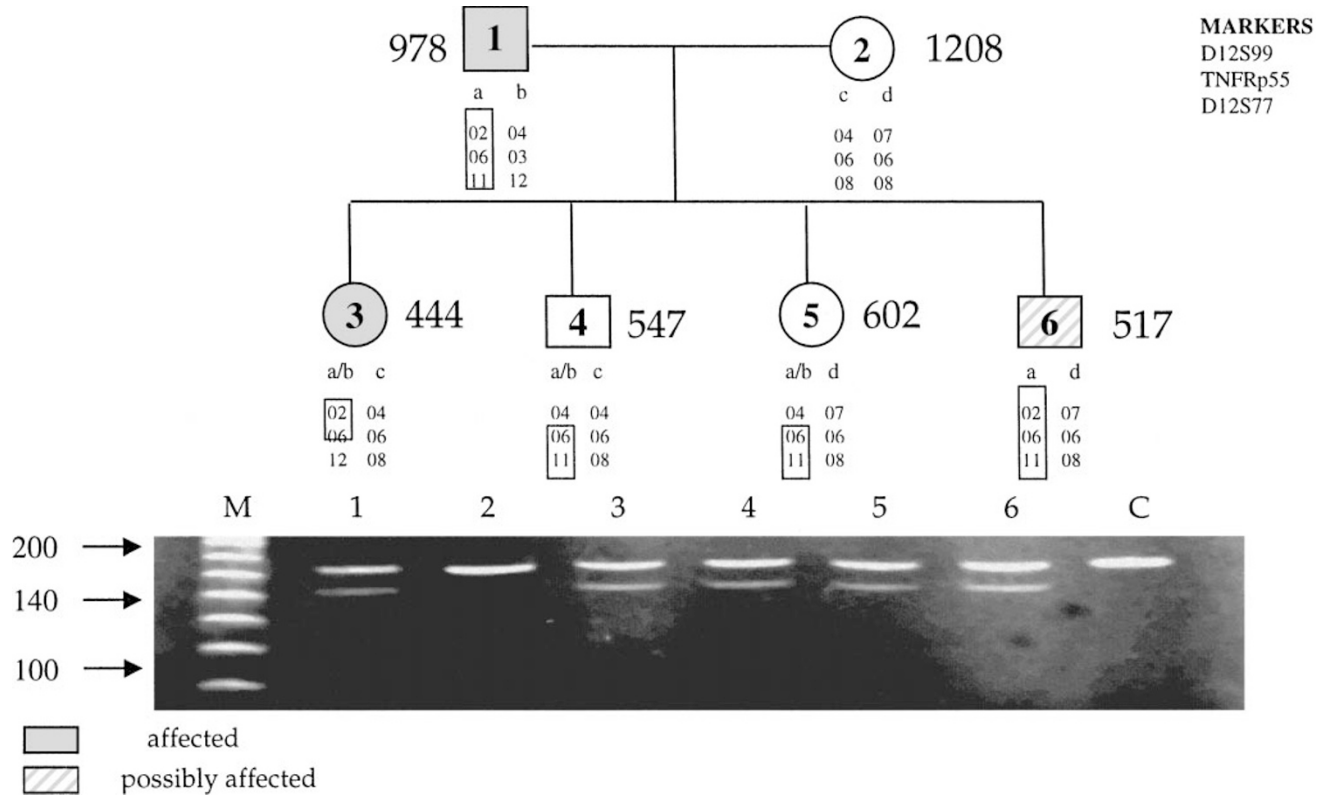

Figure 1 Pedigree of the Dutch family with genotypes of three markers spanning the *TNFRSF1A* locus on chromosome 12p13 and a picture of the *Sma*I RFLP assay for the R92P mutation. Samples were loaded on 16% polyacrylamide gels (Novex, UK) and stained with ethidium bromide. Positive and negative controls were included in each run. The numbers besides each symbol correspond to soluble TNFRSF1A levels (pg/ml). The normal range is 746–1966 pg/ml.

level in the affected father was towards the lower limit of normal (978 pg/ml) (normal range 746–1966 pg/ml). Soluble TNFRSF1B levels were normal in all family members.

Discussion

Recent developments in the molecular genetics of periodic fevers have facilitated the diagnoses of these patients. The novel R92P mutation found in two unaffected carriers suggests that this mutation is less penetrant and further supports the concept that even in 'simple' Mendelian disorders the disease phenotypes are, in fact, complex traits.[8] Among the possible modifiers of the TRAPS phenotype in this family include gene environment interactions, background genes, and varying thresholds for modifier protein function. Some possible background genes that may be involved include metalloprotease cleavage enzymes involved in TNF and TNF receptor shedding, in addition to disintegrin docking molecules which interact with these enzymes. The approximately half normal plasma TNFRSF1A levels found in the two unaffected carriers aged 35 and 38 years respectively, plus one mildly affected individual, compared with the normal levels in the affected father may reflect the compensatory increase in TNFRSF1A levels found during attacks of fever in the more severely affected members. It is also notable that the low plasma TNFRSF1A levels segregate completely with the mutation in all the children, including those who are unaffected.

Other cases of non-penetrant *TNFRSF1A* mutations have been described;[1] some members of the original T50M Irish family have mild symptoms in comparison with the severely affected proband, and one woman with this mutation remains asymptomatic in her mid-50s. Likewise, one male member of the Finnish family with the C88Y mutation is asymptomatic in his early 20s. It is notable that all these unaffected individuals have low plasma levels of soluble TNFRSF1A, as is the case with the asymptomatic members of this Dutch family and it is possible that low levels of soluble TNFRSF1A in combination with particular environmental insults may be necessary to produce the fullblown TRAPS phenotype.

There was no genetic evidence to support a diagnosis of either FMF or HIDS in affected members of this family in view of the dominant pattern of inheritance (parent–child transmission) and lack of haplotype sharing in the offspring. Furthermore, the clinical presentation (long duration of attacks and no lymphadenopathy) did not fit the clinical picture of either FMF or HIDS.

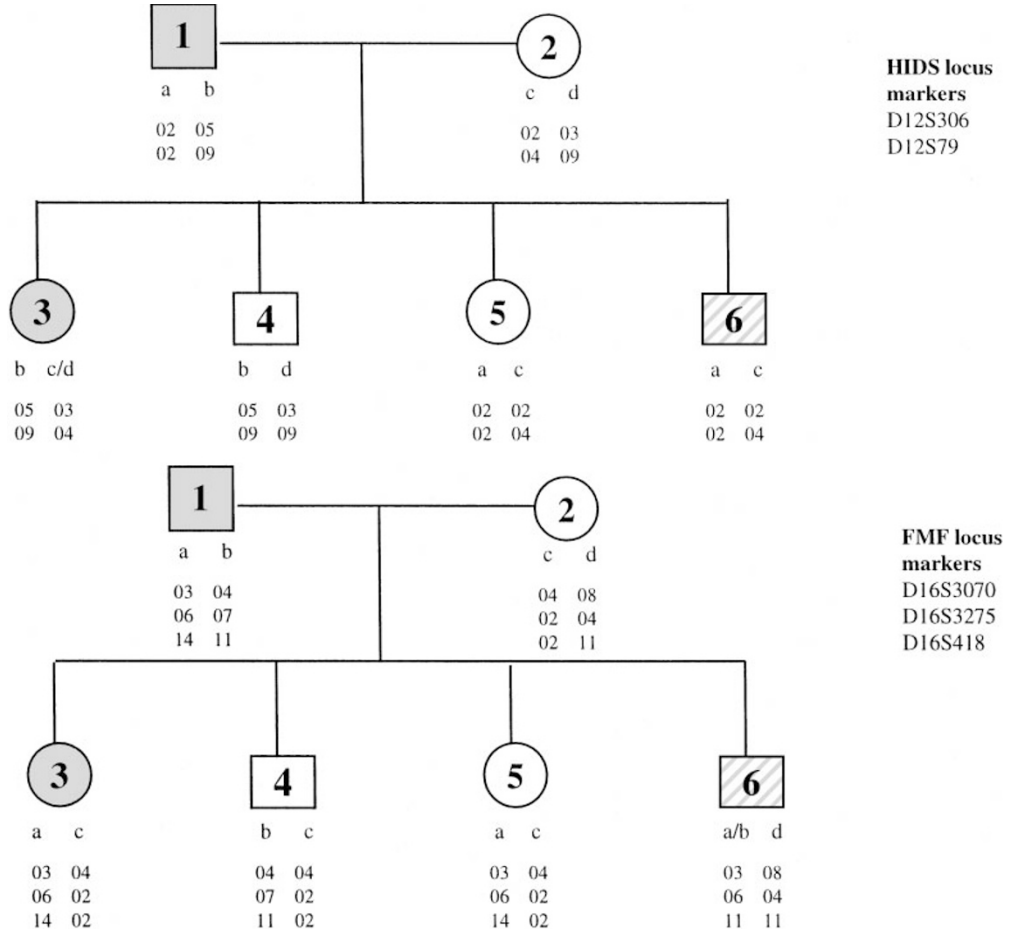

Figure 2 The Dutch pedigree showing markers spanning the HIDS and FMF loci; there is no evidence of haplotype sharing in subjects 3 and 6.

This result also raises the question as to whether *TNFRSF1A* mutations may be present in mildly symptomatic or indeed asymptomatic members of the overall community. In this and previous studies we have not found mutations in unaffected controls but clearly a larger study is required to exclude formally this possibility.

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References

1 McDermott MF, Aksentijevich I, Galon J *et al*: Germline mutations in the extracellular domains of the 55 kDa TNF receptor (TNF-R1) define a family of dominantly inherited autoinflammatory syndromes. *Cell* 1999; **97**: 133–144.
2 McDermott MF: Autosomal dominant recurrent fevers – clinical and genetic aspects. *Rev Rhum Engl Ed.* 1999; **66**: 484–491.
3 Williamson LM, Hull D, Mehta R, Reeves WG, Robinson BHB, Toghill PH: Familial hibernian fever. *Quart J Med* 1982; **204**: 469–480.
4 Kastner DL: Intermittent and periodic arthritis syndromes. In Koopman W (ed). *Arthritis and Allied Conditions: a Textbook of Rheumatology*, Williams & Wilkins: Baltimore, 1997, 13th edn, pp 1279–1306.
5 Zweers EJ, Erkelens DW: Een Nederlandse familie met familiale mediterrane koorts. (A Dutch family with familial Mediterranean fever). *Ned Tijdschr Geneeskd* 1993; **137**: 1570–1573.
6 Eskdale J, Turestkaya RL, Armstrong C, Kuprash DV, Nedospasov SA, Gallagher G: A polymorphic microsatellite marker in the human p55 receptor, CD120a. *Genes Immun* 2000; **1**: 228–230.
7 Drenth JPH, Cuisset L, Grateau G, Vasseur C, van de Velde-Visser SD, de Jong JGN and contributing members of the International Hyper-IgD Study Group: Mutations in the gene encoding mevalonate cause hyper-IgD and periodic fever syndrome. *Nat Genet* 1999; **22**: 178–181.
8 Dipple KM, McCabe ERB: Phenotypes of patients with 'simple' Mendelian disorders are complex traits: thresholds, modifiers, and systems dynamics. *Am J Hum Genet* 2000; **66**: 1729–1735.