Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome

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Hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS; MIM 260920) is a rare, apparently monogenic, autosomal recessive disorder characterized by recurrent episodes of fever accompanied with lymphadenopathy, abdominal distress, joint involvement and skin lesions[1]. All patients have high serum IgD values (>100 U/ml) and HIDS 'attacks' are associated with an intense acute phase reaction whose exact pathophysiology remains obscure[2–4]. Two other hereditary febrile disorders have been described. Familial Mediterranean fever (MIM 249100) is an autosomal recessive disorder affecting mostly populations from the Mediterranean basin and is caused by mutations in the gene *MEFV* (refs 5,6). Familial Hibernian fever (MIM 142680), also known as autosomal dominant familial recurrent fever, is caused by missense mutations in the gene encoding type I tumour necrosis factor receptor[7–10]. Here we perform a genome-wide search to map the HIDS gene. Haplotype analysis placed the gene at 12q24 between *D12S330* and *D12S79*. We identified the gene *MVK*, encoding mevalonate kinase (MK, ATP:mevalonate 5-phosphotransferase; EC 2.7.1.36), as a candidate gene. We characterized 3 missense mutations, a 92-bp loss stemming from a deletion or from exon skipping, and the absence of expression of one allele. Functional analysis demonstrated diminished MK activity in fibroblasts from HIDS patients. Our data establish *MVK* as the gene responsible for HIDS.

Using a dense map of fluorescent markers, we established that HIDS is linked to the subterminal region of the long arm of chromosome 12q24. A maximum 2-point lod score of 3.02 at a recombination fraction (θ) of 0.058 was detected with marker *D12S306*. Further refinement mapped the gene between *D12S306* and *D12S79* (Table 1). There was no linkage with all other markers tested. The clinical presentation of HIDS and analysis with the HOMOG program suggested homogeneity, and haplotype analysis suggested 10 informative recombination events in our families. There were four recombinations in four families (HIDS 1-2, 3-4, 13-5, 15-6), which allowed us to place the gene centromeric to *D12S79*, and the recombination in one family (HIDS 2-5) placed the gene telomeric to *D12S330*. This narrowed the candidate region to a 9-cM interval. All other recombinations were consistent with this localization. Analysis of this interval in the Human Transcript Map (http://www.ncbi.nlm.nih.gov/genemap/) identified *MVK* as a plausible functional candidate gene. MK follows 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in the synthetic pathway of cholesterol and is expressed in peroxisomes, which are present in all mammalian cells. Complete deficiency of MK causes mevalonic aciduria (MIM 251170), which is associated with high concentrations of mevalonic acid present in all body fluids[11].

Cultured lymphoblastic cells were available from 6 affected subjects belonging to 4 different families and from all the members of family 13. Sequence analysis of *MVK* cDNA identified four mutations in *MVK* in patients with HIDS. Subject 7 of family 5 (5-7) was a compound heterozygote for two missense mutations at positions 591 and 894 (Fig. 1*a*,*b*). These mutations replace proline by leucine at codon 165 (P165L) and isoleucine by threonine at codon 268 (I268T). The first mutation affects the secondary structure of the polypeptide chain, and may affect enzyme activity. Segregation analysis shows that the P165L mutation was inherited from the mother and I268T from the father, each parent being a heterozygous carrier of the respective mutation. Sequencing of *MVK* cDNA revealed that affected subjects 1-4, 2-3, 2-6, 2-8 and 3-6 are homozygotes for a missense mutation at position 1220 (Fig. 1*c*), which results in the replacement of valine by isoleucine at codon 377 (V377I). This mutation abolishes a *Bsm*AI site, and the subjects appear heterozygous on examination of genomic

Table 1 • Two-point linkage analysis data for the HIDS gene

Marker	Lod score at a recombination fraction (θ) of								
	0.00	0.01	0.05	0.1	0.2	0.3	0.4	Z_{max}	θ
D12S92	—	–5.39	–1.46	–0.17	0.48	0.39	0.13	0.491	0.220
D12S81	—	–7.31	–2.4	–0.79	0.11	0.16	0.05	0.26	0.183
D12S101	—	–9.59	–3.31	–1.19	0.07	0.22	0.08	0.28	0.28
D12S306	—	2.20	3.01	2.87	2.01	1.05	0.30	3.02	0.058
D12S330	—	–4.17	–0.40	0.76	1.13	0.74	0.23	1.15	0.178
D12S79	—	–0.80	1.89	2.39	1.95	1.07	0.31	2.49	0.108
D12S366	—	–3.83	–0.29	0.69	0.93	0.57	0.17	0.964	0.167

Markers span a sex-averaged interval of 40 cM on chromosome 12q24.

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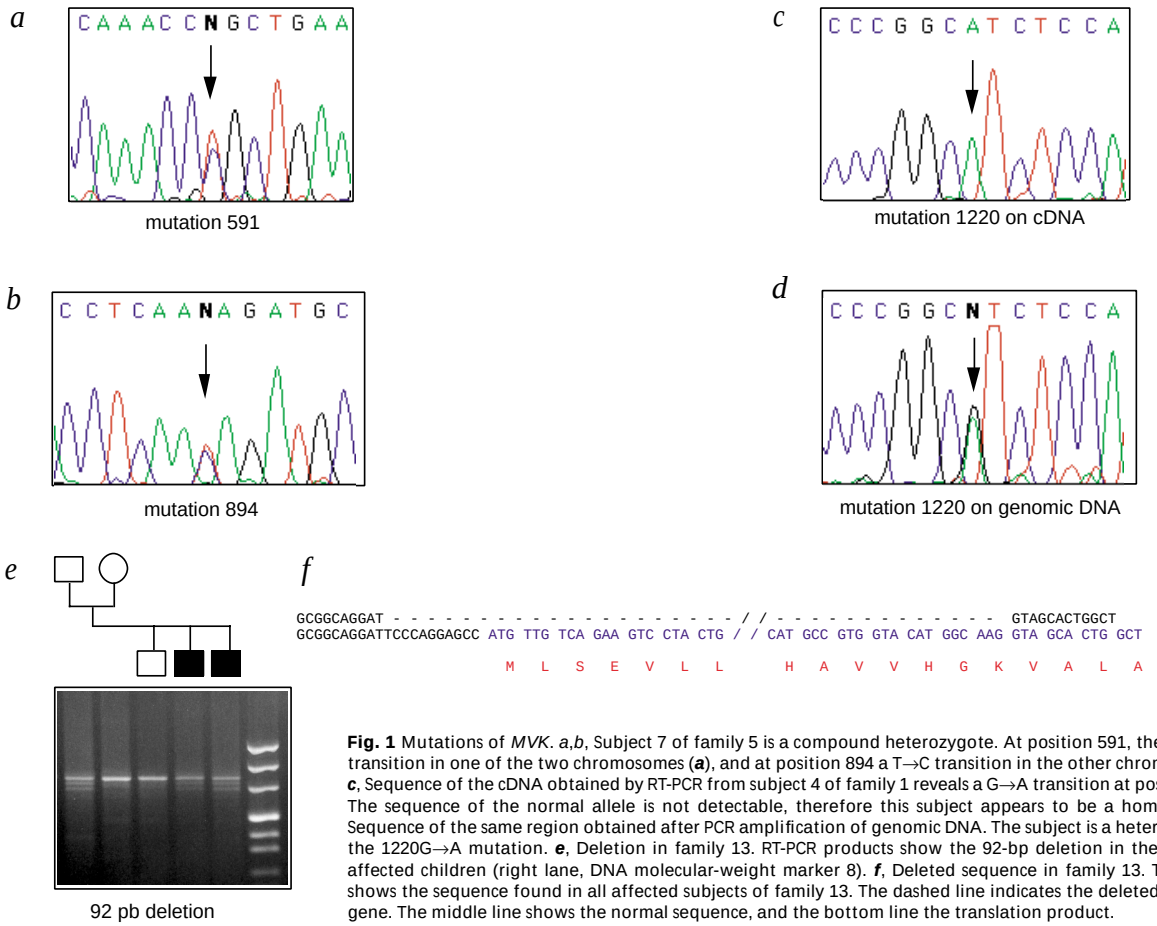

Fig. 1 Mutations of *MVK*. *a*,*b*, Subject 7 of family 5 is a compound heterozygote. At position 591, there is a C→T transition in one of the two chromosomes (***a***), and at position 894 a T→C transition in the other chromosome (***b***). ***c***, Sequence of the cDNA obtained by RT-PCR from subject 4 of family 1 reveals a G→A transition at position 1,220. The sequence of the normal allele is not detectable, therefore this subject appears to be a homozygote. ***d***, Sequence of the same region obtained after PCR amplification of genomic DNA. The subject is a heterozygote for the 1220G→A mutation. ***e***, Deletion in family 13. RT-PCR products show the 92-bp deletion in the father and affected children (right lane, DNA molecular-weight marker 8). ***f***, Deleted sequence in family 13. The top line shows the sequence found in all affected subjects of family 13. The dashed line indicates the deleted part of the gene. The middle line shows the normal sequence, and the bottom line the translation product.

DNA (Fig. 1*d*). Southern-blot analyses using *MVK* cDNA as probe and a variety of restriction endonucleases failed to show any genomic DNA rearrangement in these subjects. This indicates these subjects are compound heterozygotes carrying a missense mutation (V377I) and a second, unknown mutation that results in the absence of mRNA. Possible causes for this may be: (i) a mutation in the promoter which inhibits the expression of the gene; (ii) a null (nonsense or frameshift) or any other mutation that induces high mRNA instability; (iii) a short insertion or deletion in the corresponding region; or (iv) a mutation affecting the splicing of the transcript. In all events, such a mutation would result in the absence of MK protein.

We also found by genomic analyses that affected members of 5 additional families, including family 13, were heterozygous for the same mutation, which suggests that this is the most frequent mutation in HIDS. Analysis of the gene sequence shows that this site does not correspond to a known mutational hot spot. Further proof that *MVK* is responsible for HIDS was provided by analysis of family 13. cDNA sequence analyses of the RT-PCR products obtained from the affected subjects of family 13 using oligonucleotides 29 and 788 show the presence of a paternally inherited 92-bp loss beginning 11 bp upstream from the ATG translation initiation codon (Fig. 1*e*). This cDNA deletion may result from a genomic deletion mutation or, more likely, from an exon-skipping mutation, because the end of the deleted fragment is a potential splicing acceptor site. This deletion is considered a null mutation. The next in-frame ATG is approximately 200 bp downstream, resulting in truncation of the active site and half of the protein. For other reading frames, the translated protein will not encode mevalonate kinase. Thus, this deletion predicts an absence

Table 2 • Biochemical analysis

	Mevalonic acid in urine[a]			MK	
Patient[b]	remission	attack	Patient[b]	μU/mg of protein	(as % of mean controls)
22	<0.5	8	22	48	9
52	<0.5	2	52	3	0.5
55	<0.5	3	55	48	9
4	1	<0.5	17	66	12.4
10	<0.5	8	21	30	5.6
27	1	11			
30	5	11			
35	1	6			

[a]Arbitrary units. [b]Patient numbers refer to the number in the Nijmegen HIDS registry. Clinical details on these patients have been published[1].

of enzyme activity. We did not find this 92-bp loss in the cDNAs of subjects 1-4, 2-3, 2-6, 2-8 and 3-6. We characterized a mutation in 14 independent heterozygote carriers of a total of 53 chromosomes studied. No mutations in *MVK* were detected in a set of more than 200 control chromosomes, excluding the possibility that the mutations described here represent polymorphisms.

The mutations described here are not located in known functional sites of MK such as the ATP-binding[12] and catalytic sites[13]. The V377I and I268T mutations are located in highly conserved positions of orthologous gene products in human, rat and *Schizosaccharomyces pombe*. Because the conservation is found only at the protein level and not at the nucleic acid level, it is very likely that this region is essential for the function of the enzyme. We obtained evidence of this with the detection of low MK activity in fibroblasts from five patients from additional families. We observed a residual enzyme activity in HIDS cultured fibroblasts of 7.3%±4.5% of controls (3–66 μU/mg of protein), which is different from mevalonic aciduria, in which patients exhibit values less than 1% (Table 2). These data indicates that *MVK* mutations associated with HIDS result in a functional defect of MK. To differentiate HIDS from mevalonic aciduria, we quantified excretion of mevalonic acid in urine. On the basis of the peak height, estimated concentrations in all samples were below 20 mmol/mol creatinine (versus >56,200 mmol/mol in mevalonic aciduria), whereas no excretion was observed in control samples. This suggests that the diminished enzyme activity does not result in accumulation of mevalonic acid in body fluids. There was a variation in urine mevalonic acid excretion with disease activity, and higher values were observed during attacks compared with remissions. The observed higher excretion with fever suggests that during crises MK enzyme activity is further suppressed, or there is a higher demand of this metabolic pathway during attacks.

Despite the fact that mevalonic aciduria and HIDS arise from mutations in the same gene, the residual MK activity in fibroblasts is higher in HIDS than in mevalonic aciduria[14], and mevalonic acid excretion in HIDS patients is very low. In addition, the phenotype of HIDS is very different from that of mevalonic aciduria. For example, HIDS patients do not suffer from myotonia, hypotonia or ataxia and survival appears to be unaffected. There are also no dysmorphic features or cataracts in HIDS patients. Furthermore, IgD concentrations have not been reported to be increased in mevalonic aciduria. The sequence variations seen in mevalonic aciduria are missense mutations that are in the same region as the variants described in HIDS. To explain this genetic heterogeneity, we propose two hypotheses. First, *MVK* mutations may cause different disease severity due to the level of residual MK activity. A similar concept is applicable for the Hurler/Scheie syndrome (MIM 252800), in which an α-L-iduronidase deficiency causes a cellular accumulation of mucopolysaccharides. Multiple mutant alleles have been found in compound heterozygotes as well as homozygotes. Homozygosity for the R89Q mutation causes a mild phenotype, compound heterozygosity for both mutations produces an intermediate phenotype, and homozygosity for the 704ins5 mutation is associated with a severe phenotype[15]. Second, because mevalonate is a substrate implied in various important metabolic pathways, it is possible that the MK region harbouring the HIDS mutations is also responsible for other interactions with cofactors and cell structures. The mutations causing mevalonic aciduria may interfere with some of these pathways, and HIDS mutations with others, resulting in symptoms common to the two diseases and symptoms specific of each clinical entity. Finally, it is noteworthy that a gene responsible for cholesterol metabolism causes a periodic and inflammatory disease such as HIDS. The most likely explanation is that mutations in *MVK* result in alterations of metabolism (affecting levels of dolichol, ubiquinol, haem A) using cholesterol pathway intermediates or post-translational protein modifications like farnesylation, which may in turn cause a pathogenic inflammatory response.

Methods

Subjects. We obtained samples from 34 patients and 44 unaffected members from 16 HIDS families. The pedigrees for the first 10 families have been reported[16]. The families originated from The Netherlands (8), France (5), United Kingdom (1), Spain (1) and Czech Republic (1). All patients underwent a thorough clinical and laboratory evaluation. The HIDS diagnosis was made according to set criteria and biochemical proof of affected status was obtained by measurement of the IgD content in serum[1]. The study was approved by the Medical Ethical Committee (CWOM) of the University Hospital St. Radboud.

DNA analysis. We extracted genomic DNA and mRNA from leukocytes (DNA) from fresh drawn blood or from Epstein-Barr immortalized cell lines (DNA and mRNA) according to established protocols. We performed a genome-wide linkage analysis using 276 fluorescent microsatellite markers from the AFM/Généthon panel spaced at an average interval of 12–14 cM (ref. 17). Genotyping was performed on an Applied Biosystems (ABI) 373A automated DNA sequencer. We calculated allele sizes using an internal size standard (500 Tamra, Perkin Elmer ABI). Data were analysed by GeneScan 2.1.1. (ABI) and Genotyper 1.1.1. (ABI). We verified mendelian segregation with Marksyn software (Généthon).

Linkage analyses. We tested the feasibility of a random genome-wide search using SLINK (ref. 18). We calculated pairwise lod scores using MLINK of the FASTLINK package[19]. We used ILINK to detect the optimal value of recombination factors and maximum lod scores. An autosomal recessive model was assumed and the frequency of the abnormal allele was set at 0.00001 in view of the rarity of the disorder. We used isofrequencies and specific allele frequencies estimated by calculating their occurrence in the non-carrier chromosomes from our cohort of families. We assumed a complete penetrance. The recombination frequency (θ) was stated to be equal for both sexes. We constructed and assigned haplotypes to minimize the number of crossovers in each family. We tested homogeneity using the HOMOG program[20].

Screening of *MVK* mutations. We prepared total RNA from lymphoblastic cell lines and peripheral blood lymphocytes using the RNA B reagent (Bioprobe) according to the manufacturer's instructions. We synthesized cDNA starting from total RNA (4 μg) with Superscript reverse transcriptase II (Gibco BRL), followed by PCR using Platinium *Taq* DNA polymerase (Gibco BRL) in a GeneAmp PCR system 9600 (Perkin Elmer). We determined the nucleotide sequences of the amplified fragments by standard semi-automated methods on an ABI PRISM 377 (Perkin Elmer).

We used forward primers: 29, 5′–CGGCTTCGGCGCGGAGGGG–3′; 381, 5′–TTGCCTGACGACTGTGCT–3′; 539, 5′–TGCGTGTGTCTGGCAGCA–3′; 591, 5′–CGCTGAAGGACGGGGATTG–3′; 825, 5′–CCCTTGTGGCTGGCGTC–3′; 921, 5′–GCGTGCTGGGAGAGAGATG–3′; and 1176, 5′–GCTGTGGCTTTGACTGC–3′. We used reverse primers: 535, 5′–CCAGACACACCGAGTAG–3′; 602, 5′–CCTGTTGACGCAATCCCC–3′; 736, 5′–AAATCTTCCCTTGATGGTAT–3′; 788, 5′–CTTTGGTGTTGGTCAGCAG–3′; 899, 5′–ACACTCCAGGGAGATGGC–3′; 1000, 5′–CGAGGGCATTCAGATGG–3′; and 1381, 5′–GCCTCTCCAGCAGTGTCAG–3′.

We amplified the cDNA from patients 1-4 (subject 4 of family 1), 2-3, 2-6, 2-8, 3-6 and 5-7 in two parts using primers 29/788 and 591/1381. Their sequences were determined using primers 381, 535, 591, 736, 921, 1000 and 1381. Screening for the presence of the mutations found in subject 5-7 was performed in other members of the family by amplification of their genomic DNA (cells were not available), SSCP and sequencing using primers 539/602 and 825/899 for mutations P165L and I268T, respectively. We searched the V377I mutation by amplification of either cDNA or genomic DNA and digestion with *Bsm*AI or sequencing. We found the 92-bp deletion in family 13 by cDNA amplification (primers 29/788) and

sequencing with primers 29 and 535. We sequenced cDNA from 120 normal subjects of various ethnic origins.

Mevalonate pathway. We assayed MK activity in lysates from cultured fibroblasts derived from skin biopsies from five HIDS patients[21]. Urine samples corresponding to 4 µmol creatinine were acidified with HCl to pH 1, saturated with sodium chloride and extracted with ethylacetate. We analysed organic acids as their trimethylsilyl-esters by gas chromatography and flame ionization detection (FID) or mass spectrometry. Mevanololacton was measured by FID and corrected for the internal standard. Content of mevalonolacton, eluting just before 2,3-dihydroxybutyric acid, was estimated on basis of the peak height. In normal urines a peak at this position was absent. The excretion of mevalonic acid in HIDS patients was below 20 mmol/mol creatinine.

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