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ARTICLE

Organization of the mevalonate kinase (*MVK*) gene and identification of novel mutations causing mevalonic aciduria and hyperimmunoglobulinaemia D and periodic fever syndrome

Sander M Houten[1], Janet Koster[1], Gerrit-Jan Romeijn[1], Joost Frenkel[2], Maja Di Rocco[3], Ubaldo Caruso[3], Pierre Landrieu[4], Richard I Kelley[5], Wietse Kuis[2], Bwee Tien Poll-The[1,2], K Michael Gibson[6], Ronald JA Wanders[1] and Hans R Waterham*[,1]

[1]*Departments of Pediatrics and Clinical Chemistry, Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands;* [2]*Departments of General Pediatrics, Immunology and Metabolic Disorders, University Children's Hospital 'Het Wilhelmina Kinderziekenhuis', Utrecht, The Netherlands;* [3]*Department of Pediatrics, Instituto 'G. Gaslini', Genova, Italy;* [4]*Centre Hospitalier Universitaire Paris-Sud-Bicêtre, Laboratoire de Biochemie et Service de Neuropédiatrie, Le Kremlin-Bicêtre, France;* [5]*Kennedy Krieger Institute and John Hopkins University School of Medicine, Baltimore, MD, USA;* [6]*Oregon Health Sciences University, Department of Molecular and Medical Genetics and Biochemical Genetics Laboratory, Portland, OR, USA*

Mevalonic aciduria (MA) and hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS) are two autosomal recessive inherited disorders both caused by a deficient activity of the enzyme mevalonate kinase (MK) resulting from mutations in the encoding *MVK* gene. Thus far, disease-causing mutations only could be detected by analysis of *MVK* cDNA. We now describe the genomic organization of the human *MVK* gene. It is 22 kb long and contains 11 exons of 46 to 837 bp and 10 introns of 379 bp to 4.2 kb. Three intron-exon boundaries were confirmed from natural splice variants, indicating the occurrence of exon skipping. Sequence analysis of 27 HIDS and MA patients confirmed all previously reported genotypes based on cDNA analysis and identified six novel nucleotide substitutions resulting in missense or nonsense mutations, providing new insights in the genotype/phenotype relation between HIDS and MA. *European Journal of Human Genetics* (2001) **9,** 253–259.

Keywords: gene structure; mevalonate kinase; inborn errors; mevalonic aciduria; hyperimmunoglobulinaemia D and periodic fever syndrome; mutations

Introduction

The mevalonate pathway provides cells with isoprenoids that are vital for diverse cellular processes. The main end-products include prenylated proteins, heme A, dolichol, ubiquinone-10, isopentenyl tRNAs and sterols.[1] Currently, six inherited disorders have been described caused by defects in this pathway. Four of these, Smith-Lemli-Opitz syndrome,[2–4] desmosterolosis,[5] X-linked dominant chondrodysplasia punctata[6,7] and CHILD syndrome[8,9] only affect the sterol biosynthesis. The two other disorders, mevalonic aciduria (MA; MIM 251170) and hyperimmunoglobulinaemia D and periodic fever syndrome (HIDS; MIM 260920) are caused by a deficient activity of mevalonate kinase (MK; E.C. 2.7.1.36).[10–12] MK is the first enzyme to follow 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) in the mevalonate pathway and converts mevalonate into 5-phosphomevalonate. In MA, the MK enzyme activity is usually virtually absent when measured in cultured skin

*Correspondence: Hans R Waterham, Departments of Clinical Chemistry and Pediatrics (F0-224), Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands
Tel: +31 20 566 2618; Fax: +31 20 696 2596;
E-mail: h.r.waterham@amc.uva.nl
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fibroblasts of patients.[10] In HIDS, however, a residual MK activity varying from 1 to 7% can be measured both in fibroblasts and leukocytes from patients.[12] As a result of the MK deficiency, excretion of mevalonic acid in urine occurs, although the level of excreted mevalonic acid varies significantly between both syndromes. MA is characterised by a massive excretion (1–56 mol/mol creatinine), while in HIDS the mevalonic aciduria is moderate (0.004–0.028 mol/mol creatinine) and particularly observed during febrile crises.[10,12] In controls, excretion of mevalonic acid in urine is less than 0.001 mol/mol creatinine.

MA is a severe and often fatal multi-systemic disease, characterised by psychomotor retardation, failure to thrive, hepatosplenomegaly, anemia and recurrent febrile episodes.[10] HIDS is a relative benign condition, in which patients suffer, as in MA, from recurrent fever episodes associated with lymphadenopathy, arthralgia, gastrointestinal problems and skin rash.[13] Both syndromes have a recessive mode of inheritance and are rather rare. So far, approximately 20 MA and 150 HIDS patients have been reported.

Both MA and HIDS are caused by mutations in the *MVK* gene encoding MK. In 12 of the 13 HIDS patients reported so far, one particular missense mutation, 1129G>A (V377I), has been found.[11,12] Most patients were compound heterozygotes for this common mutation and a second missense mutation that has been identified in both MA and HIDS patients,[11,12,14–17] strongly suggesting that the 1129G>A mutation is responsible for the HIDS phenotype.

Since the identification of *MVK* as the disease-causing gene in both MA and HIDS most mutations could be detected only at the cDNA level due to the fact that the genomic structure of the *MVK* gene was unknown. Analysis of cDNA isolated from lymphocyte preparations, however, often appeared troublesome probably due to instability of the *MVK* mRNA. Here, we report the genomic organisation of the *MVK* gene, which now enables mutation analysis at the genomic DNA level. In addition, we confirmed our previously reported mutations at the genomic DNA level and identified six novel mutations in the *MVK* open reading frame (ORF).

Materials and methods

Resolution of the genomic structure of human *MVK*

In order to identify the intron-exon boundaries, primer sets were designed on the *MVK* cDNA sequence to amplify potential intron sequences from genomic DNA by PCR. Care was taken that primers did not span potential splice junctions (consensus sequence A/CAG G/AG/T). Each PCR reaction contained 0.4 μM of each primer (synthesized by Pharmacia), 10 mM Tris/HCl pH 8.4, 50 mM KCl, 0.01% w/v BSA, 0.2 mM dNTP (Pharmacia) and 1.5 U Taq DNA polymerase (Promega). Each primer set was tested with 1.0, 1.5 and 2.0 mM $MgCl_2$. Two different PCR programmes were used to amplify specific *MVK* genomic DNA sequences. The 'standard' DNA amplification programme started with 2 min of denaturation at 96°C, followed by 5 cycles of 30 s at 96°C, 30 s at 55°C and 4 min at 72°C, and 25 cycles of 30 s at 94°C, 30 s at 55°C and 4 min at 72°C with a final extension step of 15 min at 72°C. The 'touchdown' PCR programme was used for increased specific primer annealing and started with 2 min of denaturation at 96°C, followed by 18 cycles during which the annealing temperature was lowered with 1°C per cycle from 72°C to 55°C. Every cycle was initiated with 30 s of denaturation at 94°C followed by 30 s of annealing and 4 min of extension at 72°C. These 'touchdown' cycles were followed by 20 cycles with 30 s at 94°C, 30 s at 55°C and 4 min at 72°C with a final extension step of 15 min at 72°C. For amplification of large sequences we also used the Advantage cDNA polymerase mix according to the instructions of the supplier (Clontech). PCR products were sequenced from both ends by means of fluorescent labelled terminators on an ABI 377A automated DNA sequencer according to the manufacturer's protocol (Perkin-Elmer) and subsequently analysed for intron-exon boundaries. Intron sizes were estimated after electrophoresis of the PCR products on standard TBE agarose gels stained with ethidium bromide.

Cloning of *MVK* cDNA

The open reading frame of the *MVK* cDNA was amplified by PCR using either primer set MVK_{10-29} (5′-cg ata gga tcc GAA GTC CTA CTG GTG TCT GC-3′) and $MVK_{1195-1177}$ (5′-cga tag gta CCT CTC AGA GGC CAT CCA G-3′) or primer set MVK_{-21--3} (5′-tgt aaa acg acg gcc agt GCG GCA GGA TTC CCA GGA G-3′) and $MVK_{1238-1219}$ (5′-cag gaa aca gct atg acc ATC CAG AAA GGG GCA TCT GG-3′). The first primer set introduced a 5′ *Bam*HI and 3′ *Kpn*I (underlined) and the second primer set a 5′ -21M13 and 3′ M13rev sequence (lower-case letters). The PCR products were ligated into the pGEM-T vector (Promega) and sequenced to characterise splice variants.

Patients and mutation analysis of *MVK* cDNA

All patients studied, were diagnosed clinically with HIDS or MA and had strongly decreased activities of MK as measured in fibroblasts and/or lymphocytes making use of ^{14}C-labelled mevalonate.[18] Mutation analysis of *MVK* cDNA was performed as described before.[17]

Mutation analysis of human *MVK* on genomic DNA

Genomic DNA was extracted from fibroblasts, lymphocytes or lymphoblasts using the Wizard Genomic DNA Purification Kit according to the instructions of the supplier (Promega). Five sets of *MVK* specific primers with -21M13 or M13rev extensions were used for amplification of the entire *MVK* ORF encoded by exon numbers 2 to 11. The sequences of these primers, the sizes of the resulting PCR fragments and the amplified exons are displayed in Table 1. For PCR amplification a 'touchdown' approach was used, in which the annealing temperature was lowered in 15 cycles with 1°C per cycle from

Table 1 Primer sets used for PCR amplification of coding exons of the *MVK* gene

Primer set	*Exons*	*Size*	*Sequence (5′ > 3′)*
MVK-IVS1$_{-79--55}$			tgtaaaacgacggccagtAACTAGGTGTTCTAAGATCTCTTCC
MVK-IVS3$_{27-48}$	2–3	~1.5 kb	caggaaacagctatgaccCCTCTCTGTAGGCTCTTAGCAC
MVK-IVS3$_{-85--61}$			tgtaaaacgacggccagtCTAGTCGATTTTCTGTGTTCTGTTG
MVK-IVS5$_{50-69}$	4–5	~2.0 kb	caggaaacagctatgaccCAGTGAGAGAAGCCACGTCC
MVK-IVS5$_{-132--112}$			tgtaaaacgacggccagtAGTGGACTTGTTCTTTCTGAG
MVK-IVS7$_{57-79}$	6–7	1023 bp	caggaaacagctatgaccTTCCCAAAGGTGTAGAGCTCTGC
MVK-IVS7$_{-132--110}$			tgtaaaacgacggccagtAATCCAGTGTCACCTCTTGTGCC
MVK-IVS9$_{63-85}$	8–9	840 bp	caggaaacagctatgaccTTCTGAGCACAGCCAGATTGCAG
MVK-IVS9$_{-60--41}$			tgtaaaacgacggccagtTGGGCATAGGACCTTGGCC
MVK$_{1238-1219}$	10–11	~1.6 kb	caggaaacagctatgaccATCCAGAAAGGGGCATCTGG

72°C to 58°C as described above. The PCR fragments were sequenced by means of −21M13 and M13rev fluorescent primers on an ABI 377A automated DNA sequencer according to the manufacturer's protocol (Perkin-Elmer).

Northern blot analysis

Total RNA was extracted from fibroblasts using the RNeasy mini kit according to the instructions of the supplier (Qiagen). After determining the RNA concentration, 15 μg of total RNA was precipitated and used for electrophoresis on a formaldehyde agarose gel as described.[19] The RNA was blotted onto a positively charged nylon membrane (Boehringer Mannheim) and visualised with α-^{32}P-labelled DNA probes. Hybridisation was performed overnight at 65°C in ExpressHyb hybridisation solution (Clontech).

Results

Resolution of the genomic structure of the *MVK* gene

Previously, Schafer *et al*[14] performed Southern blot analysis using a small radiolabelled *MVK* cDNA probe and reported that *MVK* was a single copy gene. Segregation analysis on chromosomal test panel blots and fluorescence *in-situ* hybridisation (FISH) mapped *MVK* on chromosome 12 position q24.[14,20] Using four different cDNA probes together spanning the entire *MVK* ORF we confirmed the existence of only one gene encoding MK (data not shown). In order to identify intron-exon boundaries in the *MVK* gene, primer sets were designed on the *MVK* cDNA sequence to amplify potential intron sequences from genomic DNA. Each primer set was tested under different PCR conditions. Amplified PCR products were sequenced and analysed for intron-exon boundaries. We found 11 exons ranging from 46 to 837 bp, with all intron-exon boundaries following the GT-AG rule (Table 2). Exon 1 encodes most of the 5′-untranslated region (UTR), exon 2 contains the ATG start codon and exon 11 contains the stop codon and the entire 3′-UTR. The introns vary in size from 379 bp to approximately 4.2 kb. The sizes of two introns were determined from the actual nucleotide sequence (intron 6 and 8). All other intron sizes were estimated after gel-electrophoresis. Sequences are available at GenBank (accession numbers AF217527-35).

Splice variants in MVK cDNA

Amplification by PCR of *MVK* cDNA using different primer sets typically yielded not only a fragment of the expected size, but also two smaller specific PCR products (Figure 1). In order to test whether these are *MVK* splice variants, the PCR products were subcloned and sequenced. Both products contained deletions in the *MVK* open reading frame, which introduce frameshifts resulting in truncated proteins. In the first product nucleotides 227 to 371 were deleted, which corresponds to the skipping of exon 4. In the second product nucleotides 227 to 527 were deleted, corresponding to skipping of both exon 4 and exon 5. In the course of our studies two additional rare splice variants were cloned. One of these variants contained an insert of 118 bp at the position of intron 3 in the *MVK* cDNA (GenBank accession no. AF217536). This splice variant is identical to a sequence in the expressed sequence tag (EST) database and contains an Alu repeat (GenBank accession no. AA403187). The mouse EST database also contains two clones with an in frame insertion of 36 bp at the same position in the *MVK* cDNA (GenBank accession nos. AI121069 and AI121148). The second splice variant contained both this 118 bp insert and a 91 bp deletion in the *MVK* open reading frame from position 678 to 768. This deletion corresponds to exon 8.

Mutation analysis of *MVK* at the genomic DNA level

Most previously reported mutations were identified after analysis of *MVK* cDNA. The resolution of the *MVK* gene structure enabled us to perform mutation analysis at the genomic DNA level. To this end the coding exons 2–10 and the coding part of exon 11 are amplified in five fragments using the primer sets displayed in Table 1. Chromosomal DNA was extracted from fibroblasts, lymphocytes or lymphoblasts of 27 patients of whom 17 displayed the HIDS phenotype and 10 the MA phenotype (Table 3). Table 3 includes only data of one affected member per family, in those cases that more members were identified. All 27 patients had strongly decreased activities of MK as measured in fibroblasts and/or

Table 2 Positions and sizes of the exons and introns of the *MVK* gene and sequences of the intron-exon boundaries

Exon no.	*Size (bp)*	*cDNA position*	*Splice acceptor*	*Splice donor*	*Intron size*
1	77	−91-−15		GCGGCGGCAG/gtgagaggcc	~1.3 kb
2	92	−14-78	tccctttag/GATTCCCAGG	ACATGGCAAG/gtacaaagcc	~1.1 kb
3	148	79-226	tattctatag/GTAGCACTGG	AGCTTTCTGG/gtgagtgcaa	~3.5 kb
4	145	227-371	tgtggaacag/AGCAAGGTGA	GGAAGCAGAG/gtgtgtgcgt	~1.5 kb
5	156	372-527	tgtgtttcag/GGCCCTGCCG	GCGTCAACAG/gtaaccatgg	~2.1 kb
6	104	528-631	gtgtcttcag/GTGGACCAAG	AGCACCTGGG/gtaggtgtgg	626 bp
7	46	632-677	ttctctttag/GAGGAGCCCT	CCTTAAAGAG/gtaacctggg	~4.2 kb
8	91	678-768	caccctgcag/GTCGCCAGCT	GCTGCTCAAG/gtgactcttg	379 bp
9	117	769-885	ttttctccag/TTCCCAGAGA	CGTGCTGGAA/gtaagagcct	~3.8 kb
10	154	886-1039	tctcccctag/GAGCTCATTG	CTCAAGCCAG/gtatcccggg	~1.2 kb
11	837	1040-1876	ctccccgcag/GGCTGGAGCA		

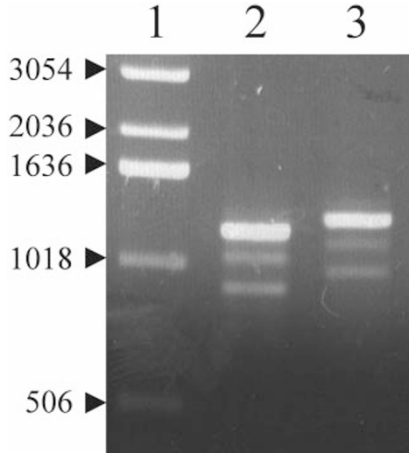

Figure 1 Amplification by PCR of *MVK* cDNA using two different primer sets revealed various splicing variants. Lane 1, DNA molecular weight standard; lane 2, *MVK* cDNA after amplification with primer set MVK_{10-29} and $MVK_{1195-1177}$; lane 3, *MVK* cDNA after amplification with primer set MVK_{-21--3} and $MVK_{1238-1219}$. The expected sizes of the fragments as calculated from the *MVK* cDNA sequence are 1186 and 1259 basepairs, respectively.

lymphocytes (Table 3). Most patients (17) had been sequenced also at the cDNA level and when the then determined genotypes were compared with the genotypes at the genomic DNA level no differences were found. Our analysis identified six novel point mutations, detected in five patients displaying the HIDS phenotype and one patient with the MA phenotype. Five of these mutations were found in patients that were heterozygotes for the novel mutation and the 1129 G>A (V377I) mutation. These mutations include an A>T transversion at nucleotide 37, which changes the lysine at position 13 into a stop codon (K13X), a T>C transition at nucleotide 116, which changes the leucine at position 39 into a proline (L39P), a G>A transition at nucleotide 185, which changes the tryptophan at position 62 into a stop codon (W62X), a C>T transition at nucleotide 404, which changes the serine at position 135 into a leucine (S135L), and a G>C transversion at nucleotide 447, which changes the tyrosine at position 149 into a stop codon (Y149X). The patient carrying the K13X and V377I allele was described previously as a mild MA patient with considerable residual MK activity in lymphoblasts and lymphocytes.[21] The presence of the V377I allele in conjunction with only moderate excretion of mevalonic acid in urine (0.051–0.069 mol/mol creatinine)[21] and the clinical data suggest that this patient in fact is a HIDS patient. The sixth novel mutation was a heterozygous G>T transversion at nucleotide 442, which changes the alanine at position 148 into a threonine (A148T). The HIDS patient carrying this allele was also a heterozygote for the I268T allele, which has been identified in several MA patients.[16,17] The residual MK activity in fibroblasts from this patient (1.6%) suggests that the A148T mutation, like the V377I mutation, is responsible for the HIDS phenotype.

Expression of *MVK* mRNA in fibroblasts of patients with different genotypes

To examine whether one of the identified mutant alleles in *MVK* affect mRNA expression or stability, Northern blot analysis was performed using mRNA isolated from fibroblasts of patients with different genotypes and phenotypes (Figure 2). None of the different genotypes resulted in lower expression levels when compared with mRNA isolated from fibroblasts of control subjects, indicating that the identified point mutations have no effect on *MVK* mRNA expression or stability.

Discussion

Here, we report the genomic organisation of the gene encoding MK. The intron-exon boundaries were identified by amplification of intron sequences from genomic DNA using primer sets designed on the *MVK* cDNA. PCR products were sequenced and analysed for intron-exon boundaries. We found 11 exons ranging in size from 46 to 837 bp, with all intron-exon boundaries following the GT-AG rule. Exon 1 encodes most of the 5′-untranslated region (UTR), exon 2 contains the ATG start codon and exon 11 contains the stop codon and the entire 3′-UTR.

Table 3 Mutations in the *MVK* gene and corresponding MK activities of patients with HIDS or MA

Patient	*Phenotype*[3]	*MK activity*[3]		*Mutation (coding effect)*[3]			
		Fibroblasts	*Lymphocytes*	*Allele 1*		*Allele 2*	
1[1]	HIDS[a]	ND	4.8%[a]	1129 G>A[4]	(V377I)[a]	1129 G>A[4]	(V377I)[a]
2	HIDS[a]	2.6%	1.7%[a]	59 A>C[4]	(H20P)[a]	1129 G>A[4]	(V377I)[a]
3	HIDS[a]	4.4%	2.3%[a]	59 A>C	(H20P)[a]	1129 G>A	(V377I)[a]
4	HIDS	4.0%	2.6%	59 A>C[4]	(H20P)	1129 G>A[4]	(V377I)
5	HIDS	1.5%	1.4%	59 A>C	(H20P)	1129 G>A	(V377I)
6	HIDS	1.9%	1.2%	59 A>C	(H20P)	1129 G>A	(V377I)
7	HIDS[a]	2.3%	4.9%[a]	803 T>C[4]	(I268T)[a]	1129 G>A[4]	(V377I)[a]
8	HIDS	6.9%	4.8%	803 T>C[4]	(I268T)	1129 G>A[4]	(V377I)
9	HIDS	1.5%	6.7%	803 T>C	(I268T)	1129 G>A	(V377I)
10	HIDS	2.9%	3.6%	803 T>C	(I268T)	1129 G>A	(V377I)
11	HIDS	4.2%	ND	803 T>C	(I268T)	1129 G>A	(V377I)
12	HIDS	4.5%	3.4%	116 T>C[4]	(L39P)	1129 G>A[4]	(V377I)
13	HIDS	1.4%	ND	185 G>A[4]	(W62X)	1129 G>A[4]	(V377I)
14	HIDS	ND	1.3%	404 C>T	(S135L)	1129 G>A	(V377I)
15	HIDS	ND	ND	447 C>G	(Y149X)	1129 G>A	(V377I)
16	HIDS	1.6%	ND	442 G>T[4]	(A148T)	803 T>C[4]	(I268T)
17	MA[b]	ND	1.6%[b]	37 A>T	(K13X)	1129 G>A	(V377I)
18	MA[c]	0.3%[c]	ND	59 A>C[4]	(H20P)[c]	1000 G>A[4]	(A334T)[c,e]
19[2]	MA[d]	0.4%	ND	790 C>T[5]	(L264F)[f]	790 C>T[5]	(L264F)[f]
20[1]	MA	0.0%	ND	790 C>T[5]	(L264F)	790 C>T[5]	(L264F)
21	MA[c]	0.2%[c]	ND	803 T>C[5]	(I268T)[c]	803 T>C[5]	(I268T)[c]
22	MA	0.1%	ND	803 T>C[5]	(I268T)	803 T>C[5]	(I268T)
23	MA[c]	0.3%[c]	ND	928 G>A[5]	(V310M)[c]	928 G>A[5]	(V310M)[c]

[1]Two patient sibship.
[2]Three patient sibship.
[3]Previously reported data are indicated by superscript: [a]Houten *et al*[12]; [b]Gibson *et al*[21]; [c]Houten *et al*[17]; [d]Mancini *et al*[22]; [e]Hinson *et al*[15]; [f]Hinson *et al*.[16]
[4]Zygosity confirmed either by sequencing of parental material or independent subcloning of the different cDNAs.
[5]Apparent homozygote but not confirmed in parents.
ND denotes not done.

The introns vary in size from 379 bp to approximately 4.2 kb. The total size of the *MVK* gene is approximately 22 kb.

Exon 1 and 2 have been reported in two previous studies. Bishop *et al*[23] studied the *MVK* promoter region and found consensus sequences for a sterol regulatory element (SRE) and the Sp1 transcription factor (GenBank accession no. AF033345). The presence of an SRE in the *MVK* promoter region suggests a coordinate regulation of genes functioning in sterol biosynthesis. In their study, intron 1 was identified 15 bp upstream of the *MVK* ATG start codon. In addition, Graef *et al*[24] reported the insertion of hepatitis B virus DNA in intron 1 of the *MVK* gene in a human hepatoma cell line. This led to the over-expression of hybrid transcripts (HBmk; GenBank accession no. X75311) arising from an HBV promoter and, as a consequence, the over-production of functionally active MK.

Three intron-exon boundaries also were identified after analysis of *MVK* splice variants. In fibroblast and lymphocyte cDNA, deletions of exon 4 or exon 4 and 5 are rather frequent and sometimes even preferentially amplified in certain lymphocyte cDNA preparations (unpublished data), which greatly hampers cDNA mutation analysis of patient material. This exon skipping is most probably due to the presence of a weak splice acceptor site and/or branch point sequence. At the position of intron 3 also sometimes an insertion containing an Alu repeat was identified. The sequence of this insertion was not observed in the sequenced 5′ and 3′ ends of intron 3, suggesting that it is the result of a cryptic splice site in the unsequenced parts of this intron. All these splice variants encode truncated proteins. In mice, however, an in frame insertion occurs at the same position introducing 12 additional amino acids. Screening of the mouse EST database revealed that 2 of 5 identified EST sequences in this region contained this insert. This indicates that these splice variants could be of functional importance at least in mice. Finally, we also identified an mRNA with a deletion of exon 8, while Graef *et al*[24] identified several clones with a deletion of exon 5. A deletion of exon 5 does not interrupt the reading frame of the gene, but results in an inactive MK protein.[25]

Most *MVK* mutations in patients with MK deficiency have only been determined at the cDNA level. We sequenced 17 HIDS and 10 MA patients at the genomic DNA level. In all patients previously analysed at the cDNA level the genotype could be confirmed. In addition, we identified six novel point mutations in the *MVK* ORF, three of which were nonsense mutations resulting in early stop codons and truncated proteins. The other three missense mutations affect conserved amino acids. The A148T mutation was identified in a HIDS patient together with

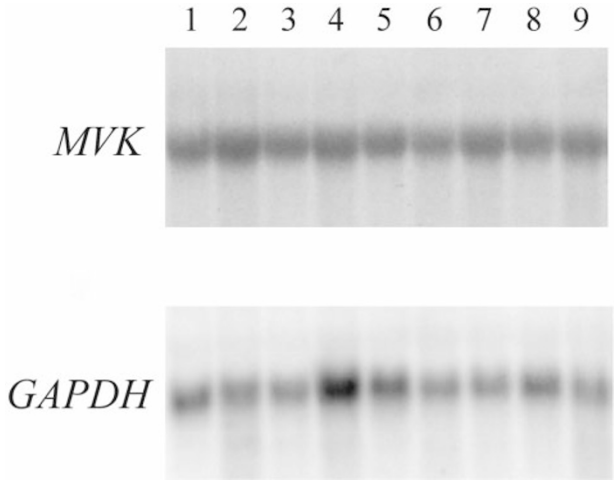

Figure 2 Northern blot analysis of *MVK* transcripts from control, HIDS and MA fibroblasts. A blot containing 15 μg of total fibroblast RNA from each subject was probed with α-^{32}P-labelled human *MVK* cDNA, subsequently stripped and reprobed with α-^{32}P-labelled glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA. Lanes 1 – 3, control subjects; lanes 4 – 9, patient 18, 23, 7, 9, 10 and 13, respectively.

the I268T mutation that has been identified in several MA patients. This suggests that the A148T mutation also leads to the HIDS phenotype as has been shown for the V377I mutation. Thus far, only one other HIDS patient has been described without the V377I mutation. This patient was a heterozygote for a mutation changing the proline at position 165 into a leucine (P165L) and the I268T mutation,[11] which suggests that the P165L mutation also leads to the HIDS phenotype. The alanine at position 148 is part of a spatially conserved motif, which is present in all MK sequences, but also in several other metabolite kinases including galactokinase, homoserine kinase and phosphomevalonate kinase (ERG8)[26] and has the characteristics of an ATP binding site ($GXGXXAX_{(13-26)}K$).[14,26,27] Expression studies in *E. coli* will be necessary to evaluate the effect of this mutation, for example on ATP binding. One patient sibship diagnosed with HIDS was confirmed to be a homozygote for the V377I allele by sequencing of both parental and patient material at the genomic DNA level. This shows that homozygosity for this mild allele also leads to the HIDS phenotype, suggesting a complete penetrance.

The residual MK activity in our HIDS patients varied from 1 to 7%, whereas residual MK activity in almost every MA patient was virtually undetectable ($<0.5\%$). Only one mild MA patient appeared to have considerable MK activity.[21] The fact that this patient had a moderate excretion of mevalonic acid in urine, the clinical characteristics of HIDS and the V377I allele suggests that this patient is a HIDS patient.

So far, only one HIDS patient has been described with a potential splice site mutation.[11] In addition to the 1129 G > A mutation this patient was a heterozygote for a 92 bp deletion in his *MVK* cDNA corresponding to exon 2. The authors' suggestion that this deletion was the result of exon skipping due to a mutation in the splice site, is supported by the elucidation of the genomic organisation as reported in this paper. However, the actual mutation that leads to this skipping remains to be identified.

Northern blot analysis of mRNA from fibroblasts of patients with different genotypes revealed no effect of any of the tested mutations on mRNA stability or expression. This is in accordance with previous studies, which showed that although most mutations result in proteins with reduced enzyme activity when expressed in *E. coli*, they predominantly affect the stability of the MK protein as shown by immunoblot analysis.[12,16,17]

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