Molecular Cloning of Human Mevalonate Kinase and Identification of a Missense Mutation in the Genetic Disease Mevalonic Aciduria*


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Molecular cloning of human mevalonate kinase and identification of a missense mutation in the genetic disease mevalonic aciduria is described.

Cholesterol is an essential component of cellular membranes as well as a precursor for steroid hormones, vitamin D, and bile acids. Regulation of cholesterol biosynthesis occurs principally at the step catalyzed by 3-hydroxy-3-methylglutaryl-CoA reductase. However, other enzymes in the pathway are also involved in regulation of cholesterol biosynthesis. Recent evidence indicates that mevalonate kinase (EC 2.7.1.36; ATP(R)-mevalonate-5-phosphotransferase), the first enzyme in the pathway to follow 3-hydroxy-3-methylglutaryl-CoA reductase, can play an important regulatory role because its activity may be controlled by feedback inhibition (2-5). Geranyl pyrophosphate and farnesyl pyrophosphate (FPP)1 are distal intermediates in the cholesterol biosynthetic pathway that inhibit mevalonate kinase activity by binding competitively at the ATP-binding site on the enzyme (2, 3). This feedback inhibition is thought to function in the regulation of the intracellular concentration of FPP. When intracellular levels of FPP are elevated, feedback inhibition would block mevalonate kinase activity and curtail further FPP synthesis. Regulation of the intracellular concentration of FPP is important because FPP is a key intermediate at a branch point in the pathway. In mammalian cells, FPP is a precursor for the biosynthesis of many vital cellular components such as cholesterol, dolichols, ubiquinones, farnesylated proteins, and heme A (1, 6). Therefore, feedback regulation of mevalonate kinase activity may serve to maintain the size of one or more intracellular pools of FPP that are used by multiple biosynthetic pathways.

A mutation in the gene coding for mevalonate kinase is presumed to cause the genetic disease mevalonic aciduria, which is the first proposed inherited disorder of the cholesterol/isoprene biosynthetic pathway in humans (7, 8). Mevalonic aciduria is transmitted as an autosomal recessive trait, and there are at least seven reported cases of this genetic disease (7, 9, 10). Cells taken from subjects with mevalonic aciduria have <10% of the normal levels of mevalonate kinase activity (9, 11), and all subjects have massive accumulation of mevalonate in both their serum and urine (7, 8, 10). In spite of the marked reduction in mevalonate kinase activity, subjects with mevalonic aciduria have nearly normal levels of serum cholesterol (7, 8). Cells taken from these subjects also appear to synthesize nearly normal amounts of cholesterol (11, 12). These data suggest that either the small residual amount of mevalonate kinase activity in these cells is capable of maintaining normal rates of cholesterol biosynthesis or that a mutation, in the enzyme causes rapid denaturation and loss of enzyme activity when cells are disrupted for enzymatic analysis. The molecu-

1 The abbreviations used are: FPP, farnesyl pyrophosphate; SDS, sodium dodecyl sulfate; bp, base pair(s); kb, kilobase(s); PCRs, polymerase chain reactions.
lar defect causing this genetic disease is still unknown. As a first step in elucidating the molecular basis of this inherited disorder, we have isolated a cDNA clone coding for human mevalonate kinase, and we have used the DNA sequence to identify a mutation in the mevalonate kinase gene from a subject with mevalonic aciduria.

**Experimental Procedures**

**Materials**—A human skin fibroblast Agt11 cDNA library, a human B-lymphocyte Agt11 library, and Escherichia coli Y1090 were purchased from Clontech. A Agt11 cDNA library (MK1T) derived from mRNA purified from fibroblasts of the mevalonic aciduria index case (Z. W.) was prepared by Invitrogen. The plasmid vectors were purchased from the manufacturers as follows: A.S.A.P. genomic DNA isolation kit, Boehringer Mannheim; oligo(T)-cellulose, human genomic DNA, and M13mp18 and M13mp19 sequencing vectors, Pharmacia LKB Biotechnolog Inc.; chromosomal test panel blots and SpeedHyb solution, Bio-Start; Sequenase kit, U. S. Biochemical Corp.; GeneAmp DNA hybridization reagent kit, Perkin-Elmer Cetus Instruments; custom-synthesized gel-purified oligonucleotides, The Midland Certified Reagent Co.; PGM vectors and T7 DNA polymerase sequencing system kits, Promega Biotec; minimal essential medium, Opti-MEM, lipopolysaccharin, and DHA5F² competent cells, Life Technologies Inc., fetal calf serum, HyClone Laboratories; [α-³²P]ATP (7000 Ci/mmol); [α-³²P]dATP (3000 Ci/mmol), and (R)-[5-¹⁴C]mevalonic acid 5-phosphate (58 Ci/mmol), Amersham Corp.; and [³H]mevalonolactone (27.5 Ci/mmol), DuPont-New England Nuclear. The SV40-A2d construct was supplied by Yakov Gluzman (Cold Spring Harbor Laboratory), and the pCMV5 vector was a gift from David W. Russell (University of Texas Southwestern Medical Center, Dallas).

**Cell Lines**—Normal fibroblasts and fibroblasts from the mevalonic aciduria index case (Z. W.) and the proband's family (mother, father, and brother) were transformed with the SV40-A2d construct (13). Cells were grown at 37°C in humidified air containing 5% CO₂ in minimal essential medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Confluent monolayers of cells were rinsed twice with 5 ml of ice-cold phosphate-buffered saline, scraped into 2 ml of ice-cold phosphate-buffered saline, and collected by centrifugation at 1200 x g for 10 min at 4°C. Cell pellets were frozen in dry ice/ethanol and stored at −70°C. COS-7 cells were grown under the same conditions in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin and then twice at 65°C with 2× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate (pH 7.0)) containing 0.1% SDS and washed twice at 0.1× SSC containing 0.1% SDS. Nine cDNA clones were isolated from the human skin fibroblast library, and six cDNA clones were isolated from the B-lymphocyte library. Approximately 1× 10⁶ recombinants from both a human skin fibroblast cDNA library and a B-lymphocyte cDNA library were screened with a full-length rat mevalonate kinase cDNA clone (14) radiolabeled by random priming (15) to a specific activity of 5× 10⁶ cpm/μg. Plaque hybridization was performed as described (16). Plaque hybridization filters were washed twice at 68°C in 1× SSC, 1% SDS, 0.5% SDS, 0.1 M NaH₂PO₄, 0.25 M NaCl, 1 mM EDTA, and 100 μg/ml salmon sperm DNA; and then hybridized overnight at 42°C with a radiolabeled 350-bp EcoRV/BglII DNA fragment (specific activity = 2× 10⁸ cpm/μg) from the human mevalonate kinase cDNA clone. The membrane was washed twice at 65°C in 0.25 M NaHPO₄ (pH 7.2), 0.5% SDS, and 1 mM EDTA; followed by two washes in 0.05 M NaHPO₄ (pH 7.2), 0.5% SDS, and 1 mM EDTA at 65°C. The radiolabeled bands were visualized by autoradiography.

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**DNA Sequencing of cDNA Clones**—The DNA sequence was determined by the dideoxynucleotide chain termination method (16). Sequencing reactions using Sequenase's modified T7 DNA polymerase were performed following the manufacturer's protocol. The DNA and protein sequences were aligned and compared to rat mevalonate kinase cDNA and protein sequences using the Intelligenetics and PCGene computer programs. The Swiss Protein and FDR data bases were also searched for other proteins that show amino acid sequence similarity to human mevalonate kinase.

**Southern Blot Analysis**—Normal human genomic DNA (10 μg) and genomic DNA isolated from fibroblasts from the mevalonic aciduria index case (Z. W.) were digested with restriction endonucleases. DNA fragments were separated by electrophoresis through a 1.0% agarose gel, transferred to nylon membranes, and were autoradiographed. DNA samples prepared from both normal and mevalonic aciduria skin lines. PCR amplification of the DNA region containing the putative mutation was performed as described above. Approximately 200 ng
of the DNA was dried down to a volume of 10 μl, and 1 μl of 5 M NaOH was added to denature each sample. The samples were then spotted onto BioTrans nylon membranes and dried. Duplicate membranes were prepared for hybridization with either the radiolabeled normal or mutant oligonucleotides. The membranes were hybridized for 4 h at 55 °C in 0.9 M NaCl, 0.2 M Tris (pH 8.0), 5 mM EDTA, 0.5% dried milk, and 1% SDS with a 1.3 × 10^6 cpm/ml concentration of either the normal oligonucleotide (5'-ATTGACATGACGACCACT-3') or the mutant oligonucleotide (5'-ATTGACATGACGACCACT-3') radiolabeled at the 5'-terminus with °P. The blots were washed in 1 × SSC, 1% SDS at room temperature to remove background. The wash temperature was then increased to 60 or 65 °C for the normal and mutant probes, respectively. The radiolabeled bands were visualized by autoradiography.

Transfection Analysis—Standard methods were used to construct the normal and mutant mevalonate kinase expression plasmids. Briefly, a 1.3-kb EcoRI/PstI fragment containing the coding sequence of the normal human mevalonate kinase cDNA was inserted into the eukaryotic expression vector pCMV5, a derivative of pCMV4 (18). The mutant expression construct was made by substituting into the normal expression construct a 621-bp EcoRI/PstI fragment from one of the mutant cDNA clones obtained through the cDNA library screening. The sequence of the mutant insert was verified by automated sequencing using fluorescence-labeled primers (19) and a DNA Sequencer (Model 373A, Applied Biosystems, Inc.). Liposome-mediated transfection of COS-7 cells was performed according to the manufacturer. Subconfluent cultures in 100-mm dishes were transfected with 26 μg of the vector alone, the normal mevalonate kinase construct, or the mutant construct for 16 h in 7 ml of serum-free media. Following 40 h of expression in minimal essential medium and 10% fetal calf serum, the cells were harvested in 250 μl of 100 mM KH₂PO₄, 10 mM dithiothreitol, 10 mM KF and lysed by sonication. Cellular debris was removed by centrifugation at 1000 × g for 5 min, and supernatant protein concentrations were determined (20). The supernatant was used to measure mevalonate kinase activity by radioactive assay (21). SDS-polyacrylamide gel electrophoresis and immunoblotting were as described (2) using 300 μg of protein and a polyclonal antibody generated against purified rat mevalonate kinase. The rat mevalonate kinase used as a standard was purified as described (2).

RESULTS

Isolation and DNA Sequence Analysis of cDNA Clone Coding for Human Mevalonate Kinase—Six human mevalonate kinase cDNA clones (pHMK1-pHMK6) were initially isolated after screening 10⁶ recombinants from a human B-lymphobocyte cDNA library with the radiolabeled rat mevalonate kinase cDNA. The sizes of the cDNA inserts in these clones ranged from 3.0 to 1.6 kb. All of the cDNAs were subcloned into sequencing vectors, and the DNA sequence was determined. The resulting DNA sequences were compared to the known nucleotide sequence for rat mevalonate kinase (14); and although there was strong conservation of the DNA sequence, each of the isolated clones contained a deletion ranging in size from 150 to 300 bp within the 5'-coding region. To obtain a full-length cDNA clone, a 1.6-kb cDNA insert from one of these clones was used to screen a human skin fibroblast cDNA library. Nine clones were isolated after screening 1 × 10⁷ recombinants, and the sizes of the cDNA inserts in these clones ranged from 2.0 kb to 500 bp. The DNA sequences of two different 2.0-kb cDNA inserts (pHMK101 and pHMK104) were determined (Fig. 1). The cDNAs contained a 1188-bp open reading frame and coded for a 396-amino acid polypeptide with a predicted M₀ of 42,450. Assuming translational initiation at the first methionine codon, the 689-bp 3'-untranslated region contained a consensus polyadenylation signal (AATAAA), and a poly(A) tail was located 17 bp downstream from this site. One of the 3.0-kb cDNA clones (pHMK4) isolated from the B-lymphocyte cDNA library was also subcloned into a sequencing vector, and the nucleotide sequence was determined. There was no sequence conservation to the human mevalonate kinase cDNA sequence observed for the first 2101 nucleotides until a SacI restriction site was reached. The DNA sequence of the remaining 950 nucleotides from the SacI site to the poly(A) tail of the 3.0-kb clone was identical to the full-length human cDNA sequence from nucleotides 886 to 1972. The presence of a consensus splice site signal immediately 5' of the SacI site suggests that the first 2101 nucleotides are within the intron sequence derived from incomplete splicing of the mRNA (see Fig. 7). The DNA sequence of the intron region was used in later studies to produce primers for PCR amplification.

Analysis of Deduced Amino Acid Sequence of Human Mevalonate Kinase—With a single residue gap inserted in the deduced amino acid sequence of rat mevalonate kinase, there was an 82% homology between the human and rat amino acid sequences (Fig. 1). The deduced molecular weight of human mevalonate kinase (42,450) is only slightly larger than the molecular weight of the rat enzyme (41,990). Based on the hydropathy profile, human mevalonate kinase, like rat mevalonate kinase, is a very hydrophobic protein, with an overall index of hydropathy of +0.06 (22); and hydrophobic amino acids compose 45% of the total amino acids. The consensus sequence for a putative ATP-binding site previously identified in rat mevalonate kinase is also present in the human enzyme (14).

Protein Sequence Similarities—Computer-assisted searches of the Swiss Protein and PIR data bases identified three regions in the protein sequence of human mevalonate kinase that showed strong identity to the amino acid sequences reported for galactokinase (23-26), the yeast RAR1 protein (27), the yeast GAL3 protein (28), and the yeast phospho- mevalonate kinase protein (29). Region A spans 27 amino acids, region B spans 22 amino acids, and region C spans 12 amino acids in length (Fig. 2). Consensus sequences were identified in each region. When compared over the length of the protein, the relative locations of these motifs were also found to be spatially conserved (Fig. 3), suggestive of a close evolutionary linkage for these proteins.

Chromosomal Localization of Mevalonate Kinase—The chromosomal localization of the mevalonate kinase gene was determined by hybridizing a radiolabeled PetI/EcoRI DNA fragment of the human mevalonate kinase cDNA to a Southern blot containing DNA purified from 25 different hamster-human somatic cell hybrids. The PetI/EcoRI DNA fragment that derived from the 3'-untranslated region of the cDNA did not cross-hybridize to the hamster gene homolog. The radiolabeled cDNA hybridized to an ~3-kb DNA fragment on Southern blots from four hamster-human hybrid cell lines. Comparison of the content of human chromosomes in these hybrid cell lines versus cell lines that did not hybridize to the radiolabeled probe indicated that the mevalonate kinase gene is located on human chromosome 12 (Table I). There were no discordant hybrid cell lines.

Southern Hybridization Analysis of Genomic DNAs from Normal and Mevalonic Aciduria Subjects—To determine if the mevalonic aciduria index case has a major rearrangement of the mevalonate kinase gene, genomic DNA was isolated from either normal fibroblasts or fibroblasts from the proband and digested with eight separate restriction endonucleases. Southern blot hybridization studies showed identical restriction fragments in both DNA samples, indicating that the reduced level of mevalonate kinase activity in the index case does not result from a major rearrangement of the mevalonate kinase gene (Fig. 4). Data using a smaller (122-bp) fragment of radiolabeled cDNA for Southern blot analysis also suggests that the genomic DNA coding for mevalonate kinase is a single copy gene (data not shown).
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**Mevalonate Kinase mRNA**—To determine the size and relative levels of mevalonate kinase mRNA, a Northern blot was prepared using poly(A)^+^ RNA isolated from either normal fibroblasts or fibroblasts from the mevalonic aciduria index case. The size of human mevalonate kinase mRNA from normal and mevalonic aciduria samples (Fig. 5, lanes 1 and 2, respectively) is ~2.0 kb, which is identical to the size reported in the normal fibroblast line. However, the relative abundance of mevalonate kinase mRNA was similar in both normal and mevalonic aciduria cells, respectively.

**Isolation and DNA Sequence Analysis of Mevalonate Kinase cDNA Clone from Subject with Mevalonic Aciduria**—A cDNA library was prepared from mRNA purified from fibroblasts from the mevalonic aciduria index case. Twelve cDNA clones (pMK1T1—pMK1T12) were obtained after screening one × 10^6 recombinants with a radiolabeled normal human mevalonate kinase cDNA insert (pHMK101). The cDNA inserts in these clones ranged in size from 880 bp to 2.0 kb. The DNA sequences were aligned with the normal human mevalonate kinase cDNA sequence. Two of the cDNA inserts contained full-length sequence corresponding to the normal mevalonate kinase. However, 4 of the 12 cDNA inserts contained a single base pair substitution in the coding region (nucleotide 902) that replaced an adenine with a cytosine (the remaining clones contained partial sequences only). The effect of this transversion was to change an asparagine residue to a threonine residue, which resulted in the elimination of a predicted β-turn in the secondary structure of the protein (Fig. 6).

**Sequence Analysis of Normal Human Genomic DNA and Fibroblasts from Mevalonic Aciduria Patient**—To confirm the A to C nucleotide substitution and to ensure that its presence in the cDNA clones was not the result of an artifact produced during construction of the MK1T cDNA library, normal human genomic DNA as well as genomic DNA isolated from fibroblasts from the mevalonic aciduria index case were sequenced. The 208-bp region surrounding the putative mutation was amplified by genomic DNA by PCR, and the nucleotide sequence was determined (Fig. 7). The sequence obtained from the mevalonic aciduria genomic DNA confirms the presence of the missense mutation at nucleotide 902 and also demonstrates that the patient is a heterozygote for the allele containing the A to C mutation.

**Identification of Missense Mutation by Allele-specific Oligonucleotide Hybridization**—To determine whether the A to C
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nucleotide substitution was present as a common polymorphism in normal human genomic DNA and whether it was present in other mevalonic aciduria subjects, an allele-specific polymorphism in normal human genomic DNA and whether it was from genomic DNA by the PCR technique, and the product of the amplicon 208-bp region surrounding the mutation was amplified by oligonucleotide hybridization screen was employed. The analysis included the following:

- **Regions A-C**:
  - **Region A**:
    - RAT MEV KINASE
    - HUMAN MEV KINASE
    - E. coli GAL KINASE
    - S. cerevisiae GAL KINASE (GAL 1)
    - K. marxianus GAL KINASE
    - S. cerevisiae RAR1
    - S. cerevisiae P-MEV KINASE

- **Region B**:
  - RAT MEV KINASE
  - HUMAN MEV KINASE
  - E. coli GAL KINASE
  - S. cerevisiae GAL KINASE (GAL 1)
  - Streptomyces sp. GAL KINASE
  - K. marxianus GAL KINASE
  - S. cerevisiae RAR1
  - S. cerevisiae GAL3
  - S. cerevisiae P-MEV KINASE

- **Region C**:
  - RAT MEV KINASE
  - HUMAN MEV KINASE
  - E. coli GAL KINASE
  - S. cerevisiae GAL KINASE (GAL 1)
  - Streptomyces sp. GAL KINASE
  - S. cerevisiae RAR1
  - S. cerevisiae GAL3
  - YPMK

**CONSENSUS**

- **Region A**
  - **RAT MEV KINASE**
    - HUMAN MEV KINASE
    - E. coli GAL KINASE
    - S. cerevisiae GAL KINASE (GAL 1)
    - K. marxianus GAL KINASE
    - S. cerevisiae RAR1
    - S. cerevisiae P-MEV KINASE

**CONSENSUS**

- **Region B**
  - **RAT MEV KINASE**
    - HUMAN MEV KINASE
    - E. coli GAL KINASE
    - S. cerevisiae GAL KINASE (GAL 1)
    - Streptomyces sp. GAL KINASE
    - K. marxianus GAL KINASE
    - S. cerevisiae RAR1
    - S. cerevisiae GAL3
    - S. cerevisiae P-MEV KINASE

**CONSENSUS**

- **Region C**
  - **RAT MEV KINASE**
    - HUMAN MEV KINASE
    - E. coli GAL KINASE
    - S. cerevisiae GAL KINASE (GAL 1)
    - Streptomyces sp. GAL KINASE
    - S. cerevisiae RAR1
    - S. cerevisiae GAL3
    - YPMK

**CONSENSUS**

**Fig. 2.** Protein sequence conservation found in the three regions of human mevalonate kinase. Shown are the three regions of protein sequence homology, regions A-C. Amino acids are shown in single letter code, and the numbers in parentheses represent the positions in the amino acid sequence for each protein. Amino acids present at a given position in 50% or more of the sequences are boxed, and the consensus amino acid sequence is shown below each region. MEV, mevalonate; GAL, galactokinase; P-MEV, phosphomevalonate.

**Fig. 3.** Spatial arrangement of three conserved regions of protein sequence. Regions A-C of conserved protein sequence are represented by solid, hatched, and checkered boxes, respectively. The approximate residue number for each protein is shown at the top. HMK, human mevalonate kinase; RMK, rat mevalonate kinase; E. coli galactokinase; YGK, yeast galactokinase; SGK, Streptomyces galactokinase; RAR1, yeast RAR1 protein; YGAL3, yeast GAL3; YPMK, yeast phosphomevalonate kinase.

The amino acid sequence shown for **Kluyveromyces marxianus** represents only a fragment of the galactokinase.

was spotted onto duplicate BioTrans nylon membranes. The membranes were then hybridized with allele-specific end-labeled oligonucleotide probes to discriminate between the normal sequence or the sequence containing the A to C substitution. As shown in Fig. 8, seven different samples of normal genomic DNA samples were negative for the mutation in addition to genomic DNA samples from four different subjects with mevalonic aciduria. However, hybridization of the oligonucleotide with the mutation to genomic DNAs from the proband (Z. W.) and the proband’s father and brother confirmed the presence of the point mutation in these subjects, whereas the proband’s mother was negative. Hybridization of the oligonucleotide with the normal sequence to genomic DNA from the proband indicated that the index case is a compound heterozygote.

**Transient Expression of Normal and Mutant Mevalonate Kinases**—Although the mutation identified in the patient with mevalonic aciduria displayed mendelian inheritance, verification of this defect as the causative agent required expression analysis. To this end, mutant and normal cDNAs were inserted into the expression vector pCMV5 and expressed transiently in COS-7 cells (18). Fig. 9 shows the typical enzyme activity and protein levels (inset) expressed from the two constructs. Using a polyclonal antibody generated against purified rat mevalonate kinase, immunoblot analysis was performed on equivalent amounts of protein from cell lysates of each transfection. Human mevalonate kinase enzyme activity was determined by a radiochemical assay of the same cell lysates. It is clear that whereas each construct expressed an equal amount of mevalonate kinase protein, the activity associated with the mevalonic aciduria defect was greatly diminished. Control and mock-transfected cells displayed...
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**TABLE I**

Segregation of the human mevalonate kinase gene with human chromosome 12 in hamster-human somatic cell hybrids

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*+, presence of the human chromosome in >45% of the cells; --, absence of the human chromosome.

**+, presence of the mevalonate kinase DNA sequence; --, absence of the mevalonate kinase DNA sequence.

nearly undetectable levels of mevalonate kinase protein and activity. Whereas in this particular experiment the mutant showed a reduction in activity that was ~20% that of the normal, these values varied between 5 and 20% in five separate experiments. Interestingly, in each of these experiments, a variation in the electrophoretic mobility was noted between the normal and mutant enzymes. This is possibly a reflection of the perturbation in protein secondary structure caused by the amino acid substitution.

**DISCUSSION**

The protein sequences of human and rat mevalonate kinases are highly conserved, and there is an 82% sequence identity in the amino acid sequences of both enzymes. Similar to rat mevalonate kinase, the human enzyme displays the motif (G-X-G-X-G-X-G-X-G-X-K, where X represents any amino acid) for the ATP-binding site identified in the amino acid sequence of protein kinases (30, 31). The relative position of this potential ATP-binding site is also conserved in both human and rat mevalonate kinases. Currently, it is unknown whether this putative ATP-binding site on mevalonate kinase is functional.

Mevalonate kinase also displays protein sequence homology to galactokinase and the yeast RAR1 protein. The three regions of amino acid sequence homology (regions A–C) (Figs. 2 and 3) shared by mevalonate kinase and these other proteins as well as the conservation of the relative spatial arrangement of these regions suggest that these genes may belong to a common gene family. Mevalonate kinase and galactokinase have similar characteristics, even though galactokinase (EC 2.7.1.6, ATP:α-D-galactose 1-phosphotransferase) is an enzyme involved in carbohydrate metabolism and catalyzes the phosphorylation of galactose. Both proteins are cytosolic enzymes with similar subunit masses that utilize ATP as a cofactor for phosphorylating low molecular weight substrates (mevalonic acid and galactose). The function of these conserved regions of amino acid sequence is not known, but they may represent canonical sequences for either the catalytic site or the nucleotide-binding site on both enzymes. The yeast RAR1 protein shares the same regions of amino acid sequence homology as described for mevalonate kinase and galactokinase, but the identity of the RAR1 protein is unknown. The RAR1 gene was isolated by screening for mutations that increase the mitotic stability of plasmids whose replication is dependent on weak origins of DNA replication (autonomously replicating sequence elements).
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**Fig. 4.** Southern blot analysis of genomic DNAs from normal and mevalonic aciduria subjects. Genomic DNAs isolated from normal and mevalonic aciduria fibroblasts were digested with the indicated restriction endonucleases. Southern blots were prepared, and the nitrocellulose membranes were hybridized to radiolabeled probes corresponding to the entire coding sequence of the human mevalonate kinase cDNA. The position of molecular size standards are shown to the left.

**Fig. 5.** RNA blot analysis of levels of mevalonate kinase mRNA in normal and mevalonic aciduria fibroblasts. RNA blots were prepared using poly(A)' RNA purified from either normal human fibroblasts (lanes 1 and 2) or fibroblasts from the mevalonic aciduria patient (lanes 3 and 4). The nitrocellulose membranes were hybridized to a 539-bp radiolabeled fragment from the 3'-untranslated region of the full-length human mevalonate kinase cDNA clone, pHMK101 (lanes 1 and 2). The RNA blot was stripped and rehybridized to an actin probe (lanes 3 and 4). The relative positions of molecular size standards are shown to the left. The mevalonate kinase activities in normal and mevalonic aciduria fibroblasts were 19.9 and 0.28 pmol/min/mg of protein, respectively.

Two other proteins that only contain amino acid sequence homology to regions A and B (Fig. 2) are the proteins encoded by the yeast GAL3 gene (28) and the yeast phosphomevalonate kinase gene (29). The amino acid sequence of the GAL3 protein is similar to the sequences of yeast and *E. coli* galactokinases, but the GAL3 protein does not exhibit galactokinase activity (28, 32). Amino acid sequence homology in regions A and B indicates that the GAL3 and phosphomevalonate kinase proteins may be related to the gene family of mevalonate kinase, galactokinase, and the RAR1 protein. For clarity, we propose to name this new family of genes the mevalonate kinase gene family.

Isolation of a cDNA clone coding for human mevalonate kinase allowed us to determine the chromosomal localization of the mevalonate kinase gene and to characterize the mutation in the genetic disease mevalonic aciduria. Results from Southern hybridization analysis using genomic DNA either from normal subjects or from hamster-human hybrid cell lines indicated that mevalonate kinase is a single copy gene located on chromosome 12. The chromosomal locations of only two other enzymes (3-hydroxy-3-methylglutaryl-CoA synthase the pyrophosphorylation of 5-phosphomevalonate to mevalonate 5-pyrophosphate. This product is used in the synthesis of a variety of essential compounds including sterols, dolichols, and ubiquinone. The GAL3 gene is required for rapid induction of the galactose-melibiose regulon genes in *Staphylococcus cerevisiae*. The amino acid sequence of the GAL3 protein is similar to the sequences of yeast and *E. coli* galactokinases, but the GAL3 protein does not exhibit galactokinase activity (28, 32). Amino acid sequence homology in regions A and B indicates that the GAL3 and phosphomevalonate kinase proteins may be related to the gene family of mevalonate kinase, galactokinase, and the RAR1 protein. For clarity, we propose to name this new family of genes the mevalonate kinase gene family.

**Fig. 6.** β-Turn probability determination for normal mevalonate kinase and mevalonic aciduria mevalonate kinase. The deduced amino acid sequences for normal mevalonate kinase and mevalonic aciduria mevalonate kinase were analyzed by the Chou-Fasman algorithm for β-turn probability. Mevalonic aciduria mevalonate kinase differs by a single base pair substitution at nucleotide 902 (residue 301) in the coding region, resulting in an asparagine to threonine substitution.
Identification of a Missense Mutation in Mevalonic Aciduria

Fig. 7. Nucleotide sequence of genomic DNA from patient with mevalonic aciduria. Genomic DNA from either normal human fibroblasts or fibroblasts from a mevalonic (MEV.) aciduria patient (Z. W.) was amplified by PCR, and the nucleotide sequence was determined. Upper, a portion of the autoradiograph is shown for the sequences from bp 899 to 910, reading in a 5' to 3' direction from the top of the gel, of the normal DNA and of the patient's DNA. The heterozygous state of the patient's DNA is shown. The intron-exon junction immediately underlined, letters.

Fig. 8. Identification of mevalonic aciduria mutation by allele-specific hybridization. Genomic DNAs were isolated from control lymphoblasts and fibroblasts as well as fibroblasts from a number of mevalonic aciduria patients, including the patient (Z. W.) and the patient's mother, father, and brother. The region surrounding the A to C nucleotide substitution was amplified by PCR, and the product was spotted onto duplicate filters. The filters were hybridized with either an oligonucleotide corresponding to the normal sequence in the region of the A to C change (normal allele) or to an oligonucleotide from the same region incorporating the A to C change (mevalonic (MEV.) aciduria allele). Lane 1, human mevalonate kinase clone DNA; lanes 2 and 3, purchased normal human genomic DNA; lanes 4-8, normal genomic DNA from control fibroblasts/lymphoblasts; lane 9, patient Z. W.; lane 10, Z. W.'s mother; lane 11, Z. W.'s father; lane 12, Z. W.'s brother; lanes 13-16, four individual mevalonic aciduria patients.

and reductase) in the cholesterol biosynthetic pathway are known, and both genes are located on human chromosome 5 (33-36). In situ hybridization studies are needed to determine the regional mapping of human mevalonate kinase on chromosome 12.

Mevalonic aciduria is postulated to be caused by a mutation in the gene coding for mevalonate kinase. This hypothesis was based on the observation that there is a marked reduction in mevalonate kinase activity in cells from mevalonic aciduria subjects, and there is a large increase in the levels of mevalonate in the serum and urine of these subjects. These data are consistent with having a mutation in the mevalonate kinase gene, but they are not conclusive. By isolating a cDNA clone coding for human mevalonate kinase, we were able to identify a missense mutation in the mevalonate kinase gene from a subject with mevalonic aciduria. The missense mutation changed the protein sequence of mevalonate kinase by replacing an asparagine residue with a threonine. This substitution caused a significant alteration in the predicted secondary structure of the enzyme. Analysis of the protein sequence of mevalonate kinase using the Chou-Fasman algorithm indicated that this amino acid substitution eliminated a β-turn in the secondary structure of the enzyme. The marked effect of this amino acid substitution on protein structure may explain the loss of mevalonate kinase activity associated with this genetic disease. The transfection studies support this conclusion. COS-7 cells transfected with the mutant cDNA construct exhibited a severe reduction in the level of mevalonate kinase activity when compared to the normal expression construct, even though equivalent amounts of protein were produced from the two vectors. Thus, the missense mutation resulted in a substantial inactivation of enzyme activity.

This mutation was identified in the DNA sequences of both a cDNA clone and a PCR-amplified region of genomic DNAs.
Identification of a Missense Mutation in Mevalonic Aciduria

A genetic defect in the proband's mother has not yet been identified. Two full-length cDNA clones isolated from the mevalonic aciduria cDNA library contained the normal coding sequence for mevalonate kinase; and based on results from allele-specific oligonucleotide hybridization, this normal DNA sequence was inherited from the proband's mother. The presence of a normal coding sequence for mevalonate kinase seems incompatible with the marked reduction in mevalonate kinase activity measured in the proband and, to a lesser degree, in the proband's mother. However, both of the cDNA clones with the normal coding sequence for mevalonate kinase did not contain the complete 5′-untranslated region. Therefore, it is possible that a mutation in the 5′-untranslated region of the mevalonate kinase mRNA may inhibit translation. It is unlikely that this allele contains a mutation that alters gene transcription since cells from the proband contained normal levels of mevalonate kinase mRNA (Fig. 5). To date, the molecular basis for the mevalonate kinase deficiency in the proband's mother is not known, and additional studies are needed to determine the complete nucleotide sequence of the 5′-untranslated region for the mevalonate kinase mRNA.

Our study provides the first evidence that a mutation in the gene coding for mevalonate kinase can cause the genetic disease mevalonic aciduria, and this is the first identified mutation in the cholesterol biosynthetic pathway in humans. Moreover, the fact that the missense mutation identified in our study was not detected in the proband's mother as well as four other subjects with mevalonic aciduria indicates that this genetic disease may be caused by different types of mutations in the gene coding for mevalonate kinase.

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Fig. 9. Transient expression of mutant and normal mevalonate kinases in COS-7 cells. Expression constructs containing either normal (normal MK) or mutant (Meu Aciduria, MA) mevalonate kinase were transfected into COS-7 cells and expressed for 40 h. Cell lysates were analyzed for enzyme activity by a radiochemical assay (histogram) and for mevalonate kinase expressional levels by immunoblotting (inset). Equivalent amounts of protein were used from each sample in both the enzyme assay (21 μg) and in each lane of the SDS gel (300 μg). Molecular mass standards (STD) are indicated to the left of the autoradiograph.
Identification of a Missense Mutation in Mevalonic Aciduria