Am. J. Hum. Genet. 58:1205–1211, 1996

Mutations and Phenotype in Isolated Glycerol Kinase Deficiency

Ann P. Walker,[1,*] Françoise Muscatelli,[1] Amanda N. Stafford,[1] Jamel Chelly,[2] Niklas Dahl,[3] Hans K. Blomquist,[4] Joris Delanghe,[5] Patrick J. Willems,[6] Beat Steinmann,[7] and Anthony P. Monaco[1,†]

[1]ICRF Laboratories, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford; [2]Institut Cochin de Genetique Moleculaire, INSERM Unité 129, Paris; [3]Department of Clinical Genetics and Pediatrics, University Hospital, Uppsala; [4]Department of Pediatrics, Umeå University, Umeå, Sweden; [5]Department of Clinical Chemistry, University Hospital, Gent; [6]Department of Medical Genetics, University of Antwerp, Antwerp; and [7]Division of Metabolic and Molecular Diseases, Department of Paediatrics, University Children's Hospital, Zurich

Summary

We demonstrate that isolated glycerol kinase (GK) deficiency in three families results from mutation of the Xp21 GK gene. GK mutations were detected in four patients with widely differing phenotypes. Patient 1 had a splice-site mutation causing premature termination. His general health was good despite absent GK activity, indicating that isolated GK deficiency can be silent. Patient 2 had GK deficiency and a severe phenotype involving psychomotor retardation and growth delay, bone dysplasia, and seizures, similar to the severe phenotype of one of the first described cases of GK deficiency. His younger brother, patient 3, also had GK deficiency, but so far his development has been normal. GK exon 17 was deleted in both brothers, implicating additional factors in causation of the severe phenotype of patient 2. Patient 4 had both GK deficiency with mental retardation and a GK missense mutation (D440V). Possible explanations for the phenotypic variation of these four patients include ascertainment bias; metabolic or environmental stress as a precipitating factor in revealing GK-related changes, as has previously been described in juvenile GK deficiency; and interactions with functional polymorphisms in other genes that alter the effect of GK deficiency on normal development.

Introduction

Glycerol kinase (GK) deficiency has been described in three major clinical forms, with infantile, juvenile, and adult presentation (McCabe 1995). The infantile form results from an Xp21 contiguous gene–deletion syndrome, in which the adrenal hypoplasia congenita (AHC), GK, and sometimes part or all of the Duchenne muscular dystrophy (DMD) locus, are deleted. There is a high frequency of neonatal and infant deaths in these families, resulting from undiagnosed primary adrenal insufficiency. The clinical features include growth and psychomotor retardation, hypogonadotropic hypogonadism (HH), and osteoporosis, plus muscular dystrophy if the DMD locus is deleted (reviewed by Wise et al. 1987; McCabe 1995). A candidate gene for AHC has recently been cloned, and point mutations have been shown to result in both AHC and HH (Muscatelli et al. 1994; Zanaria et al. 1994). A contiguous gene–deletion syndrome causing GK deficiency, mental retardation (MR), and DMD in the absence of adrenal deficiency has been described in two brothers, associated with a syndrome of vomiting, acidosis, and febrile illness (Davies et al. 1988).

Both juvenile and adult GK deficiency are referred to as "isolated GK deficiency," indicating that these patients do not have AHC or DMD. No deletions have been detected previously in isolated GK deficiency when either genomic or GK gene probes have been used (Francke et al. 1987; Walker et al. 1992). Juvenile GK deficiency has been described in four unrelated patients with episodes of serious illness progressing from vomiting and acidemia to stupor and unconsciousness, presenting in the first few years of life. Two patients had abnormal EEGs, and one had grand mal epileptic seizures. Hypotonia and a Reye-like illness may also occur (Eriksson et al. 1983; Ginns et al. 1984; Howell et al. 1989; Bonham and Crawford 1992). The patients were susceptible to acute clinical episodes under conditions that increase lipid mobilization, such as fasting or exercise. These cases indicate that metabolic state and environmental factors may influence the effect of GK deficiency. The clinical episodes of these juvenile patients are similar to those of the GK-DMD contiguous gene–deletion brothers reported by Davies et al (1988).

Adult GK deficiency has been reported in nine affected males from five families (Rose and Haines 1978; Goussault et al. 1982; Pometta et al. 1984; Wirth et al. 1985;

Received March 22, 1995; accepted for publication March 28, 1996.

Address for correspondence and reprints: Dr. Ann P. Walker, Lecturer in Molecular Genetics, Academic Department of Medicine, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, United Kingdom. E-mail: a_walker@rfhsm.ac.uk

* Present address: Academic Department of Medicine, Royal Free Hospital School of Medicine, London.

† Wellcome Trust Centre for Human Genetics, Oxford.

0002-9297/96/5806-0013$02.00

Table 1

Isolated-GK-Deficiency Patients

	Age (years)	Clinical Finding(s)	Serum Glycerol (mM)	Urine Glycerol (mM)	GK (% of Normal)	GK Mutation	RNA Expression
Patient 1	61	Chronic pancreatitis	8.19	210	~0 (leukocyte)	IVS6 G-1→C	Deletion of AG at nucleotides 553–554, frameshift, reduced levels of GK transcripts
Patient 2	7	Retarded growth and psychomotor development, fractures and abnormal teeth, seizures	4.5	283	4 (fibroblast)	Exon 17 deleted	Exon 17 absent, reduced levels of GK transcripts, additional transcript of 4.7 kb
Patient 3 (brother of patient 2)	3	Normal growth and psychomotor and tooth development	7.9	84	4 (fibroblast)	Exon 17 deleted	Exon 17 absent; additional transcript of 4.7 kb
Patient 4	13	MR (IQ = 70)	8.6	93.6	10 (fibroblast)	D440V	D440V, reduced levels of GK transcripts
Normal range	...	...	.08–.23	<.2	100	...	...

NOTE.—Key clinical and biochemical findings for patient 1 are summarized from the report by Delanghe et al. (in press); and those for patients 2 and 3 are summarized from the report by Blomquist et al. (in press).

Delanghe et al., in press). These patients ranged in age from 39 to 76 years and were in good health, indicating that GK deficiency can be benign. Adult GK deficiency may be detected incidentally as pseudohypertriglyceridemia, during routine metabolic assessment. However, one atypical case of isolated GK deficiency has been described with neonatal hypotonia, apnea, and mild developmental delay in the absence of adrenal insufficiency or myopathy (Lewis et al. 1994).

In isolated GK deficiency, no mutations have been reported previously. We describe a unique cohort of four isolated GK-deficiency patients and demonstrate that all these cases of GK deficiency arise from intragenic mutation at the Xp21 GK locus.

Patients and Methods

Patients

Patient 1 was male, of Belgian origin, 61 years of age, and in good health. He was referred because of "refractory hypertriglyceridemia" unresponsive to sustained combined therapy with diet and gemfibrozil. Laboratory investigation revealed hyperglycerolemia and glyceroluria. Leukocyte GK activity was not detectable (table 1). Subsequent ultrasound and laboratory investigations revealed chronic pancreatitis; glucose metabolism, however, was normal. Alcohol abuse and gall stones were excluded as the cause of the pancreatitis (Delanghe et al., in press).

Patient 2 was male, 7 years of age, and of Swedish/Finnish maternal origin. He had psychomotor and growth retardation and sustained multiple fractures from minor trauma, which were not preventable by treatment with vitamin D or phosphate supplements. He had both early loss of abnormal primary teeth and hypophosphatemia, but, unlike the situation in hypophosphatemic vitamin D–resistant rickets, serum alkaline phosphate was normal. At 2 years of age he had generalized seizures, which responded to treatment, although the electroencephalogram results remained pathological. Laboratory investigations revealed hyperglycerolemia, glyceroluria, and fibroblast GK activity at 4% of control levels (table 1). His younger brother, patient 3 (3 years of age), had the same deficiency of fibroblast GK activity, but so far his development has been normal (Blomquist et al., in press).

Patient 4 was male and 13 years of age, with maternal origin in the former Yugoslavia. He was referred at age 7 years, because of MR (IQ = 70). There was no known family history of MR. He was found to have hyperglycerolemia and glyceroluria, as well as decreased fibroblast GK activity (10% of control; table 1).

Patient 5 was included in this study as a control. He has AHC, GK deficiency, and DMD ("infantile GK deficiency") and has an Xp21 deletion, which has been well characterized (Récan et al. 1992; Walker et al. 1992, 1993; Muscatelli et al. 1994).

Nucleic Acid Extraction and Blotting

DNA extraction, Southern and northern blotting, and hybridization were performed by standard techniques (Sambrook et al. 1989). PolyA RNA extraction was performed by using oligo (dT) cellulose type 3, according to the manufacturer's instructions (Becton Dickinson). The northern blot was controlled for RNA loading, by hybridization with an aldolase A cDNA probe (Chelly et al. 1988). Pulsed-field gel electrophoresis was performed after digestion with *Not*I, *Bss*HII, *Sfi*I, *Nru*I, and *Mlu*I, with a resolution of 0.2–1.6 Mb (Ho and Monaco 1995). Cosmid partial digestion restriction mapping was as described by Muscatelli et al. (1994).

Reverse Transcriptase (RT)–PCR and Direct Sequence Analysis

GK was reverse transcribed and amplified from 100 ng lymphoblastoid or fibroblast polyA RNA, by using rTth DNA polymerase (Perkin Elmer Cetus). The entire coding region was amplified by using three overlapping primer pairs, in which the forward (first) primer of each pair was 5′-biotinylated (Hultman et al. 1989): Primers S1B (GCCGGCCGACCTGAAGCTG) and S2 (CACCTCCATTGACTCCTCCTG) gave a 605-bp product. Primers S3B (CTTTTTGGGACTATTGATTCATGG) and S4 (CAATAAGGTGCATATAACCCCG) gave products of 596 or 614 bp. Primers S5B (TACTTCTTATGGCTGCTACTTCG) and S6 (TGGGAATCCATGAGTTGGTAGG) gave products of 541 or 628 bp. The RT-PCR product was gel purified and reamplified in 20–25 cycles of PCR. Direct sequencing was performed as described by Muscatelli et al. (1994).

Allele-Specific Oligonucleotide (ASO) Hybridization

A 750-bp genomic fragment containing exons 15 and 16 was amplified by using primers P34 (intronic; CACCCGAGATTTATTCAG) and P16 (exonic; CTCCGCATTAATCTGAGG). Duplicate dot blots of PCR products were prepared. ASOs (Ikuta et al. 1987)—ASO-N (CTACAAGCAGACATTCTGTA) for the normal sequence and ASO-M (CTACAAGCAGTCATTCTGTA) for the mutation of patient 4—were radiolabeled by using T4 polynucleotide kinase and were hybridized and washed as described elsewhere (Sambrook et al. 1989; Lemna et al. 1990).

Results

Southern Analysis

Southern blot analysis of genomic DNA from patients 1 and 4 revealed normal patterns with cDNA probes spanning the entire GK gene. The brothers, patients 2 and 3, were both demonstrated to have a 5-kb deletion removing exon 17 (fig. 1). This was detected by identification of specific junction fragments with GK probes 1 and 3 and by absence of Xp21-specific hybridization with an exon 17 probe (probe 2). Analysis of genomic DNA by pulsed-field gel electrophoresis and hybridization with GK, DXS708 (JC-1), and DXS1075 (1A-5) probes revealed a normal pattern for patient 2 (not shown).

Northern Analysis

Patient 1 had decreased levels of all three GK transcripts (fig. 2), which were only visible after prolonged exposure. Patients 2 and 4 had reduced GK mRNA transcripts, but patient 3 had normal transcript levels. The brothers, patients 2 and 3, however, had identical GK activity (table 1). The brothers both had an additional transcript of 4.7 kb. No transcript was detected in patient 5, whose deletion removes the Xp21 GK gene.

Mutation Analysis

The cDNA sequence of patient 1 contained a 2-bp deletion of AG at nucleotides 553–554, the first two nucleotides of exon 7. Genomic primers (Sargent et al. 1994) were used to amplify exon 7, revealing the underlying splice-site mutation to be a single nucleotide change, G→C, at intronic nucleotide 553-1, designated "IVS6 G-1→C" (fig. 3 and table 1). The cDNA sequence of the brothers, patients 2 and 3, revealed that exon 16 was spliced directly to the penultimate exon 18, consistent with the mapping data, which revealed a deletion removing exon 17 (fig. 1 and table 1).

For patient 4, a single-base-pair change in exon 15 was found in the cDNA and also was detected in genomic DNA. The mutation identified in this patient was an aspartate-to-valine substitution at codon 440 (GAC→GTC) and is designated "D440V." ASO hybridization (Ikuta et al. 1987) revealed that this mutation was not present in any of the 100 normal X chromosomes tested (fig. 4 and table 1). ASO hybridization also revealed that the mother of patient 4 is heterozygous for the D440V mutation.

Partial cDNA sequence from the Xp21-deletion patient 5 indicated illegitimate transcription from GK homologous loci on chromosome Xq and chromosome 1 and/or from the cDNA 142–like locus on chromosome 4 (Chelly et al. 1989; Sargent et al. 1994). The Xq and chromosome 1 loci are pseudogenes, and it is unlikely that very rare transcripts from the chromosome 4 locus have any functional significance in fibroblasts.

Alternative Splicing of an 18-bp Exon?

Primers S3B/S4 amplified at least two closely spaced bands from lymphoblastoid and fibroblast RNA. The smallest product had normal sequence, and a slightly

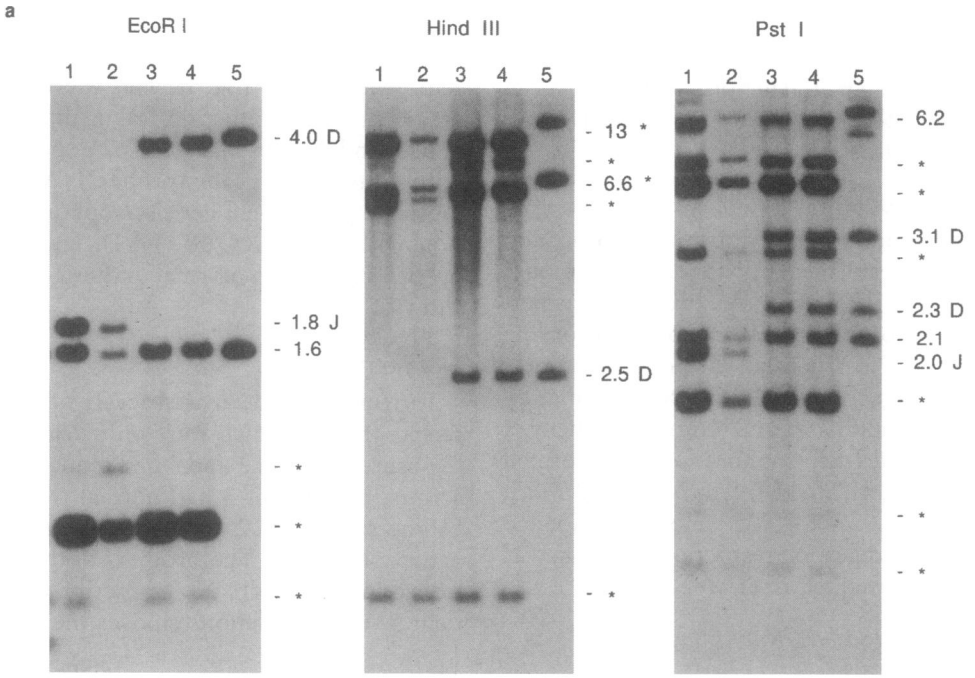

Figure 1 Map of the GK deletion in the brothers, patients 2 and 3. *a,* Hybridization of probe 1, a cDNA fragment containing exons 15–17 and 19, to genomic and YAC DNA digested with *Eco*RI, *Hin*dIII, and *Pst*I. Lane 1, Patient 2. Lane 2, Patient 3. Lanes 3 and 4, Normal male controls. Lane 5, YAC 7072 (ICRFy900A0269; Walker et al. 1992, 1993). Only Xp-specific bands are seen in the YAC, and the size of these (in kb) is indicated at the right of each panel. Additional bands, denoted by an asterisk (*), are due to homologous loci (Sargent et al. 1994). In some cases, an Xp21 band comigrated with a band from the homologous locus. Bands deleted in patients 2 and 3 are marked "D," and junction fragments (J) seen on *Eco*RI and *Pst*I digests are indicated; no junction fragment was seen with *Hin*dIII. Hybridization of probe 2 (exon 17) to the same blot did not detect any Xp21-specific fragments in patients 2 or 3, and probe 3 (exons 15 and 16) detected the same *Eco*RI and *Pst*I junction fragments as did probe 1 (not shown). *b,* Cosmid partial digestion restriction map of the deletion region in normal DNA. Cosmid 22 (Muscatelli et al. 1994) was subjected to partial digestion with three enzymes: *Hin*dIII (H), *Eco*RI (E), and *Pst*I (P). The positions of probes 1–3 (described above) are shown above the map. Restriction-enzyme sites are shown as vertical lines. The positions of GK exons 15–17 and 19 are indicated by solid bars; these were deduced by hybridizations to digests of cosmid, YAC, and genomic DNA and by interexon PCR of genomic DNA. All the mapping and deletion data are consistent with a 5-kb deletion removing exon 17 in both brothers (patients 2 and 3), as shown schematically at the bottom of the figure.

larger product contained the additional 18mer AAAATCTCTCATAGCGTG between exons 8 and 9. Amplification from GK cosmid 21 (Muscatelli et al. 1994) with the forward 18mer sequence and a reverse primer from exon 9 yielded a 1.9-kb product, mapping the 18mer between exons 8 and 9 in genomic DNA.

An 18-bp insertion had been reported at this same position in one cDNA clone from fetal brain; however, it subsequently was thought to be an artifact, because it could not be detected in a YAC clone spanning this region (Sargent et al. 1993, 1994). The 18mer is not present in the *Escherichia coli* or *Bacillus subtilis* GK genes or in known human GK homologues/pseudogenes (Sargent et al. 1994). The sequence of the 18mer, the position of the splice junction between the exons 8 and 9, and the partial splice-donor sequence of exon 8 (Sargent et al. 1994) indicate that the 18mer is not contiguous with either exon 8 or exon 9—and that therefore it does not arise by use of a cryptic splice site. It seems likely that the 18mer is an addi-

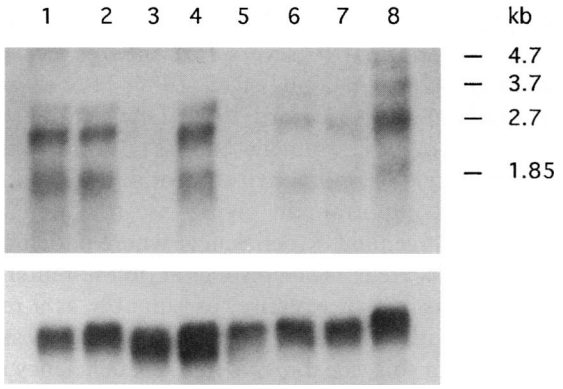

Figure 2 Northern blot hybridized with GK probe 1 (*upper panel*) and aldolase A (*lower panel*). Lanes 1 and 2, Control lymphoblastoid cell lines. Lane 3, Patient 1 lymphoblastoid cell line. Lane 4, MRC-5 control fibroblasts. Lane 5, Patient 5 fibroblasts (Xp21 deletion). Lane 6, Patient 4 fibroblasts. Lane 7, Patient 2 fibroblasts. Lane 8, Patient 3 fibroblasts. No hybridization to RNA from the deletion patient 5 was detected, even after prolonged exposure. After prolonged exposure, all other cell lines show expression of the previously reported 1.85-, 2.7-, and 3.7-kb GK transcripts (Walker et al. 1993), although they were present at decreased levels in patients 1, 2, and 4. The brothers, patients 2 and 3, had an additional 4.7-kb transcript.

tional small alternative exon with limited tissue expression.

Discussion

The mutation found in patient 1 is within the invariant AG dinucleotide of the exon 7 splice-acceptor site (Mount 1982). Mutations at these invariant sites typically abolish or greatly decrease correct splicing (Krawczak et al. 1992). The next available AG, at positions +1 and +2 of the splice-acceptor site, was used as the functional splice site. Exon 7 was thus retained in the transcript, with the loss of two nucleotides, causing frameshift and the first predicted stop codon at nucleotide 607. Premature stop codons are generally associated with significantly decreased transcript levels (McIntosh et al. 1993), consistent with the northern blot results. Despite absence of enzyme activity, patient 1 was in good general health at 61 years of age. Ultrasound and laboratory examinations, however, did demonstrate chronic pancreatitis. Alcohol abuse, gallstones, and hypertriglyceridemia were excluded as the cause of the pancreatitis, which may therefore be related to chronic and sustained hyperglycerolemia (Delanghe et al., in press).

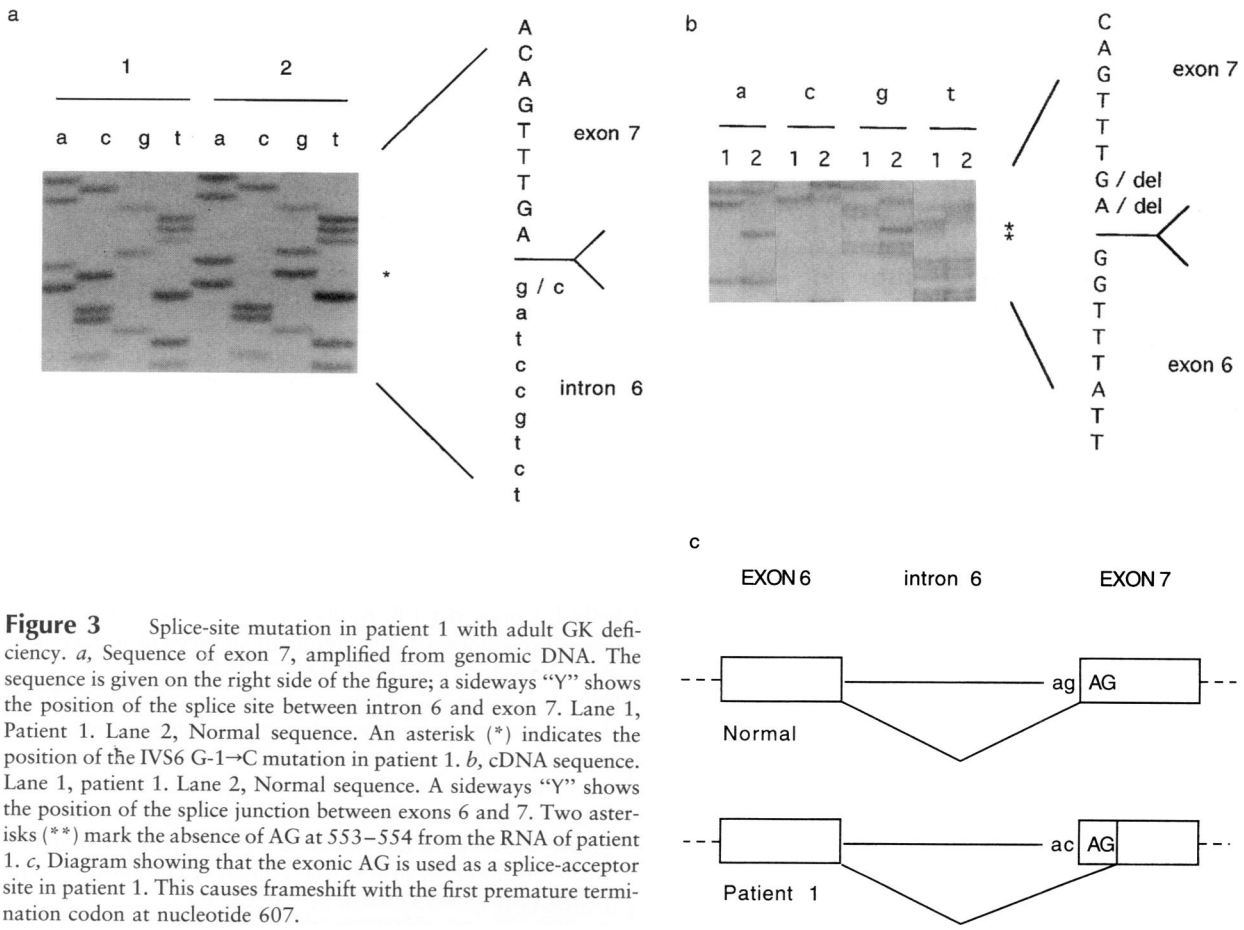

Figure 3 Splice-site mutation in patient 1 with adult GK deficiency. *a*, Sequence of exon 7, amplified from genomic DNA. The sequence is given on the right side of the figure; a sideways "Y" shows the position of the splice site between intron 6 and exon 7. Lane 1, Patient 1. Lane 2, Normal sequence. An asterisk (*) indicates the position of the IVS6 G-1→C mutation in patient 1. *b*, cDNA sequence. Lane 1, patient 1. Lane 2, Normal sequence. A sideways "Y" shows the position of the splice junction between exons 6 and 7. Two asterisks (**) mark the absence of AG at 553–554 from the RNA of patient 1. *c*, Diagram showing that the exonic AG is used as a splice-acceptor site in patient 1. This causes frameshift with the first premature termination codon at nucleotide 607.

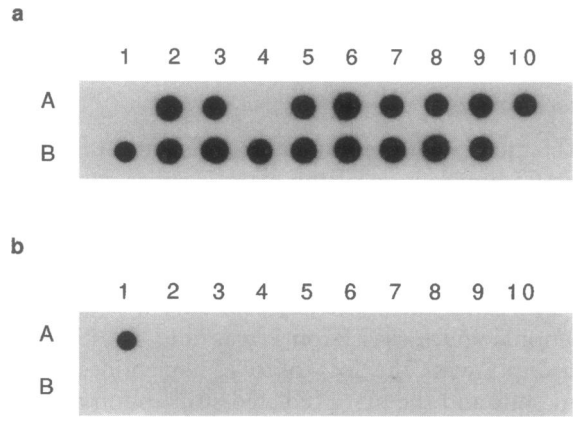

Figure 4 Mutation (D440V) in patient 4. This mutation is not seen in 100 normal X chromosomes. Genomic amplification products containing exons 15 and 16 were used to prepare identical dot blots. *a*, Hybridization with the normal sequence ASO, ASO-N. Dot blots are as follows: A1, patient 4; A2, patient 2; A3, patient 1; A4, patient 5; A5–A10 and B1–B9, unrelated normal females; and B10, no DNA control. An additional 35 normal female samples were also tested (not shown). ASO-N did not hybridize either to products amplified from patient 4 or to products amplified from Xp21-deletion patient 5 (no amplification), but it did hybridize to all 50 normal female samples. *b*, Hybridization with the patient 4 mutation–specific ASO-M. ASO-M hybridized specifically to DNA amplified from patient 4 but not to that of other GK-deficiency patients or to that of any of the 50 normal female individuals tested.

The severe phenotype of patient 2 bears a strong resemblance to that in an earlier report of two brothers with GK deficiency and psychomotor and growth retardation, spasticity, osteoporosis, and pathologic bone fractures; however, unlike patient 2, these brothers also had adrenal insufficiency and myopathy resembling DMD (McCabe et al. 1977; Guggenheim et al. 1980). In that earlier report, the family history of abnormal dental enamel and multiple bone fractures was not consistent with X-linked inheritance (Guggenheim et al. 1980), indicating that GK deficiency was not the cause of the bone disease. In the present study, two brothers had the same mutation, a deletion of exon 17, implicating additional factors in the severe phenotype of patient 2. The possibility that the deletion may predispose to a second Xp21 rearrangement only in patient 2 seems unlikely, on the basis of both the RT-PCR analysis across the GK gene and the normal genomic pulsed-field gel profile.

GK deficiency in patient 4 was detected by laboratory investigations following presentation with MR at age 7 years. His mutation, D440V, predicts substitution of the acidic amino acid aspartate by neutral valine. This aspartate residue is conserved in both *E. coli* and *B. subtilis* (Guo et al. 1993; Walker et al. 1993). It is unlikely that this missense mutation could cause MR directly by a dominant, gain-of-function mechanism, since the patient's mother was heterozygous for the D440V mutation yet was not mentally handicapped.

The phenotype of isolated GK deficiency patients is variable from normal to severe (McCabe et al. 1977; Eriksson et al. 1983; Ginns et al. 1984; Howell et al. 1989; Bonham and Crawford 1992; Lewis et al. 1994; present study). Ascertainment bias may contribute to this variability. Second, environmental or metabolic stress may be important in giving rise to GK-related disease, as in juvenile GK deficiency, where patients are susceptible to acute clinical episodes at times of intercurrent illness, fasting, or exercise, presumably as a result of lipolysis with release of glycerol. A third possible explanation for this variability is that GK deficiency may contribute to phenotype in multifactorial disease. Interaction with functional polymorphisms in other genes may change the effect of GK deficiency on normal development. The effects of sustained hyperglycerolemia during growth and development are not known, and consequences may be variable depending on contributions of both environment and genetic background. Previous deletion analysis of the Xp21 region containing the GK gene has suggested the presence of a gene that may give rise to MR (reviewed in Fries et al. 1995; McCabe 1995). The present report has described mutations in four isolated GK deficiency patients, two of whom have MR. Further analysis will elucidate whether GK deficiency may contribute to this MR.

Acknowledgments

We thank Mac Ho, Alain Hovnanian, Marita Pohlschmidt, Günther Zehetner, Emmanuel Huguet, Kay MacDermot, and Phil Wilkinson for support. This work was supported by the Imperial Cancer Research Fund.

References

Blomquist HK, Dahl N, Gustafsson L, Hellerud C, Holme E, Holmgren G, Matsson L, et al (1996) Glycerol kinase deficiency in two brothers with and without clinical manifestations. Clin Genet (in press)

Bonham JR, Crawford M (1992) The effect of isolated glycerol kinase (EC2.7.1.30) deficiency on glucose homeostasis—a cause for concern in affected patients. Abstract OC8.5. Abstracts of the Vth International Congress Inborn Errors of Metabolism. CRC Press, Boca Raton

Chelly J, Concordet J-P, Kaplan J-C, Kahn A (1989) Illegitimate transcription: transcription of any gene in any cell type. Proc Natl Acad Sci USA 86:2617–2621

Chelly J, Kaplan J-C, Maire P, Gautron S, Kahn A (1988) Transcription of the dystrophin gene in human muscle and non-muscle tissues. Nature 333:858–860

Davies KE, Patterson MN, Kenwrick SJ, Bell MV, Sloan HR, Westman JA, Elsas LJ, et al (1988) Fine mapping of glycerol kinase deficiency and congenital adrenal hypoplasia within Xp21 on the short arm of the human X chromosome. Am J Med Genet 29:557–564

Delanghe J, De Slypere JP, De Buyzere M, Willems P, Walker

A. Pseudohypertriglyceridemia due to benign glycerol kinase deficiency. Eur J Lab Invest (in press)

Eriksson A, Lindstedt S, Ransnäs L, von Wendt L (1983) Deficiency of glycerol kinase (EC2.7.1.30) Clin Chem 29:718–722

Francke U, Harper JF, Darras BT, Cowan JM, McCabe ERB, Kohlschütter A, Seltzer WK, et al (1987) Congenital adrenal hypoplasia, myopathy, and glycerol kinase deficiency: molecular genetic evidence for deletions. Am J Hum Genet 40: 212–227

Fries MH, Lebo RV, Schonberg SA, Golabi M, Seltzer WK, Gitelman SE, Golbus MS (1995) Mental retardation locus in Xp21 chromosome microdeletion. Am J Med Genet 46: 363–368

Ginns EI, Barranger JA, McClean SW, Sliva C, Young R, Schaefer E, Goodman SI, et al (1984) A juvenile form of glycerol kinase deficiency with episodic vomiting, acidemia, and stupor. J Pediatr 104:736–739

Guggenheim MA, McCabe ERB, Roig M, Goodman SI, Lum GM, Bullen WW, Ringel SP (1980) Glycerol kinase deficiency with neuromuscular, skeletal and adrenal abnormalities. Ann Neurol 7:441–449

Guo W, Worley K, Adams V, Mason, J, Sylvester-Jackson D, Zhang Y-H, Towbin JA, et al (1993) Genomic scanning for expressed sequences in Xp21 identifies the glycerol kinase gene. Nat Genet 4:367–372

Guossault Y, Turpin E, Neel D, Dreux C, Chanu B, Bakir R, Rouffy J (1982) 'Pseudohypertriglyceridemia' caused by hyperglycerolemia due to congenital enzyme deficiency. Clin Chim Acta 123:269–274

Ho MF, Monaco AP (1995) PFGE in physical mapping. In: Monaco AP (ed) Pulsed field gel electrophoresis: a practical approach. Oxford University Press, Oxford, pp 21–43

Howell RR, Grier R, Dominguez B, Draehn DK (1989) Neurological manifestations of juvenile glycerol kinase deficiency: improvement with nutritional therapy. Brain Dysfunction 2:126–130

Hultman T, Stahl S, Hornes E, Uhlén M (1989) Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. Nucleic Acids Res 17:4937–4946

Ikuta S, Takagi K, Wallace RB, Itakura K (1987) Dissociation kinetics of 19 base paired oligonucleotide-DNA duplexes containing different single mismatched base pairs. Nucleic Acids Res 15:797–811

Krawczak M, Reiss J, Cooper DN (1992) The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: causes and consequences. Hum Genet 90:41–54

Lemna WK, Feldman GL, Kerem B-S, Fernbach SD, Zevkovitch EP, O'Brien WE, Riordan JR, et al (1990) Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. N Engl J Med 322:291–296

Lewis B, Harbord M, Keenan R, Carey W, Harrison R, Robertson E (1994) Isolated glycerol kinase deficiency in a neonate. J Child Neurol 9:70–73

McCabe ERB (1995) Disorders of glycerol metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 7th ed. McGraw Hill, New York, pp 1631–1652

McCabe ERB, Fennessey PV, Guggenheim MA, Miles BS, Bullen WW, Sceats DJ, Goodman SI (1977) Human glycerol kinase deficiency with hyperglycerolemia and glyceroluria. Biochem Biophys Res Commun 78:1327–1333

McIntosh I, Hamosh A, Dietz HC (1993) Nonsense mutations and diminished mRNA levels. Nat Genet 4:219

Mount SM (1982) A catalogue of splice junction sequences. Nucleic Acids Res 10:459–472

Muscatelli F, Strom TM, Walker AP, Zanaria E, Récan D, Meindl A, Bardoni B, et al (1994) Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia and hypogonadotropic hypogonadism. Nature 372:672–676

Pometta D, Suenram A, von der Weid N, Widmann JJ (1984) Liver glycerokinase deficiency in man with hyperglycerolaemia and hypertriglyceridaemia. Eur J Clin Invest 14:103–106

Récan D, Chafey P, Leturcq F, Hugnot J-P, Vincent N, Tomé F, Collin H, et al (1992) Are cysteine-rich and COOH-terminal domains of dystrophin critical for sarcolemmal localization? J Clin Invest 89:712–716

Rose CI, Haines DSM (1978) Familial hyperglycerolemia. J Clin Invest 61:163–170

Sambrook J, Fritsch EF, Maniatis T (eds) (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sargent CA, Affara NA, Bentley E, Pelmear A, Bailey DMD, Davey P, Dow D, et al (1993) Cloning of the X-linked glycerol kinase deficiency gene and its identification by sequence comparison to the bacillus subtilis homologue. Hum Mol Genet 2:97–106

Sargent CA, Young C, Marsh S, Ferguson-Smith MA, Affara NA (1994) The glycerol kinase gene family: structure of the Xp gene, and related intronless retroposons. Hum Mol Genet 3:1317–1324

Walker AP, Chelly J, Love DR, Ishikawa-Brush Y, Récan D, Chaussain J-L, Oley CA, et al (1992) A YAC contig in Xp21 containing the adrenal hypoplasia congenita and glycerol kinase deficiency genes. Hum Mol Genet 1:579–585

Walker AP, Muscatelli F, Monaco AP (1993) Isolation of the human glycerol kinase gene by positional cloning. Hum Mol Genet 2:107–114

Wirth A, Heuck CC, Bieger W, Schlierf G (1985) Pseudohypertriglyceridamie bei glycerokinase-mangel. Dtsch Med Wochenschr 110:843–847

Wise JE, Matalon R, Morgan AM, McCabe ERB (1987) Phenotypic features of patients with congenital adrenal hypoplasia and glycerol kinase deficiency. Am J Dis Child 141: 744–747

Zanaria E, Muscatelli F, Bardoni B, Strom TM, Guioli S, Guo W, Lalli E, et al (1994) An unusual member of the nuclear hormone receptor superfamily responsible for X-linked adrenal hypoplasia congenita. Nature 372:635–641