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## AN UNEXPECTEDLY HIGH FREQUENCY OF HYPERGALACTOSEMIA IN AN IMMIGRANT BOSNIAN POPULATION REVEALED BY NEWBORN SCREENING

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*Original paper*

*Abstract*

In galactokinase (GALK) deficiency, galactose cannot be phosphorylated into galactose-1-phosphate, which leads to cataract formation. Neonatal screening for hypergalactosemia in Berlin has been performed by thin-layer chromatography since 1978, which detects classical galactosemia and GALK deficiency. Until 1991, GALK deficiency has not been identified in a total of  $\approx 260,000$  samples. In contrast, from 1992 to 1999, nine patients were detected in a total of  $\approx 240,000$  screened newborns. One Turkish patient was homozygous for two novel S142I/G148C GALK mutations in close proximity to the putative ATP-binding site of the enzyme. The other eight children were born to five families belonging to the Bosnian refugee population consisting of approximately 30,000 individuals who have arrived in Berlin since 1991. In two of these families, GALK deficiency was subsequently diagnosed in siblings who had cataract surgery at 4 and 5 y of age, respectively. In all these 10 Bosnian patients, a homozygous P28T mutation located near the active center of the enzyme was identified. We propose that neonatal screening of populations with a significant proportion of Bosnians and possibly other southeastern Europeans, e.g. Romani, should be particularly directed toward GALK deficiency, an inborn error of metabolism that is readily amenable to effective treatment.

**Abbreviations:** GALK, galactokinase

## INTRODUCTION

GALK is the first of three enzymes involved in the conversion of galactose to glucose. It catalyzes the phosphorylation of galactose to galactose-1-phosphate. When infants with homozygous GALK deficiency are fed with milk, galactose accumulates and is converted to galactitol. Within weeks, the deposition of galactitol in the lens leads to cataract formation (1–3), which may also affect heterozygous carriers, albeit at a later age (4–6). Importantly, the development of cataracts can be avoided if a galactose-free diet is initiated early (7). GALK deficiency is inherited in an autosomal recessive fashion and the incidence of the disease has been estimated to be as low as 1:100,000 to 1:1,000,000 in most populations (8, 9). A higher frequency has been suggested in southeastern Europe generally and in Romani in particular (10–12). The human *galk* gene maps to chromosome 17q24 and codes for a protein of 392 amino acids and a molecular mass of 42 kD (13). The gene contains eight exons and spans about 7.3 kb (14). The *galk* gene has also been cloned from bacteria [*Escherichia coli* (15)] and yeast [*Saccharomyces cerevisiae* and *Streptomyces lividans* (16, 17)]. All known galactokinases share three highly conserved regions containing a GALK signature sequence encoded by exon 1 (13, 15), and two separate ATP-binding motifs encoded by exons 3 and 7 (15, 16, 18).

Complementation analyses in yeast suggested a second putative human *galk*

locus that was designated *gk2* (19). The *gk2* gene maps to chromosome 15 and codes for a protein with a 34.6% sequence identity with GALK1. However, only GALK1 exhibits significant enzyme activity in transfected mammalian cells, which indicates that *galk1* represents the only functional human gene (20). In GALK deficiency, 18 different mutations have been described to date.

Most of the reported mutations were private mutations found in single families (13, 21, 22), with the exception of two mutations: the Q382X mutation found in six Costa Rican families (22) and the P28T missense mutation found in a Turkish newborn (22), as well as in five Romani families from Bulgaria, where a founder effect has been suggested (23).

In this report, we describe an unexpectedly high incidence of GALK deficiency in the Berlin neonatal screening program since 1991. One patient of Turkish origin was homozygous for a novel mutant genotype characterized by two distinct base changes at codons 142 and 148, respectively. All other affected neonates were of Bosnian descent and carried the homozygous P28T mutation. Based on these results, we discuss the relevance of neonatal GALK deficiency screening.

## METHODS

Screening for hypergalactosemia is included in the national neonatal screening program. Genotype analysis

was performed as part of the routine diagnostic work-up after written informed consent was obtained from the parents of all probands.

### Screening methods.

Patients with GALK deficiency were identified by neonatal screening that is based on the detection of increased amounts of galactose in dried blood spots by thin-layer chromatography (23).

### Measurements of enzyme activity.

Quantitative estimation of enzymatic activity in erythrocytes of the patients and their parents was performed by monitoring the conversion of <sup>14</sup>C-labeled galactose to (<sup>14</sup>C)-galactose-1-phosphate (24).

### Mutation analysis.

Genomic DNA of the probands, their parents, and normal controls were isolated from peripheral blood. DNA sequencing was performed using biotinylated amplification primers that have been described previously (13). For the separation of the double-stranded PCR products, Dynabeads (Dynal, Lake Success, NY, U.S.A.) were used (25). The presence of the P28T mutation was confirmed by *Ava*I restriction analysis of a 346-bp PCR fragment that was

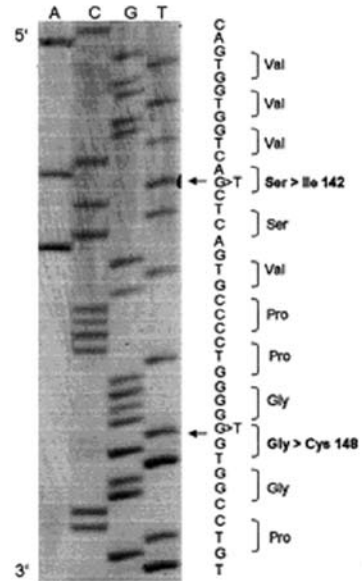
amplified from the genomic DNA. The S142I/G148C double mutation was confirmed by allele-specific oligonucleotide hybridization [S131I: 5'-TGGTGGTCAGCTCAGTGCC-3' (wild type) and 5'-TGGTGGTCA~~T~~CTCAGTGCC-3' (mutation G 392 to T); G137C: 5'-CCCTGGGGGGTGGCCTGT-3' (wild type) and 5'-CCCCTGGGG~~T~~GTGGCCTGT-3'(mutation G409 to T)].

## RESULTS

Newborn screening for hypergalactosemia by thin-layer chromatography has been carried out in Berlin since 1978. Between 1978 and 1990, about 20,000 samples were analyzed annually. Since 1991 (1 y after the reunification of East and West Germany), this annual number increased to approximately 32,000 samples. Up to 1991, GALK deficiency was not detected among 260,000 neonates. In contrast, between 1992 and 1999, increased galactose concentrations were found in 9 of 240,000 newborns (Table 1). The diagnosis of severe GALK deficiency was confirmed by demonstrating absent GALK activity in erythrocytes of the patients. One patient was Turkish and eight were born to 5 non-consanguineous Bosnian families. Two of these families reported a Romani background. All patients were clinically unaffected at the time of diagnosis and a galactose-restricted diet was initiated.

Cataracts did not develop in the next 25–105 mo. Two further unrelated siblings born in Bosnia, where no neonatal screening for hypergalactosemia is performed, had cataract surgery at 4 and 5 y of age, respectively. The diagnosis of GALK deficiency in these siblings was confirmed by measuring enzyme activity in erythrocytes. No clinical evidence for cataract was found in the parents of the affected children and the measurement of GALK activity was consistent with a heterozygous status.

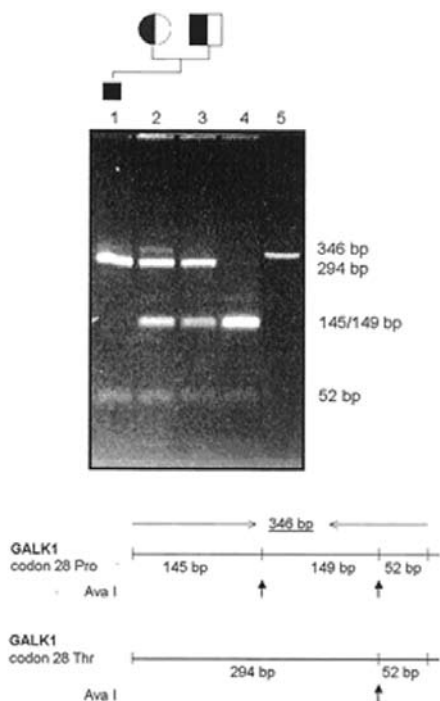
DNA analysis of the GALK-deficient Turkish patient revealed compound heterozygosity for missense mutations at codons 142 (AGC>ATC, serine>isoleucine) and 144 in exon 3 of the *galk1* gene, respectively (GGT>TGT, glycine>cysteine) (Fig. 1). Both parents were heterozygous for this double mutation, which was confirmed by allele-specific oligonucleotide hybridization (not shown). This double mutation was not detected in 20 healthy probands of different ethnic backgrounds.



**Figure 1** DNA sequence analysis of the *galk1* gene in the Turkish patient homozygous for the S142I/G148C double mutation. The arrows indicate the positions of the G>T mutations at positions 425 and 442 of the *galk1* cDNA. Val, valine; Ser, serine; Ile, isoleucine; Pro, proline; Gly, glycine; Cys, cysteine.

**Table 1** Study of the *galk* gene in six families with GALK deficiency detected by newborn screening in Berlin, Germany

Family No	Country of origin	No. patients detected	Siblings with cataracts	Mutation
1	Bosnia	2	-	P28T
2	Bosnia	2	1	P28T
3	Bosnia	2	-	P28T
4	Bosnia	1	-	P28T
5	Bosnia	1	1	P28T
6	Turkey	1	-	S142I, G148C



**Figure 2** *Ava*I restriction analysis of a 346-bp *galk1* PCR fragment encompassing the CCC>ACC (P28T) mutation in a Bosnian family. The PCR fragment carrying the normal sequence [codon 28 proline (Pro)] contains two *Ava*I restriction sites and is digested into a 52-bp fragment and two longer co-migrating fragments of 145 bp and 149 bp. The PCR fragment carrying the mutation [codon 28 threonine (Thr)] has lost one of the two *Ava*I sites and is digested into a 52-bp fragment and a single larger abnormal fragment of 294 bp. The short 52-bp fragment is not affected by the mutation and was used as a control for complete digestion by the enzyme. Lane 1: DNA of the index patient; lanes 2 and 3: maternal and paternal DNAs; lane 4: normal DNA; lane 5: undigested DNA.

DNA analysis of the eight Bosnian patients demonstrated a single CCC>ACC (proline>threonine) point mutation at codon 28 of exon 1 of the *galk1* gene (not shown). This mutation abolished a CCCGAG *Ava*I site and restriction analysis of PCR fragments amplified from genomic DNA could thus be used to confirm the status of the patients and their parents, respectively (Fig. 2).

## DISCUSSION

Since the cloning of the *galk1* gene (13), 18 mutations have been identified in patients with GALK deficiency (12, 13, 21, 22). The functional relevance of the novel S142I/G148C double mutation reported here in a Turkish family is demonstrated by the lack of enzyme activity in the erythrocytes of the affected child and by the intermediate activity in the heterozygous parents. Both mutations are located close to a highly conserved region of the enzyme (Fig. 3), which represents a putative ATP-binding motif (15, 16, 18). It is difficult to differentiate which of the two mutations is functionally more important, but it may be significant that the S142I mutation occurs two amino acid residues upstream of the putative ATP-binding domain, whereas the G148C amino acid change is directly located within the ATP-binding site. Furthermore, in the G148C mutant, the strong helix breaker glycine is substituted for cysteine, a feature that may favor the formation of a  $\beta$ -sheet and

that may also trigger the formation of atypical sulfhydryl bonds. This putative conformational modification may thus reduce the efficiency of ligand binding (26, 27) and abolish enzyme activity.

<i>galk</i> -genes				signature	
amino acids 21-50					
Human	/	<u>REEFGA</u> <u>PEL</u>	<u>AVSAPGRVNL</u>	<u>IGEHTDYN</u> <u>OG</u>	/
<i>E.coli</i>	/	<u>ANAEGYPATH</u>	<u>TIQAPGRVNL</u>	<u>IGEHTDYN</u> <u>DG</u>	/
<i>L.helveticus</i>	/	<u>EATSNKAKD</u>	<u>VFFSEGRINV</u>	<u>IGEHTDYN</u> <u>GG</u>	/
<i>K.lactis</i>	/	<u>DTD.DSRKFF</u>	<u>ITRSPGRVNL</u>	<u>IGEHDY</u> <u>CGP</u>	/
<i>S.lividans</i>	/	<u>CGRRAREPEL</u>	<u>AVSAPGRVNL</u>	<u>IGEHTDYN</u> <u>DG</u>	/

amino acids 130-160				ATP-binding I		
Human	/	A	<u>PLP.GESAVV</u>	<u>VSSVPLGGGL</u>	<u>SSSASLEVAT</u>	/
<i>E.coli</i>	/	N	<u>SFG.GVDMVI</u>	<u>SGNVPGAGL</u>	<u>SSSASLEVAV</u>	/
<i>L.helveticus</i>	/	D	<u>GIDHGENLYI</u>	<u>EANLPSGSL</u>	<u>SSSAIEMLM</u>	/
<i>K.lactis</i>	/	K	<u>GPVHGMEIYV</u>	<u>KGDIPSGGL</u>	<u>SSSAFICAV</u>	/
<i>S.lividans</i>	/	.	<u>..LTGADVHL</u>	<u>ASTVPSGAGL</u>	<u>SSSAALEVRP</u>	/

**Figure 3** Phylogenetic comparison of *galk* genes of different organisms. The figure compares the human galactokinase gene (human) with that of *E. coli*, *Lactobacillus helveticus*, *Kluyveromyces lactis*, and *S. lividans*. Amino acids 21–50 and 130–160 are shown. Phylogenetically conserved amino acids are highlighted. The amino acids affected by the mutations reported here are in bold type and underlined.

The relevance of the P28T mutation in the disease is also confirmed by the absence of enzyme activity in erythrocytes of the probands. This mutation is located just N-terminally to a region that is phylogenetically highly conserved (Fig. 3) and is thought to be the active site of the enzyme (15, 16). The amino-terminal head of this critical segment is composed almost entirely of strong helix formers, suggesting an  $\alpha$ -helical conformation in

this area. At position 28, the helical arrangement is interrupted by a proline. The rigid five-member ring of this amino acid does not fit within a straight stretch of an  $\alpha$ -helix and acts as a strong helix breaker. Because of its bulky shape, proline promotes reverse turns, so-called “hairpin bends” within proteins (28–30). The substitution of threonine for proline at this position is expected to severely disrupt the secondary structure of the N-terminal portion of the enzyme, which probably interferes with the function of the active site in this area. The critical role of this part of the enzyme is further supported by the deleterious effect of a previously described V32M mutation in a homozygous GALK-deficient patient (13). The P28T mutation was identified in all of the nine GALK-deficient Bosnian children. A Romani background was reported by two of the five families. The finding of the same P28T mutation in five Bulgarian Romani families with GALK deficiency supports the view that P28T represents a founder mutation in this population (12). The only Turkish GALK-deficient newborn reported previously also bore the P28T mutation (22).

It is impossible to determine the exact incidence of GALK deficiency in the Bosnian population. A rough estimate can be based on the number of Bosnians ( $\approx 30,000$ ) that arrived in Berlin between 1991 and 1999. Although the ethnic background of refugees is not registered by the German immigration authorities, this group is estimated to include  $\approx 5,000$  Romani. The birth rate is unknown, but

the occurrence of 8 newborns with GALK deficiency in a population of 5,000–30,000 in a period of 9 y suggests that this disorder is relatively common in this group. In comparison, during the same period, 27 patients with phenylketonuria were identified by the neonatal screening of the ethnically mixed Berlin population of 3,500,000 (Mönch E, unpublished data). These figures suggest that GALK deficiency in Bosnians may be about 35 times more frequent than phenylketonuria in the Berlin population. The relatively high incidence of GALK deficiency in Bosnians in general and in Romani in particular is supported by previous reports of GALK deficiency in Romani families from Bavaria, Austria, Switzerland, and Bulgaria (10–12, 31).

Screening for GALK deficiency is technically simple, and the identification of this disorder in the newborn period enables the prevention of cataracts (3). Therefore, it may be warranted to include investigations of GALK deficiency in newborn screening programs for high-risk populations such as those from southeastern Europe. It might be questioned

whether the expense of screening and the adherence to a galactose-free diet is preferential to cataract surgery. However, it must be considered that surgical management of cataracts results in a significant aphakic refractive error and most surgical techniques do not enable subsequent accommodation. There are also possible long-term complications of surgery such as inflammatory changes, secondary membranes, glaucoma, retinal detachment, and changes of the axial length of the eye (32, 33). The patients described here were treated with a galactose-free diet and did not develop cataracts. In conclusion, we propose that GALK deficiency should be included in the neonatal screening program of high-risk populations.

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