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CLINICAL BRIEF

A Case of Classical Galactosemia: Identification and Characterization of 3 Distinct Mutations in Galactose-1-Phosphate Uridyl Transferase (GALT) Gene in a Single Family

Ramandeep Singh · Gurjit Kaur · Babu R. Thapa · Rajendra Prasad

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Abstract Galactosemia is an autosomal recessive disorder of galactose metabolism. In the very first instance of its kind from India, the authors report the presence of three different galatose-1-phosphate uridyl transferase (GALT) gene mutations, associated with galactosemia, in a single Indian family. One of the three mutations, S307X, is a novel mutation (GenBank Accession number GQ355273) and is of nonsense nature causing the truncation of the GALT protein resulting in the decreased enzyme activity. The authors have also emphasized the importance of introduction of new born screening program for galactosemia and its genetic analysis in select settings across the country.

Keywords Galactosemia · Galatose-1-phosphate uridyl transferase (GALT) gene · Mutations · RFLP (restriction fragment length polymorphism) · SSCP (single stranded conformational polymorphism)

R. Singh · R. Prasad (✉)
Department of Biochemistry, Postgraduate Institute of Medical Education and Research (PGIMER),
Chandigarh 160012, India
e-mail: fateh1977@yahoo.com

G. Kaur
Department of Physiology, Government Medical College and Hospital, Sector-32,
Chandigarh 160032, India

B. R. Thapa
Division of Pediatric Gastroenterology, Postgraduate Institute of Medical Education and Research,
Chandigarh 160012, India

Introduction

Galactosemia is an autosomal recessive disorder caused by deficient or absent activities of any of the three enzymes involved in the galactose metabolic pathway. The predominant form is classical type galactosemia, which is due to severe reduction or absence of the GALT enzyme (MIM# 230400, EC 2.7.7.12) and presents as neonatal hepatitis with liver failure [1]. Here, the authors report a unique case of classical galactosemia with presence of three distinct mutations, including a novel one, in human GALT gene associated with galactosemia, in a single family.

Case Report

A 2.0 kg, small for gestational age, female neonate born to a primigravida mother by full term normal vaginal delivery was admitted in the hospital at day 8 of life with persistent jaundice. At 6 weeks of life she developed poor feeding and lethargy which augmented over the next 10 days. She had sun set sign and bilateral cataracts. The abdomen was distended, tense and shiny with dilated visible veins and everted umbilicus. There was hepatosplenomegaly with free fluid in the abdomen. Ultrasound of the head revealed ventriculo-hemispheric ratio of 50% with hydrocephalus. Computed Tomography (CT) scan showed dilated lateral and third ventricles with normal appearing third ventricles suggestive of aqueductal stenosis.

In view of physical examination, neonatal cholestasis syndrome, plausibly galactosemia was considered and the patient was further evaluated. Hemogram showed hemoglobin 7.9 g/dL, total leukocyte count 16.5×10^9/L with

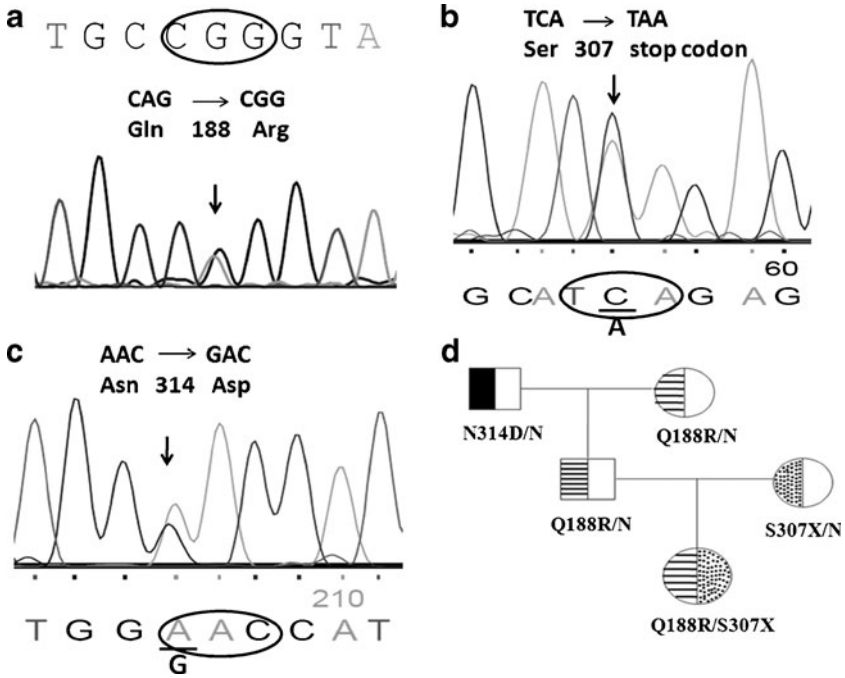

Fig. 1 Electropherogram showing **a** The GALT sequence of Q188R mutation in a heterozygous state in Exon 6 where A is replaced by G; **b** S307X mutation in a heterozygous state in Exon 10 where C is replaced by A; **c** N314D mutation in a heterozygous state in Exon 10 where A is replaced by G; **d** Showing the family pedigree

neutrophilic leucocytosis (61% neutrophils). Liver function tests showed elevated levels of serum aspartate transaminase and alanine transaminase, serum alkaline phosphatase and total and conjugated fraction of serum bilirubin besides hypoalbuminemia.

In consideration of cholestasis, as per the study protocol, the patient was then evaluated for galactosemia. A reduced GALT enzyme activity of 17%, compared to control, was observed. The galactose-1-phosphate level of patient was found to be 20.57 mg/100 ml packed erythrocyte lysate (Normal <1 mg/100 ml packed erythrocyte lysate) [2]. Mutational analysis on genomic DNA isolated from peripheral blood [3] using Polymerase Chain Reaction (PCR) [1], Restriction Fragment Length Polymorphism (RFLP) [1], Single Stranded Conformational Polymorphism (SSCP) [4] and subsequent DNA sequencing revealed the presence of Q188R mutation in exon 6 of the GALT gene of the proband (Fig. 1a). SSCP and subsequent DNA sequencing of exon 10 divulged the proband to carry a novel mutation S307X (Fig. 1b). So, the patient was found to be a compound heterozygote with genotype Q188R/S307X.

The father and mother of proband were found to have 43% and 47% GALT activity of the normal, whereas the paternal grandfather and grandmother were having 52% and 45% of GALT activity, respectively. Mutational analysis of the proband's family, as summarized in Table 1, disclosed her father and paternal grandmother to be carriers for Q188R whilst mother and grandfather were found to be carriers for S307X and N314D respectively (Fig. 1b, c). In effect, a total of 3 different mutations viz. Q188R, N314D and S307X were found to be present in the family (Fig. 1d).

The patient was put on galactose free diet on which she improved and later at the age of 4 months, underwent surgery for aqueductal stenosis.

Discussion

The most common mutations in GALT gene associated with galactosemia are Q188R and N314D and are prevalent in different populations with different frequencies. Q188R is the most common mutation observed in Caucasians [5] and is quite rare in individuals of Indian origin [6], while N314D has a widespread prevalence and is coming out to be the most common mutation in Indian population with a frequency of 38.8%. Whilst, Q188R is associated with a more severe biochemical phenotype [6], N314D is associated with a milder phenotype which is not expressed/

Table 1 Mutations and the corresponding GALT activity observed in proband and the family members

Family members of proband	GALT activity (%)[a]	Mutation	Exon
Proband	17	Q188R/S307X	6/10
Father	43	Q188R	6
Mother	47	S307X	10
Paternal Grandfather	52	N314D	10
Paternal Grandmother	45	Q188R	6

[a] GALT activity is expressed in terms of percentage with comparison to control

symptomatic every time, even when the mutation is present in homozygous state. This can be the reason why family members of the index patient, in whom the mutations were detected, were asymptomatic.

Proband in the present case was a compound heterozygote with the genotype Q188R/S307X. Both these mutations are of different nature. While Q188R is a missense mutation close to the active site of enzyme in exon 6 [7], S307X is a novel nonsense mutation creating a downstream stop codon in exon 10 which could lead to a truncated version of enzyme. Thereby, these mutations are responsible for drastically reduced GALT enzyme activity in the proband which ultimately had the marked impact on the galactose metabolism.

In view of the facts that different genotypes are associated with different biochemical phenotypes and sometimes even do not present with the clinical manifestations, genetic analysis for identification of mutations in the genes involved in the galactose metabolism should be used as screening procedure for the early diagnosis of asymptomatic galactosemia as is already established in developed countries.

Conclusions

Specific investigations and genetic analysis of the index patients and their families is necessary for early detection of galactosemia and counseling of the family members.

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Contributions RS; Carried out the genetic analysis and prepared the manuscript, GK; Estimation of enzyme activity, BRT; Clinical evaluation of the case, RP; Designed and conceptualized the study.

Conflict of Interest None.

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