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Biochemical and molecular characterization of GALT gene from Indian galactosemia patients: Identification of 10 novel mutations and their structural and functional implications

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ABSTRACT

Classical Galactosemia is an autosomal recessive disorder of galactose metabolism caused by severe reduction or absence of the galactose-1-phosphate uridyl transferase (GALT) enzyme. Till date, no reports are available on clinical and molecular spectrum of galactosemia from Indian population. The characterization of underlying GALT gene lesions was performed in 55 unrelated galactosemia patients. The GALT mutational spectrum comprised 16 distinct mutations including 10 previously unreported mutations. N314D was the most common mutation with a frequency of 40% followed by Q188R at 2.7%. The novel GALT gene mutations included 6 missense mutations *viz.* Y89H, Q103R, P166A, S181F, K285R, R333L; one nonsense mutation, S307X and 3 silent mutations — Q103Q, K210K and H319H. The functional significance of the novel GALT missense mutations was investigated using SNPs&GO and SIFT tools. Further, modeling studies using 3D models of mutant and wild type GALT proteins revealed mutations to exert their effects at the molecular level by altering H-bonds, salt bridges, secondary structure or surface features. The study highlighted the heterogeneity of classical galactosemia in the Indian population and also emphasizes the importance of GALT gene analysis in diagnosis of galactosemia. It also revealed that the Indian GALT mutational profile differs significantly from other populations studied.

1. Introduction

Classical Galactosemia (MIM# 230400) is an autosomal recessive disorder of galactose metabolism caused by mutations in the galactose-1-phosphate uridyl transferase gene (GALT; MIM# 606999). The incidence of this disorder is 1 in 30,000–60,000 but is very heterogenous among populations [1]. The human GALT gene is located on chromosome 9p13 spanning 4.3 kb of DNA arranged in 11 exons [2]. More than 230 sequence variations in the GALT gene have been reported of which the most prevalent among Caucasian populations is Q188R [3]. Classical galactosemia is also characterized by a high allelic heterogeneity, with a typical distribution of mutations among several populations and ethnic groups [4]. Loss of GALT enzyme (EC 2.7.7.12) activity results in the accumulation of galactose-1-phosphate in various organs leading to clinical manifestations like vomiting, diarrhea, weight loss, lethargy, hypotonia, jaundice, hepatomegaly, E. coli sepsis, cataracts, bleeding tendencies and liver failure during neonatal period and early infancy [5].

In contrast to the western countries, where galactosemia is routinely included in all newborn screening programs, this is not the case in India. Till date, there is no documentation of galactosemia from India. This report details our own mutation database of the GALT gene in the Indian population for the first time with identification and characterization of 16 different mutations including 10 novel mutations. Further, we also predicted the effect of novel mutations on structure and function of the GALT enzyme using different computer based applications.

2. Material and methods

2.1. Ethical clearance

The study was approved by the Ethics Committee of the Post Graduate Institute of Medical Education and Research, Chandigarh. An information sheet was provided to each patient/family member and written informed consent was obtained.

2.2. Patients and clinical evaluation

A total of 450 infants with cholestasis who attended the Pediatric Liver Clinic of Pediatric Gastroenterology ward of Postgraduate Institute of Medical Education and Research (PGIMER) were screened for

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GALT activity. Work up for cholestasis included ultrasound of abdomen, hemogram, liver function tests, PTI/INR, blood and urine culture, intrauterine infections work up (toxoplasma, rubella, CMV, syphilis, herpes) and urine succinyl acetone when indicated. Blood samples for GALT enzyme assay were taken on Neonatal Screening cards (Whatman Filter Paper 903). Blood samples for genetic analysis were collected in Acid Citrate Dextrose (ACD) vials from the patients who showed GALT activity ≤50%. After priming with ursodeoxycholic acid (UDCA) (30 mg/kg/day), mebrofenin scan was done to rule out biliary atresia when stool was clay colored or ultrasound of abdomen showed absent gall bladder or distended gall bladder but no post prandial contraction or triangular card sign was positive. Laprotomy was done once there was suggestion of biliary atresia. Liver biopsy was done when it was indicated. Age matched healthy subjects with no signs and symptoms of disease were included as controls. Infants with unconjugated hyperbilirubinemia and known etiology of cholestasis were not included in the study.

2.3. Red blood cell GALT (galactose-1-phosphate uridyl transferase) assay

GALT enzyme activity was measured using a Perkin–Elmer neonatal GALT kit (Perkin Elmer Wallac Victor 2D fluorometer, Finland) which measured the uridylphosphoglucose (UDPG) consumption on samples of dried blood eluted from the filter. The assay is an adaptation of the Beutler and Baluda procedure [6]. It is based on the enhancement of the fluorescence of nicotinamide adenine dinucleotide phosphate (NADPH) through a series of enzymatic reactions. GALT enzyme activity was calculated and expressed as units/g Hb (normal value > 3.5 units/g Hb).

2.4. Red blood cell galactose-1-phosphate assay

Quantitative estimation of galactose-1-phosphate was carried out in deproteinised hemolysates of patients and control subjects using the procedure of Kirkman and Maxwell [7]. An enzymatic reaction involved the conversion of galatose-1-phosphate in the presence of uridine diphosphate glucose (UDPG) into glucose-1-phosphate and uridine diphosphate galactose (UDPGal). Residual UDPG was measured as NADH production by measuring absorbance increase at 340 nm (Normal range: <1 mg Glactose-1-Phosphate/100 ml packed erythrocyte lysate).

2.5. Genetic analysis

Genomic DNA was isolated from whole blood by method of Daly et al. [8]. For GALT gene mutation analysis, chromosomes were first screened for the presence of most common mutations — c.563A > G (p.Q188R) in exon 6 and c.940A > G (p.N314D) in exon 10 using polymerase chain reaction (PCR) based restriction fragment length polymorphism technique (RFLP) [9]. For unknown/novel mutations in GALT gene, all the 11 exons and exon–intron boundaries were PCR amplified using specific set of primers [9] and were then subjected to single stranded conformational polymorphism (SSCP) analysis [10]. Normal controls were used in each run to prevent over interpretation of SSCP patterns as abnormal. The patients' samples exhibiting shifts relative to normal samples on SSCP were subjected to automated DNA sequencing using an ABI Prism BigDye Terminator Sequencing Ready Reaction Kit (Perkin Elmer, USA) and a DNA sequencer ABI Genetic Analyzer 3130.

2.6. Pathological predictions of novel substitution mutations

Pathological prediction of mutations was made using SNPs&GO analysis (Single nucleotide polymorphisms and Gene Ontology; http://snps-and-go.biocomp.unibo.it/snps-and-go/). SNPs&GO has been proposed to be an accurate method based on support vector machines, to predict disease related mutations from the protein sequence, scoring with accuracy = 82% and Matthews correlation coefficient = 0.63 [11]. SNPs&GO collects in an unique framework information derived from protein sequence, protein sequence profile, and protein function. The output page reports the mutations as disease related or non-disease related on a reliability index scoring from 0 (unreliable) to 10 (reliable). Pathological predictions were further confirmed by another computer based application - SIFT (Sorting Intolerant from Tolerant) [12]. The threshold value for pathological mutations was ≤0.05 (http://blocks.fhcrc.org/sift/SIFT.html). The results of SIFT analysis have been documented in Supplementary Table 1. Human Splicing Finder matrix (http://www.umd.be/HSF/) was used to predict any difference in splicing between the novel mutants and their respective wild-type reference sequences.

2.7. Analysis of effects of novel missense mutations on GALT protein

The characterized mutations were also analyzed for their effect on the structure of GALT enzyme. The mutant models of human GALT enzyme were used as the starting point to simulate the effect of mutations which were prepared using a program MODELLER and were provided by Facchiano and Marabotti, Institute of Food Science, National Council of the Researches, Avellino, Italy [13]. This facility was applicable only to the missense mutations (http://bioinformatica.isa.cnr.it/GALT).

2.8. Statistical analysis

Statistical analysis was performed using Chi Square test and Student's unpaired t test. Pearson correlation coefficient analysis was used for correlation analysis between the GALT activity and galactose-1-phosphate levels. P value of ≤0.05 was considered significant. All the values are mean ± SD. SPSS-statistical software (SPSS, version 16.0) was used for data analysis.

3. Results

Out of 450 infants having cholestasis, 55 (12.2%) patients showed a GALT enzyme activity ≤50%. These patients were then subjected to further biochemical and genetic analysis. The cut off for galactosemia was set as GALT enzyme activity ≤50% so as not to miss the patients harboring the milder mutations like N314D in which the enzyme activity get reduced to 50% of the control even when present in homozygous state [14].

3.1. Demographics

Average age at diagnosis in galactosemia subjects was found to be 2.1 ± 5.6 (SD) months. However, the range of age of presentation was from 2 days to 11 months. Male to female ratio was 37:18. Majority of the patients were from north-western states of India. History of previous sib death due to liver problems was seen in 3 families.

3.2. Clinical findings

The clinical findings at the time of diagnosis in galactosemia patients were — jaundice (100%), hepatomegaly (80%), splenomegaly (51%), coagulopathy (22%), encephalopathy (14%), cataracts (32%) and sepsis (14%) while 3 patients died during the hospital course. A significantly decreased GALT activity of 20.1% ± 7.9 was observed in galactosemia patients as compared to 87.2% ± 10.4 in controls (Fig. 1). Levels of galactose-1-phosphate were also considerably elevated in the galctosemic subjects (17.24 ± 5.13 mg/100 ml packed erythrocyte lysate) in comparison to the controls (1.14 ± 0.19 mg/100 ml packed erythrocyte lysate) (Fig. 2). Also, a significant

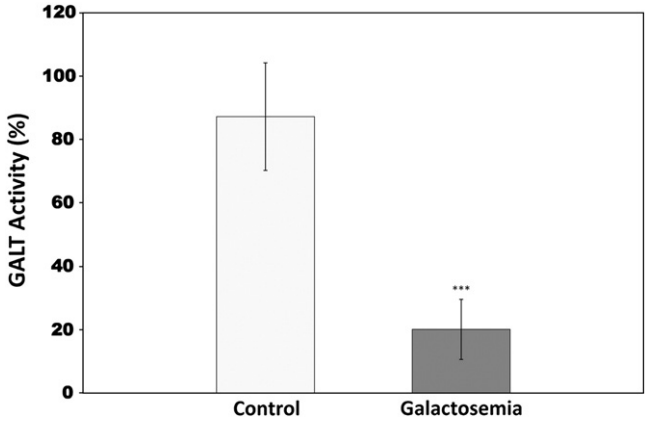

Fig. 1. Comparison of blood GALT activity between galactosemia subjects and controls. The data are expressed as proportional enzyme activity ±SD. Statistical analysis was done by means of Chi Square test. *** P<0.01 as compared to normal controls.

negative correlation (r = −0.74) was observed between GALT activity and galactose-1-phosphate levels (Fig. 3).

3.3. Identification and characterization of mutations in the GALT gene

Taken together, 16 different mutations in GALT gene were identified in 55 Indian Galactosemia patients (Table 1). N314D was found to be the most common mutation in our population with a frequency of about 40% (44 alleles) followed by Q188R at 2.7% (3 alleles). Remaining 63 GALT chromosomes were analyzed for the presence of novel/unknown mutations using RFLP, SSCP and subsequent DNA sequencing as result of which 14 different mutations were detected. Out of these 4 have been previously reported- F171S, P185L, L218L and H319Q; while 10 were the novel mutations (Table 1). These included 6 missense mutations *viz.* Y89H, Q103R, P166A, S181F, K285R, R333L; one nonsense mutation, S307X and 3 silent mutations — Q103Q, K210K and H319H.

Each novel mutation was identified in one patient only and none was found in the 55 control subjects from the population. All the novel mutations have been registered with the GenBank, National Center for Biotechnology Information (NCBI) with specific accession numbers and are freely available at http://www.ncbi.nlm.nih.gov/GenBank (Table 1). The mutations have also been submitted to the

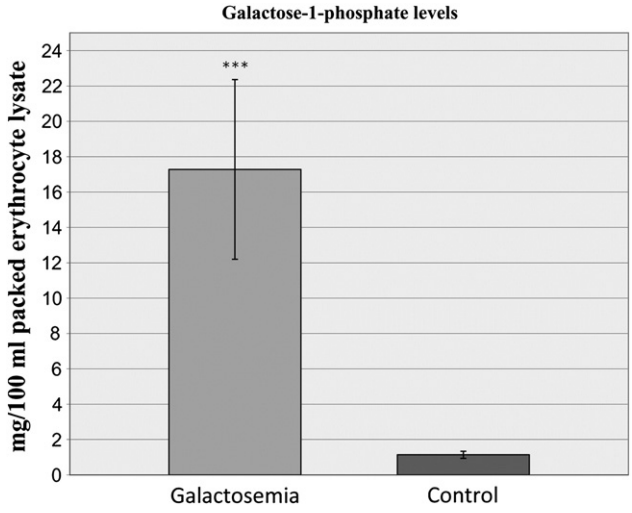

Fig. 2. Comparison of blood galactose-1-phosphate levels in galactosemia subjects and controls. The data are expressed as mean ± SD. Statistical analysis was done by means of unpaired Student's t-test. *** P<0.01 as compared to normal controls.

GALT mutation database, ARUP Online Scientific Resource (http://arup.utah.edu/database/galactosemia/GALT_welcome.php) maintained by Associated Regional and University Pathologists (ARUP) affiliated to University of Utah, USA. Mutations on both alleles were identified in 18 patients and on one allele in 31 patients. Both alleles remained unidentified in 6 subjects. The average GALT activity and levels of galactose-1-phosphate associated with different mutations/genotypes is shown in Table 2.

3.4. Novel missense/nonsense mutations and phenotypic features

SNPs&GO analysis was carried out for the pathological predictions of mutations besides determining their effects on the structure of GALT enzyme by its using computer simulated 3D models (Tables 3, 4). SNPs&GO is one of the best scoring classifiers available for predicting whether a mutation at the protein level is or is not disease-related. This facility was applicable only to the missense mutations. Results of SNPs&GO analysis were further confirmed by SIFT analysis (Supplementary Table 1).

3.4.1. Y89H (GenBank ID: HQ637434)

This mutation was present in heterozygous state in a one month old female child with persistent jaundice. The baby had distended abdomen with umbilical hernia. There was hepatomegaly, sepsis, hypoalbuminemia, coagulopathy, bilateral retinal hemorrhages and occult blood in stool. GALT activity was 15%. The second allele harbored N314D mutation. Y89H was predicted to be a disease related mutation by SNPs&GO analysis with a score of 3 on reliability index (RI) (Table 3). SIFT analysis also forecasted Y89H to be a deleterious mutation with an output prediction score of 0.00 (threshold ≤ 0.05) (Supplementary Table 1). On structural analysis using 3D models of mutant and wild type GALT proteins, it was found that substitution of tyrosine to histidine had probably modified the solvent accessibility and the hydrogen bond network of GALT enzyme thereby affecting the GALT activity (Table 4).

3.4.2. Q103R (GenBank ID: GU129564)

It was identified in a 1 month old male child in a homozygous state with cholestasis, hepatomegaly and bilateral cataracts. There was a late onset of neonatal *Klebsiella pneumoniae* sepsis. GALT activity was 20%. SNPs&GO analysis revealed Q103R to be a non-disease associated variation with a score of 9 on reliability index (Table 3). SIFT analysis, too, predicted Q103R to be a tolerable change (Supplementary Table 1). However, modeling studies predicted Q103R to affect the secondary structure and the solvent accessibility of enzyme to a significant extent besides disturbing the inter-subunit relationships (Table 4).

3.4.3. P166A (GenBank ID: GQ403671)

This mutation was found on one allele in a 2.5 months old male child alongwith a novel silent mutation H319H on another allele (GenBank ID: GQ403669). GALT activity was 21%. The child developed jaundice at day 12 of life and presented with hepatomegaly, thrombocytopenia and pneumonia. Blood culture was positive for *Klebsiella pneumoniae*. P166A mutation was predicted to be neutral or non-disease associate change by SNPs&GO with a RI score of 8 (Table 3). This prediction was further strengthened by SIFT analysis results (Supplementary Table 1). Further, structural analysis of mutant and wild type GALT proteins also did not highlight any significant difference (Table 4).

3.4.4. S181F (GenBank ID HQ412987)

The 1 month old male child was homozygous for S181F genotype and presented with icterus since day 1 and abdominal distension since day 5 of life. Ultrasound revealed hepatosplenomegaly. GALT activity was 10% of the normal. There were repeated seizures, failure to

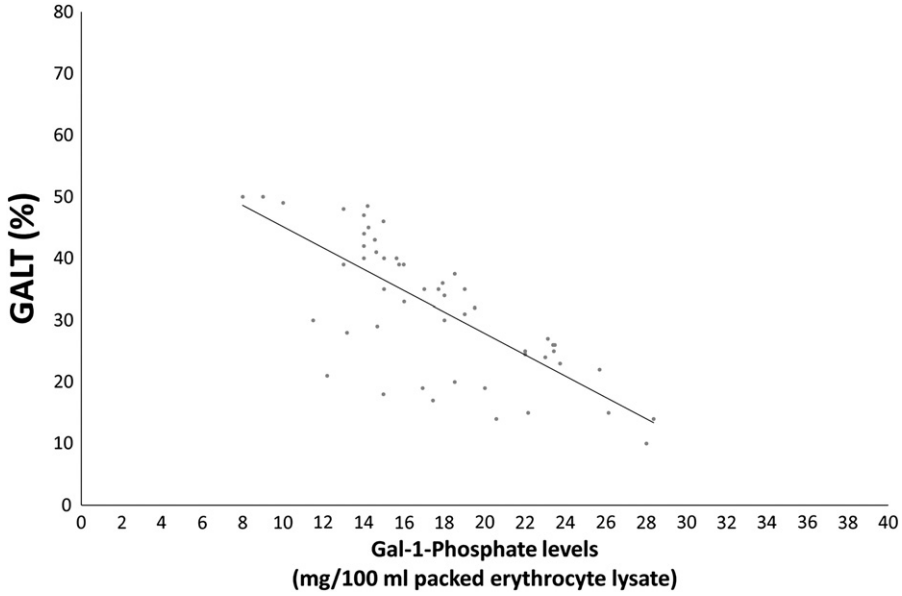

Fig. 3. Correlation between GALT activity and galactose-1-phosphate levels in galactosemia subjects. Correlation significant at P<0.01 level.

thrive with hypoglycemia and coagulopathy. Child succumbed to acute liver failure and sepsis. S181F was predicted to be a disease causing mutation with a high RI score of 7 (Table 3). Further, the mutation delivered a score of 0.00 on SIFT depicting it to be a deleterious variation and corroborating the SNPs&GO results (Table 3, Supplementary Table 1). The modeling studies predicted serine at position 181 to be involved in the inter subunit H-bond formation of GALT protein. Consequently change of serine to phenylalanine had probably modified the inter-subunit relationship. S181F was also predicted to affect the substrate binding and catalysis thus accounting for a decreased GALT activity (Table 4).

3.4.5. K285R (GenBank ID GQ857130)

The 2.5 months full term baby boy developed jaundice on day 3 of life and had a history of prolonged hyperbilirubinemia and abdominal distension. Ultrasound revealed mild hepatosplenomegaly with thick gall bladder. Intra uterine work up shows the CMV infection. K285R was identified on one allele in exon 9 of GALT gene while other allele carried N314D. GALT enzyme activity was 17%. While K285R is likely to modify the hydrogen bond network of enzyme as revealed by modeling studies, N314D is already known to affect the secondary structure of enzyme and its solvent accessibility (Table 4). K285R was envisaged to be tolerable variation by SIFT confirming its non-disease related variation status as predicted by SNPs&GO (Table 3, Supplementary Table 1).

3.4.6. S307X (GenBank ID GQ355273)

The 1.5 months old female child with a GALT activity of 19% was found to be heterozygous for S307X. Mutation on another allele was identified as N314D. She developed jaundice on day 6 of life which was progressive. There was hepatomegaly, distended abdomen with free fluid, bilateral cataracts and sun set sign. S307X was a nonsense mutation in exon 10 of GALT gene thereby leading to a truncated version of enzyme and thus affecting the enzyme activity.

3.4.7. R333L (GenBank ID GQ403670)

This missense mutation in exon 10 of GALT gene was identified in homozygous state in a 1 month old male child. Blood GALT activity was 14%. The child had a history of persistent jaundice with hepatosplenomegaly, poor feeding, lethargy, sepsis, altered sensorium and bilateral cataracts. Liver biopsy showed distorted lobular architecture and fibrosis of portal tracts with ductular proliferation. Child succumbed to acute liver failure and sepsis. SNPs&GO analysis classified R333L as a disease causing mutation with a score of 2 on RI

Table 1
Frequency of GALT gene mutations identified in Indian galactosemia patients. Total chromosomes — 110.

S. No	Mutation	Exon	Nucleotide change	Consequence	Frequency (%)	Detection method	GenBank accession number
1	Y89H[a]	3	TAC→CAC	Try to His	0.9	SSCP	HQ637434
2	Q103Q[a]	3	CAG→CAA	silent	1.8	SSCP	GU129565
3	Q103R[a]	3	CAG→CGG	Gln to Arg	1.8	SSCP	GU129564
4	P166A[a]	5	CCT→GCT	Pro to Ala	0.9	SSCP	GQ403671
5	F171S	6	TTT→TCT	Phe to Ser	1.8	SSCP	
6	S181F[a]	6	TCT→TTT	Ser to Phe	1.8	SSCP	HQ412987
7	Q188R	6	CAG→CGG	Gln to Arg	2.7	RFLP	-
8	P185L	6	CCC→CTC	Pro to Leu	1.8	SSCP	-
9	K210K[a]	7	AAG→AAA	silent	0.9	SSCP	GQ857129
10	L218L	7	CTA→TTA	silent	0.9	RFLP	-
11	K285R[a]	9	AAG→AGG	Lys to Arg	0.9	SSCP	GQ857130
12	N314D	10	AAC→GAC	Asn to Asp	40	RFLP	-
13	S307X[a]	10	TCA→TAA	Ser to Stop	0.9	SSCP	GQ355273
14	H319Q	10	CAC→CAA	His to Gln	1.8	SSCP	-
15	H319H[a]	10	CAC→CAT	silent	0.9	SSCP	GQ403669
16	R333L[a]	10	CGG→CTG	Arg to Leu	1.8	SSCP	GQ403670

[a] Denotes novel mutation; SSCP — Single strand conformation polymorphism; RFLP — restriction fragment length polymorphism.

Table 2
GALT mutations/genotypes and observed GALT enzyme activity with corresponding galactose-1-phosphate levels in galactosemia subjects (n = 55).

Mutation observed/ genotype	Number of subjects	Average GALT activity (%)[a]	Galctose-1-phosphate levels (mg/100 ml packed erythrocyte lystae)
N314D/N314D	7	41	9.12
Y89H/N314D	1	15	22.15
P185L/N314D	2	18	16.01
K285R/N314D	1	17	17.43
S307X/N314D	1	19	16.92
Q103R/Q103R	1	20	18.51
F171S/F171S	1	15	26.13
S181F/S181F	1	10	28.01
H319Q/H319Q	1	18	14.97
R333L/R333L	1	14	20.57
H319H/P166A	1	21	12.18
N314D/U	25	21	14.65
L218L/U	1	30	11.49
K210K/U	1	29	14.67
Q103Q/U	1	28	13.17
Q188R/U	3	09	23.11
U/U	6	17	15.01

[a] GALT activity is expressed in terms of percentage in comparison with control. Levels of galactose-1-phosphate in normal controls <1 mg/100 ml packed erythrocyte lystae; U — Unidentified (NCBI RefSeq for GALT: NM_000155).

(Table 3). SIFT prediction analysis also supported the SNPs&GO results (Supplementary Table 1). Modeling studies revealed arginine 333 to be present at the inter subunit surface of GALT protein. Switching over of this arginine to leucine might have attributed to the perturbed inter-subunit relationship of the enzyme, resulting in diminished enzyme activity (Table 4).

4. Discussion

Classical Galactosemia is a potentially fatal disorder of galactose metabolism. This report is first of its kind from Indian population and details the clinical, laboratory and genetic findings of galactosemia patients from North-West India. We have characterized the molecular defects in 67 out of 110 Indian GALT chromosomes. The diagnosis of galactosemia is usually made early in neonatal period. However, mean of age 2.1 months at the time of diagnosis recorded in our study is indicative of considerable delay in detection. The lack of awareness is likely to be an important reason. So, it is important to consider the diagnosis of galactosemia in neonates and infants presenting with clinical features like persistent hypoglycemia, failure to thrive, feeding difficulties, prolonged conjugated hyperbilirubinemia, hepatomegaly, ascites, invasive *E. coli* infections and galactosuria. Another reason for the delay in diagnosis can be the mild clinical phenotypes which might have delayed manifestations.

A total of 16 different mutations were observed but only two (N314D and Q188R) were found to be prevalent at a significant frequency. These data are consistent with the previous studies in other populations showing that a few mutations are frequent while others are rare [15–17]. Q188R was found on only 3 mutant alleles (2.7%) in our study. This frequency is quite low as compared other populations like 60-70% in Caucasian [18], 48% in African Americans [19] and 9% in African Negroid patients [20]. These studies together with our results strengthened the previously observed pattern of distribution of Q188R in which its frequency decreases while moving through populations in an eastern direction across the globe [21].

N314D constituted 40% of galactosemia alleles analyzed in this study and was found to be present in a homozygous state in 7 patients and in compound heterozygous state in 5 patients. However, the mutation on second allele remains unidentified in another 25 patients in which N314D was found to be present on one allele (Table 2). N314D is known to occur in two different alleles of GALT gene — Duarte 1 (D1) and Duarte 2 (D2). The GALT enzyme activity of both the variants varies: D1 alleles show 110-130% of the normal enzyme activity but D2 alleles show only 40-50% activity. D1 is known to carry a silent mutation L218L, in exon 7 in addition to N314D. In contrast, beside N314D, D2 alleles are known to carry 3 intronic base changes — G1105C, G1323A and G1391A alongwith a 4 bp deletion in 5′ untranslated region (−119delGTCA) bases upstream from the first methionine codon [22]. In our study, out of 44 N314D carrying alleles, 43 were found to constitute Duarte 2 alleles while 1 allele was not found to be associated either with D1 or with D2 variant as no associated genetic changes were observed.

Among all the identified mutations, the frequencies of mutations in GALT chromosomes were found to be 31% in exon 10, 25% in exon 6, 19% in exon 3, 12.5% in exon 7 and 6.25% each in exons 5 and 9. Thus, we report exons 6 and 10 as hotspots for mutations in the Indian galactosemia patients. Intriguingly, out of 10 novel mutations identified, only 3 mutations *viz.* Y89H, S181F and R333L were predicted to be damaging/disease related while rest were predicted to be tolerable/non-disease related mutations (Table 3).

The study of inherited disorders would be greatly benefitted by the ability to interpret the effects caused by genetic mutations not only in terms of sequence alteration, but also in term of their impact on structure - function relationship of the protein. So, we tried to

Table 3
SNPs&GO pathological prediction and splicing defect of novel substitution mutations identified in GALT gene from Indian galactosemia patients.

Wild Type	Mutant	Position	Effect	RI	Splicing defect
Y	H	89	Disease related	3	No
Q	R	103	Neutral	9	No
P	A	166	Neutral	8	No
S	F	181	Disease related	7	No
K	R	285	Neutral	8	No
R	L	333	Disease related	2	No

SNPs&GO is a web server for the prediction of human disease-related single point protein mutations (http://snps-and-go.biocomp.unibo.it/snps-and-go/). The "Effect" column indicates whether the mutation is predicted to be associated to a disease and "RI" column reports the Reliability index of the prediction, scoring from 0 (unreliable) to 10 (reliable). Potential effect on splicing was determined by human splicing finder (http://www.umd.be/HSF/HSF.html).

Table 4
Predicted Effect of novel missense mutations of GALT gene on structural features of GALT protein.

Mutation	Exon	Predicted effects on structural feature of protein	GALT activity observed (%)
Y89H	3	May modify - the solvent accessibility to a significant extent - the H-bond(s) network	15
Q103R	3	May modify - the secondary structure - the solvent accessibility to a significant extent - the inter-subunit relationships because the residue is at the inter-subunit surface	20
P166A	5	The analysis of the mutant and wild type protein, and of the structural properties of the residue, does not highlight significant differences.	21
S181F	6	May modify - the inter-subunit relationships because the residue forms inter-subunit H-bond(s) - the substrate binding and/or catalysis	10
K285R	9	May modify - the H-bond(s) network	17
R333L	10	May modify - the inter-subunit relationships because the residue is at the inter-subunit surface	14

The structures of GALT mutants were modeled using a programme MODELLER (http://salilab.org/modeller/wiki/Mutate_model) and were provided by Institute of Food Science, CNR, Italy).

analyze the effects of novel missense mutations on the structure of GALT enzyme using the computer simulated mutant models for GALT protein [13]. The different missense mutations were found to exert diverse effects on the GALT enzyme either by affecting the solvent accessibility of enzyme, perturbing the H-bond network and salt bridges or by disturbing the inter-subunit relationship of the enzyme (Table 4).

By detailed scanning of the GALT gene using SSCP, a mutation detection rate of 60.9% has been achieved in our study. Failure to detect mutations in rest of GALT alleles could be indeed due to the presence of other defects in introns or the promoter region of GALT, and also because of presence of large rearrangements such as insertions, duplications or exon deletions, which have already been found in galactosemia patients [23]. All the subjects with reduced GALT activity were put on milk (galactose) free formula, Isomil, which is soybean milk. Three patients succumbed to sepsis and acute liver failure. One of them was homozygous for S181F and other for R333L whilst we could not detect any mutation in the third patient.

Concluding, a significant higher frequency of Duarte 2 variant in our population suggests the presence of a milder form of galactosemia in India which can be well managed by early diagnosis and dietary management. However, the true incidence of galactosemia in our population is unknown as newborn screening programmes have not been introduced as yet. This study sheds light on the spectrum of GALT gene mutations in Indian population and associated clinical presentation of galactosemia. It also highlights the heterogeneity of classical galactosemia in the Indian population and emphasizes the importance of extensive GALT gene analysis in diagnosis of galactosemia. Further, the delay in diagnosis revealed by our study indicates that a better clinical vigilance is required to lower the age of diagnosis and to improve the recognition of galactosemia in India.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2012.09.017.

Conflict of interest

The authors declare no conflict of interest.

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Web Resources

http://snps-and-go.biocomp.unibo.it/snps-and-go/
http://blocks.fhcrc.org/sift/SIFT.html
http://www.umd.be/HSF/
http://arup.utah.edu/database/galactosemia/GALT_welcome.php
http://www.ncbi.nlm.nih.gov/GenBank

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