A De Novo Variant in Galactose-1-P Uridyltransferase (*GALT*) Leading to Classic Galactosemia

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Abstract Classic galactosemia (CG) is a potentially lethal genetic disease that results from profound impairment of galactose-1-P uridylyltransferase (*GALT*), the middle enzyme in the Leloir pathway of galactose metabolism. Patients with CG carry pathogenic loss-of-function mutations in both of their *GALT* alleles; the parents of patients are considered obligate carriers. We report here a first exception to that rule – a *de novo* *GALT* variant in a patient with classic galactosemia. The new variant, c.563A>C (p.Q188P), which introduces a missense substitution near the active site of the *GALT* enzyme, was found in the compound heterozygous state in a child with classic galactosemia, but not in either of her parents. Extensive genomic studies of DNA from the child and both parents confirmed the expected degrees of relationship in the trio as well as inheritance of a common c.563A>G (p.Q188R) *GALT* mutation from the mother. This result demonstrates that not all pathogenic *GALT* mutations are inherited and raises concern

that *GALT* may have a higher new mutation rate than previously believed.

Introduction

Classic galactosemia (CG) is a potentially lethal genetic disorder that affects close to 1/50,000 live births in the United States (Pyhtila et al 2014). Patients with CG demonstrate profound impairment of galactose-1-phosphate uridylyltransferase (*GALT*), the second enzyme in the Leloir pathway of galactose metabolism (Fridovich-Keil and Walter 2008). Classic galactosemia is inherited as an autosomal recessive trait. Patients carry loss-of-function mutations in both of their *GALT* alleles, and their parents are considered obligate heterozygotes for those same mutations. The carrier risk for a loss-of-function *GALT* mutation in the general population is estimated using the Hardy–Weinberg equation at about 1 in 112 people.

Sequencing the *GALT* alleles in patients with classic galactosemia reveals striking allelic heterogeneity with >250 distinct causal or ostensibly causal variants and a small number of ostensibly neutral polymorphisms reported as of January 2013 ((Calderon et al 2007), http://arup.utah.edu/database/GALT/GALT_welcome.php). Most variants are coding missense substitutions, but some are small insertions, deletions, or indels. Finally, some variants impact only noncoding sequence, and two are large deletions that remove most or all of the *GALT* gene (e.g., (Calderon et al 2007; Coffee et al 2006; Gort et al 2006; Papachristoforou et al 2014; Tyfield et al 1999), http://arup.utah.edu/database/GALT/GALT_welcome.php).

Some *GALT* mutations are common, especially in certain populations. For example, c.563A>G (p.Q188R) accounts for the vast majority of CG patient alleles in Northern

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Europe and >60% of CG patient alleles in the United States (Tyfield et al 1999). The mutation c.855G>T (p.K285N) accounts for a majority of CG patient alleles in Eastern Europe (Lukac-Bajalo et al 2007). Finally, c.404C>T (p.S135L) accounts for a majority of CG patient alleles in parts of Africa (Henderson et al 2002). Other mutations, such as c.584T>C (p.L195P), are less common, accounting for only a small percentage of reported patient alleles, and yet others are “private” – reported only in one affected patient or family. The heterogeneity of CG patient alleles identified today stands as a striking record of the many new *GALT* variants that have arisen over the course of human history, but until now there have been no reported examples of *de novo* *GALT* variants identified in a living patient or family. We report here the first such case.

Methods

Study Volunteers

The volunteers in this study were a child with classic galactosemia and both of her parents (Fig. 1). All three participated following appropriate informed consent/assent in Emory IRB Protocol # 00024933 (PI: JL Fridovich-Keil). The child was also a consented volunteer in a research study of galactosemia conducted out of Boston Children’s Hospital (IRB Protocol # NS09-07-0369 (PI: GT Berry)).

GALT Genotyping of DNA from the Child

DNA samples isolated from independent blood draws from the child were assessed by dideoxy sequencing of the full *GALT* locus first in a clinical genetics laboratory (at Boston Children’s Hospital) and subsequently in a research laboratory (at Emory University). In the research laboratory the *GALT* gene was PCR amplified as a single ~6 kb amplicon using the primers 5′-AGTACCAGGGGAG-GAATTAATTTGAATTTT-3′ and 5′-ATTCAGT-CACTGTCCAGCCTTAGTGTGATTT-3′ as described previously (Boutron et al 2012), and the relevant region was sequenced using the following custom primers: hGALT-F4(2): 5′-AAGCTTTGGTTCTGGGGAGT-3′ (3′ end anneals to position 1324), hGALT-F03(1): 5′-CCCTGGTCGG ATGTACG-3′ (3′ end anneals to position 1164), and hGALT-R3: 5′-GTGTCTGGTAGGGC-CATGTT-3′ (3′ end anneals to position 2111). Both the clinical and research labs identified the same two mutations: the common c.563A>G (p.Q188R) missense mutation and also a novel c.563A>C (p.Q188P) missense mutation, each found in the patient in the compound heterozygous state.

Fig. 1 The family described in this study. The child, FKT118, has biochemically confirmed classic galactosemia and a *GALT* genotype of c.563A>G (p.Q188R)/c.563A>C (p.Q188P). The mother, FKT118P1, is a heterozygous carrier of the c.563A>G (p.Q188R) mutation. The father, FKT118P2, does not show any *GALT* mutations in his DNA. The biological relationships illustrated were confirmed by extensive marker analysis. The couple also has another unaffected biological child (not illustrated) who has not been genotyped

GALT Genotyping of DNA from Both Parents

DNAs from parental saliva samples collected using Ora-gene kits were also genotyped by dideoxy sequencing of the relevant *GALT* locus using the following primers: hGALT-F4(2): 5′-AAGCTTTGGTTCTGGGGAGT-3′ and hGALT-R3: 5′-GTGTCTGGTAGGGCCATGTT-3′. Initial studies revealed that the mother, designated FKT118P1, was a carrier of the c.563A>G (p.Q188R) missense mutation and that the father, designated FKT118P2, carried neither mutation. Concerned that there may have been some mistake, we collected an independent saliva sample from FKT118P2 and repeated the test; again we found neither *GALT* mutation.

Affymetrix 6.0 Microarray Analysis of Genomic Markers in the Trio

To confirm the biological relationships among the three study volunteers, we subjected each DNA sample to genotyping using the Affymetrix Genome-Wide Human SNP Array 6.0 that tests more than 900,000 single nucleotide polymorphisms (SNPs) scattered across the genome (http://www.affymetrix.com/catalog/131533/AFFY/Genome-Wide-Human-SNP-Array-6.0#1_1). Briefly, to be considered for downstream analyses, each array was required to pass the Affymetrix recommended quality control (QC) parameters – namely >86% call rate, >0.4 contrast QC, and gender concordance. Genotype calling was performed using Birdseed algorithm (version 2), as implemented in the Affymetrix Power Tools software (APT 1.12.0). Estimations of alleles that are identical by state (IBS) from genotype calls were performed using an in-house script (D.J.C). In comparison with the parents, the proband had approximately 73% of genotypes that were IBS at both alleles (IBS2), 27% at one allele (IBS1) and 0%

Table 1 Percentage of >900,000 marker loci in the trio interrogated using the Affymetrix Genome-Wide Human SNP Array 6.0 showing 0, 1, or 2 alleles with identity by state (IBS)

Level of IBS	0 of 2 alleles with IBS	1 of 2 alleles with IBS	2 of 2 alleles with IBS
Expected for parent and offspring	0.0010	0.2692	0.7307
Observed for FKT118P1 and FKT118	0.0002	0.2665	0.7333
Observed for FKT118P2 and FKT118	0.0002	0.2686	0.7311

with IBS0 value, thus confirming the familial relationship (see Table 1).

Results

Case Report

The proband (FKT118, Fig. 1) is a 6-year-old girl who was born at gestational age 34 weeks and diagnosed with classic galactosemia as an infant in follow-up to an abnormal newborn screening result. On day 1 of life, she was supplemented with a proprietary formula containing lactose because of a poor sucking reflex. In the first week of life, she manifested poor feeding, emesis, loose stools, and jaundice. The serum total bilirubin concentration was 23 mg%. The newborn screening test for galactosemia was positive and the infant was switched to a soy-based formula on day 5 of life. Seizures occurred at 3 years of age, including grand mal seizures. There is a history of language delay and a speech defect. Magnetic resonance imaging (MRI) of the brain conducted at 5 years of age revealed areas of patchy subcortical white matter signal increases possibly related to non-suppressed perivascular spaces. On physical examination at age five, the proband had scars on her face due to a seizure-induced fall. There was diffuse hypotonia. There were no discernible peaks of galactitol on magnetic resonance spectroscopy (MRS). The erythrocyte GALT enzyme activity was undetectable, and the galactose-1-phosphate level was 3.67 mg% while on a lactose-restricted diet (reference range <1 mg%). The urinary galactitol excretion was 178 μ mol/mmol creatinine (reference range 2–36). By early childhood the serum follicle stimulating hormone (FSH) level was elevated and the anti-Müllerian hormone level was undetectable. A DEXA scan of bone density did not reveal any region with a Z score that was below two standard deviations of the reference mean.

Dideoxy Sequencing Reveals a Novel *GALT* Variant in a Child with Classic Galactosemia

Initial *GALT* genotyping was performed in 2008 in a clinical genetics lab (Emory Genetics Laboratory) using allele-specific primer extension (Luminex; <http://www.premierbiosoft.com/primerplex/allele-specific-primer-extension.html>) to detect the presence or absence of a panel of common mutations. The result demonstrated presence of c.563A>G (p.Q188R) and absence of the wild-type allele at that locus; no other mutations included in the panel were detected. The genotype was therefore reported, to the sensitivity of the assay, as c.563A>G (p.Q188R)/c.563A>G (p.Q188R). Of note, c.563A>C (p.Q188P) was not one of the common *GALT* mutations included in the panel.

Years later a fresh blood sample was collected (at Boston Children's Hospital) and subjected to *GALT* genotyping via full gene dideoxy sequencing. Two mutations were detected, c.563A>G (p.Q188R) and c.563A>C (p.Q188P), each in the compound heterozygous state. With permission, this result was shared with our research lab at Emory University that repeated the dideoxy sequencing on an archived DNA sample from the child; the c.563A>G (p.Q188R)/c.563A>C (p.Q188P) result was confirmed (Fig. 2). A search of the galactosemia literature and *GALT* mutation database (http://arup.utah.edu/database/GALT/GALT_welcome.php) revealed no prior reports of the c.563A>C (p.Q188P) mutation.

Dideoxy Sequencing of Parental *GALT* Loci Coupled with Comprehensive Genomic SNP Analysis of the Trio Reveals that c.563A>C (p.Q188P) is a De Novo Variant in this Family

In response to a parental request for *GALT* genotyping to determine which side of the family was at risk for carrying which *GALT* mutation, parental saliva samples were

Fig. 2 *GALT* sequencing results demonstrate the presence of mutations at *GALT* position c.563 in the mother and child, but not the father. The figure presents representative dideoxy sequencing results of a relevant segment of the *GALT* locus derived from blood from the child and saliva from both parents. All sequencing results

were confirmed with replicate reactions sequencing both strands. The three bases boxed in each sequence encode residue 188 in the *GALT* protein. The wild-type human *GALT* gene sequence can be found at NCBI accession number NG_009029 (version NG_009029.1 GI:288541293)

collected and studied. Dideoxy sequencing at the *GALT* locus in both parental samples revealed that the mother, FKT118P1, was a carrier of the common c.563A>G (p.Q188R) mutation, but no mutation was detected in the father (FKT118P2) (Fig. 2). Repeat testing of the sample confirmed the result. The family was notified and consulted regarding possible next steps, and an independent saliva sample was collected from the father and analyzed, but again no *GALT* mutation was detected.

Finally, with parental permission reaffirmed, DNA samples from the child and both parents were subjected to whole genome SNP genotyping using Affymetrix 6.0 microarrays to test the biological relationships of the samples. Comparing rates of identity by state (IBS) at each of the >900,000 SNPs genotyped for the trio confirmed, beyond question, that both parents were indeed the biological parents of the child (Table 1). This result, combined with the *GALT* genotyping result (Fig. 2),

demonstrated that c.563A>C (p.Q188P) was a *de novo* variant in the child.

Discussion

We report here what is, to our knowledge, the first case of a *de novo* pathogenic variant in human *GALT* associated with classic galactosemia: a c.563A>C transversion encoding the substitution of proline (P) in place of glutamine (Q) at residue 188 in the active site of the *GALT* enzyme. Repeated sequencing of *GALT* loci in the patient conducted in two separate laboratories using independent samples confirmed the presence of the transversion, and repeated sequencing of independent DNA samples from both parents failed to detect the transversion. Genotyping of hundreds of thousands of polymorphic markers in the child and both parents confirmed the biological relationships among the trio.

While it is notable that the *de novo* variant in this child occurred at exactly the same location, c.563, as her inherited mutation, it remains unclear whether the presence of the inherited mutation in some way predisposed the other allele to mutate early in embryogenesis. Alternatively, it is possible that c.563 in *GALT* is intrinsically vulnerable to mutation. Finally, it is possible that the occurrence of both the inherited and *de novo* mutations at exactly the same nucleotide in *GALT* in this child was simply a striking but random coincidence.

The *de novo* variant documented here is important for two reasons: first that a *de novo* mutation event in *GALT* can lead to classic galactosemia has clear implications for genetic counseling of families, especially if the proband carries a private *GALT* mutation. In this instance, parents should not be considered obligate carriers and recurrence rates for the couple should not be predicted unless both parents have been genotyped and confirmed to be carriers. Second, this result is important because it raises the possibility that new mutations may also occur in genes associated with other autosomal recessive disorders, especially those that show strong allelic heterogeneity among patients. Consistent with this possibility, *de novo* mutations have been reported previously for both phenylketonuria (Eiken et al 1992) and cystic fibrosis (White et al 1991).

Finally, whether this report of a *de novo* pathogenic mutation in *GALT* leading to classic galactosemia represents the chance observation of an extremely rare event, or the first observation of what is actually a common event, remains unknown. Until family studies or population carrier screening are commonplace for galactosemia, it will be very difficult to estimate with accuracy the new mutation rate for *GALT*.

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1-Sentence Synopsis

Genomic studies confirm a *de novo* variant in human galactose-1-P uridylyltransferase (*GALT*) leading to classic galactosemia.

Compliance with Ethical Guidelines

Conflict of Interest

Thanh-Thanh (Claire) V. Tran declares that she has no conflict of interest.

Ying Liu declares that she has no conflict of interest.

Michael Zwick declares that he has no conflict of interest.

Dhanya Ramachandran declares that she has no conflict of interest.

David Cutler declares that he has no conflict of interest.

Xiaoping Huang declares that she has no conflict of interest.

Gerard Berry declares that he has no conflict of interest.

Judith Fridovich-Keil declares that she has no conflict of interest.

Informed Consent

“All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5).”

All data from specific individuals reported here were collected following appropriate informed consent/assent and with approval by the Emory University Institutional Review Board (IRB# 00024933, PI: JL Fridovich-Keil).

Animal Rights

“This article does not contain any studies with animal subjects performed by any of the authors.”

Contributions of Each Author

Thanh-Thanh (Claire) V. Tran confirmed the *GALT* genotype of the child, performed *GALT* genotyping for both parents, and also helped to edit the manuscript.

Ying Liu helped to oversee Ms. Tran’s work, designed the *GALT* sequencing primers, and also helped to edit the manuscript.

Michael Zwick oversaw the Affymetrix 6.0 genotyping of the three DNA samples that confirmed both parent–child relationships and also helped to edit the manuscript.

Dhanya Ramachandran performed genotype calling and statistical analyses on Affymetrix SNP 6.0 data and also helped to edit the manuscript.

David Cutler assisted with the interpretation of the Affymetrix genotyping data and also helped to edit the manuscript.

Xiaoping Huang and Gerard Berry originally identified the Q188P variant in the child and also helped to write and edit the manuscript.

Judith Fridovich-Keil initiated the project, coordinated the efforts of the other authors, and wrote most of the manuscript.

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