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Galactosemia: Analzing a Familial Mutation

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Introduction

Galactosemia is a genetic metabolic disorder in which an individual is unable to break down galactose, a component of the milk sugar, lactose. There are three genes involved in the metabolism of galactose: galactokinase-1 (GALK), UDP-galactose-4-epimerase (GALE), and galactose-1-phosphate uridylyltransferase (GALT; Figure 1). A mutation in the GALT gene results in classic galactosemia, the most common and most severe form of this disorder. Persons with classic galactosemia require a milk-free diet, as several long term effects may result, such as cognitive disabilities and infertility in females. A milk-free diet is required because elevated levels of galactose in the body acts as a toxin, and the subject's liver must then continually work hard in order to try to rid the body of toxins. A less severe form of galactosemia, Duarte, is also associated with mutations of GALT.

DNA was collected from 3 subjects in a familial group exhibiting a family history of galactosemia. The two parental carriers of galactosemia have the classic form while their child's enzymatic testing has been inconsistent with the parents' testing results.

Methods and Materials

- DNA was extracted via cheek scraping and incubated in 10% Chelex solution
- The GALT gene (3,939 bp) was amplified using PCR, with the primers GALT 1-5, GALT 6-9, and GALT 10-11 (Calderon et al, 2007; Figures 2 & 3)
- PCR fragments were then cloned using an Invitrogen TOPO TA Cloning kit for sequencing
- Cloned alleles were sequenced in ABI 30XL Capillary DNA sequencer
- Contigs were aligned manually using Sequencher
- Coding sequences were translated into protein sequences and compared to ARUP Laboratory at the University of Utah database for known mutations

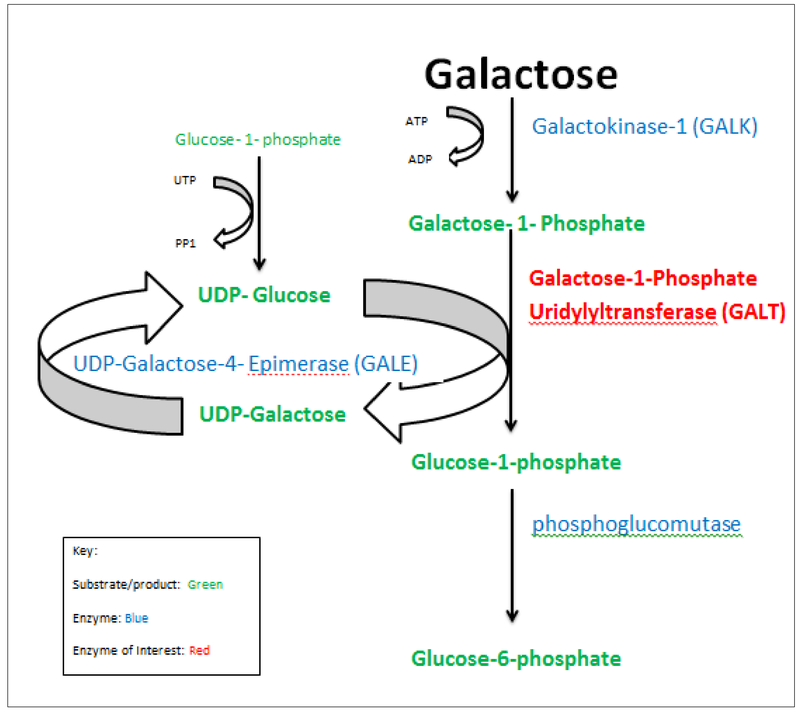

Figure 1. The GALK gene causes phosphorylation of α-D-galactose in order to produce galactose 1-phosphate (Fridovich-Keil, 2006). Phosphorylation is the process of adding a phosphate to a protein (Alberts et al., 2010). Next, the GALT enzyme performs a transfer of uridine monophosphate (UMP) from UDP-glucose to galactose 1-phosphate. This transfer results in a release of glucose 1-phosphate and ultimately produces UDP-galactose (Fridovich-Keil, 2006). Lastly the GALE gene causes an interconversion between UDP-galactose and UDP-glucose (Fridovich-Keil, 2006). Glucose-6-phosphate will ultimately go to glycolysis or to the pentose phosphate pathway.

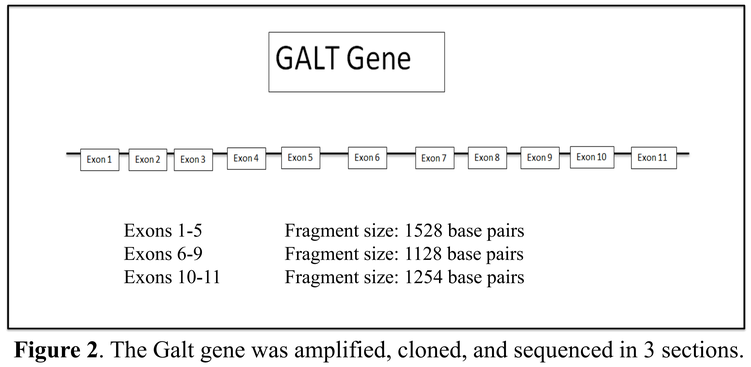

Figure 2. The Galt gene was amplified, cloned, and sequenced in 3 sections.

Results

- Coding sequence of the GALT gene is 1137 bp
- Translation of the coding sequence resulted in a protein comprised of 379 amino acids
- To date, the only remarkable mutations noted in the GALT coding sequence for the child is an insertion (771_772insC) and deletion (777delC)
- This mutation is shared by the father
- This INDEL at 771 and 777 corresponds with two amino acid substitutions 258 (Val → Arg) and 259 (Gly → Arg) (Figure 4).

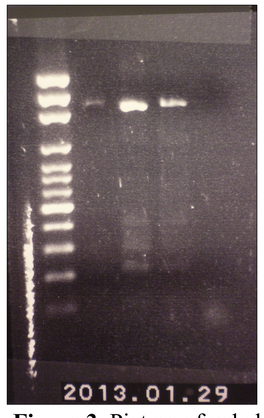

Figure 3. Picture of gel electrophoresis of a PCR run on all three subjects Exons 1-5.

Un-afflicted DNA	... CCC	GTC	GGC	CAT…
amino acid residue	257	258	259	260
	Pro	Val	Gly	His
Child and Father DNA	... CCC	CGT	CGG	CAT…
amino acid residue	257	258	259	260
	Pro	Arg	Arg	His

Figure 4. DNA sequence of the child and father compared DNA of an un-afflicted individual. The INDEL mutation of the child and father occurred on Exon 8 of the GALT gene.

Discussion and Conclusions

- An insertion/deletion mutation is shared between the father and child in exon 8, causing the reading frame to shift for 2 amino acid residues
- The INDEL at 799 corresponds with two amino acid substitutions 258 (Val → Arg) and 259 (Gly → Arg)
- This mutation has not been previously identified in the ARUP database, however, amino acid substitutions at 257, 258, and 259 have pathogenic implications (Elsas 1998; Tyfield 1999; Gort 2009)

For further information

Please contact *Smueller08@winona.edu*.
Visit ARUP website on Galactosemia
http://www.arup.utah.edu/database/GALT/GALT_welcome.php

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