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Molecular Differentiation of Congenital Lactase Deficiency from Adult-Type Hypolactasia

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A limited fraction of the human adult population retains intestinal lactase-phlorizin hydrolase (LPH) activity during adulthood, and this is called the lactase persistence phenotype. However, 95% of all adults have adult-type hypolactasia (ATH) and have difficulty digesting milk sugar. Rarely, some infants are born with an inability to digest lactase (congenital lactase deficiency or CLD) due to low levels of LPH activity, which results in severe clinical consequences if not properly diagnosed and treated by lactose avoidance. Recently, it has been shown that both recessive LPH deficiencies, CLD and ATH, are related to DNA variants affecting the lactase (LCT) gene, but they are mediated through very different molecular mechanisms. The LCT mutations resulting in childhood CLD lead to low LPH activity through nonsense-mediated LCT mRNA decay, whereas the critical nucleotide variants for the ATH phenotype represent distal enhancer polymorphisms, which regulate developmentally LCT transcript levels in intestinal cells.

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LACTASE-PHLORIZIN HYDROLASE

The small intestinal enzyme lactase-phlorizin hydrolase (LPH), encoded by the lactase gene (LCT), has two enzymatic activities: a lactase (EC 3.2.1.108) responsible for the specific hydrolysis of lactose into glucose and galactose that can be absorbed across the intestinal epithelium, and a general hydrolytic activity for cellobiose, cellotriose, and phlorizin hydrolase (EC 3.2.1.62) with a β-glycosidase activity for glycosyl ceramides.[1-3] LPH is synthesized as a polypeptide of about 220 kD,[4,5] which follows a complex intracellular processing route involving heavy glycosylation with N-linked and O-linked carbohydrates,[6,7] with intermediate forms displaying molecular weights of up to 280 kD, and one or two proteolytic steps[2,8] that render a final mature product with a molecular weight of around 160 kD.[8] The C-terminus domain is intracellular and the N-terminus is found on the luminal surface of the microvillus membrane. LPH activity is only expressed in the small intestine and is restricted to absorptive enterocytes in the villi. It is expressed along the proximal to distal axis of the intestine, with high levels in the mid-intestine and reduced activity levels in the duodenum and distal ileum.

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THE LACTASE GENE

The LCT gene is located on chromosome 2q21. Its nucleotide sequence has indicated the presence of four domains arranged with a 2-fold symmetry.[9] Two of these domains are eliminated during the maturation process, rendering a mature protein consisting of two domains containing one active site each (domains III and IV).[2,10,11] LCT mRNA is only expressed in villous enterocytes of the small intestine, but is present in very small amounts in the fetal colon. The expression of the LCT message is regulated along the length of the small intestine and the crypt-villous axis during differentiation. The distal jejunum and the proximal ileum contain the highest levels of LCT mRNA, while the levels decrease along the proximal jejunum and the distal ileum.[12] In animals, LCT mRNA remains low until just prior to birth, and then rises and remains elevated until weaning, when it declines to less than 10% of the neonatal values. This reduced post-weaning LCT mRNA expression lasts throughout adult life.[13,14]

LACTASE PERSISTENCE AND LACTASE NON-PERSISTENCE PHENOTYPES

In humans, LPH activity is low before 24 weeks of gestation and increases during the third trimester until levels in the term neonate are at or above those of infants ages 2 to 11 months. In most populations, LPH activity

decreases during mid-childhood (about 5 years of age), and this is called the lactase non-persistence phenotype. A limited fraction of the human adult population retains LPH activity during adulthood, and this is called the lactase persistence phenotype. Individuals with this phenotype can consume large quantities of milk without clinical problems.[1,15,16] The lactase persistent phenotype is very frequent in northern Europe and also in some nomadic populations in north and central Africa and Arabia.[17,18]

ADULT-TYPE HYPOLACTASIA

A small number of humans with lactase non-persistence have been shown to have an abnormality in the intracellular processing of newly synthesized LPH protein, indicating post-transcriptional control of this pathway.[19,20] However, in most the underlying mechanism for both non-persistence and persistence phenotypes relies on the developmental regulation of LCT gene transcription.[21,22] Adult-type hypolactasia (ATH) is inherited in a recessive autosomal manner[23] and is present in about 95% of all adults. ATH is characterized by low levels of the LPH enzyme activity (0–10 U/g protein) in jejunal biopsies and poor proximal lactose digestion. The intestinal mucosa has a normal histology and the activities of all other disaccharidases (maltase, sucrase-isomaltase) are normal. Studies suggest that ATH individuals are homozygous for a recessive allele, which leads to a post-weaning decline of the LPH activity, and that adults with lactase persistence are either heterozygous or homozygous for a dominant allele LCT*P, which allows LPH activity to persist.[17]

Molecular Basis for Adult-Type Hypolactasia

There have been recent insights into the molecular basis for ATH. Polymorphisms of C/T at nucleotide –13910 and of G/A at nucleotide –22018 are associated with transcriptional regulation of the human LCT gene.[24] Interestingly, the C-13910 and G-22018 polymorphisms, which are located within introns of the neighboring MCM6 gene, correlate 100% and 97%, respectively, with the ATH phenotype: all family members studied with ATH were homozygous with respect to both C-13910 and G-22018.[25] The T-13910 polymorphism is located in a transcriptional enhancer sequence, which more strongly activates the LCT promoter activity than the corresponding C-13910 variant. However, the effects of the G-22018 region on LCT promoter activity are minimal; this polymorphism seems to interfere with the enhancer activity of the C-13910 region.[26] The variant C/T-13910 is associated with ATH in both Finnish and non-Finnish populations. The molecular mechanism seems to be that the C/T-13910 variant changes a DNA-binding site for the transcription factor AP-2. The C allele, associated with ATH, is part of the AP-2 consensus-binding motif, whereas the T variant disrupts this motif.[26,27] The strong enhancer effect of the T-13910 variant compensates for the developmental changes normally occurring after weaning and is thereby able to keep the LCT gene transcriptionally active, resulting in the phenotype of LPH persistence.[26]

CONGENITAL LACTASE DEFICIENCY

Congenital lactase deficiency (CLD) is a very severe autosomal recessive, inherited gastrointestinal disorder. Since 1966, a total of 42 patients from 35 families have been diagnosed with CLD in Finland, where the estimated incidence is 1:60,000.[28,29] CLD is characterized by an almost total lack of the LPH enzyme activity (0–10 U/g protein) in jejunal biopsies, and presents clinically as watery diarrhea, severe dehydration, acidosis, and weight loss, diagnosed at a mean age of 36 days[30] after the newborn is fed breast milk or lactose-containing formula. The intestinal mucosa reveals a normal histology and activities of the other disaccharidases are normal. With a lactose-restricted diet the symptoms disappear and these infants can have normal growth and psychomotor development.[30,31]

Molecular Mechanism of Congenital Lactase Deficiency

Exciting molecular insights have become available concerning CLD. Assignment of the locus for CLD on 2q21 from 19 families was possible in 1998[32] using linkage disequilibrium. In the linkage report, Finnish investigators found that the critical DNA region for CLD was from D2S314 to D2S2385; however, initial sequence analyses of regional transcripts failed to reveal disease-causing mutations. At that time, the critical locus position excluded the LCT gene itself.[32] Because no mutations were found in the linkage region, the Finnish group reported in a recent publication a distinct molecular background of CLD. They discovered mutations within the coding region of the LCT gene in 32 patients from 24 families.[33] These investigators genotyped 15 microsatellite markers covering 5.88 cM on 2q21–2q22 in affected children. They constructed 21 different haplotypes in 48 disease chromosomes. One major haplotype, cen-5-T_{-13910}-7-5-12-9-6-11-4-15-12-9-7-5-4-4-tel, was present in 18 diseased chromosomes, representing the founder haplotype from which 15 of the remaining 20 different haplotypes were derived. Five disease mutations were found, and each carried a different haplotype that did not relate to the major haplotype. There was no

obvious relationship between CLD genotypes and clinical phenotypes. From these results, they concluded that a critical region would contain the LCT gene, and proceeded to perform sequence analysis of the gene. Three discovered CLD mutations were predicted to lead to a premature truncation of LPH, and two were missense mutations that resulted in amino acid substitutions. Their findings were as follows:

1. All of the chromosomes with the major disease haplotype carried a nonsense mutation, c.4170T→A (Fin_{major}), resulting in Y1390X, a premature stop codon in exon 9 predicting the truncation of 537 amino acids.
2. Two patients had deletion of four nucleotides, c.4998_5001del TGAG, in their paternal disease chromosome in exon 14, leading to a frameshift and premature stop codon after 55 altered amino acids (S1666fsX1722). This mutation truncates LPH in the middle of region IV.
3. The third mutation, c.653_654delCT, is a deletion of two nucleotides in exon 2, predicting a frameshift change at codon 218 and protein truncation at codon 224, S218fsX224. This mutation truncates the polypeptide early at region I.
4. The fourth mutation is a c.804G→C transversion at codon 268, leading to an amino acid substitution of histidine for glutamine, Q268H, in the last nucleotide of exon 3. This mutation affects region I.
5. The fifth mutation is c.4087G→A, a transition resulting in a missense substitution of serine for an uncharged glycine, G1363S, at codon 1363 in exon 9. This mutation is located in the mature LPH in region III at the end of the catalytically active site; it may have serious functional consequences.

The carrier frequency of Fin_{major} was determined among 556 anonymous blood donors. The highest carrier frequency of 1:35 (4/140) was seen in a little town, Nilsia, in central Finland. No carriers for other mutations (S218fsX224, S1666fsX1722, Q268H, and G1363S) were observed in any regional subpopulation screened.

The investigators used an allele-specific minisequencing method to characterize the potential impact of the mutation on the steady-state transcript level of the LCT allele carrying the nucleotide change resulting in Y1390X.[34] Using a duodenal biopsy sample from a patient who was heterozygous for the CLD nonsense mutation Y1390X and heterozygous for C/T-13910, the SNP associated with adult-type hypolactasia, they found that the transcript level of the Y1390X allele carrying the premature stop codon was at the same level as the transcript of the C-13910 allele. The C-13910 allele typically accounts for about 8% of the expressed LCT mRNA in ATH.[35] It was concluded that the Y1390X mutation led to nonsense-mediated mRNA decay of at least 90% of the disease alleles.

CONCLUSIONS

In this report, the Finnish group has confirmed that both human autosomal recessive LPH deficiencies, CLD and ATH, are related to DNA variants affecting the LCT gene, but that these are mediated through very different molecular mechanisms. The mutations resulting in childhood CLD have direct consequences for the level of LPH polypeptide because the mutation causes nonsense-mediated mRNA decay of the disease alleles,[33] whereas the critical nucleotide polymorphism resulting in the ATH phenotype represents distal enhancer polymorphisms regulating developmental LCT transcripts levels in intestinal cells.[24-27] This discovery also facilitates direct DNA-based molecular diagnosis and carrier identification of CLD, previously based on clinical symptoms supplemented by the finding of low LPH activity in assays from intestinal biopsy specimens.

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