Report

Mutations in the Translated Region of the Lactase Gene (*LCT*) Underlie Congenital Lactase Deficiency

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Congenital lactase deficiency (CLD) is a severe gastrointestinal disorder characterized by watery diarrhea in infants fed with breast milk or other lactose-containing formulas. We initially assigned the CLD locus by linkage and linkage disequilibrium on 2q21 in 19 Finnish families. Here we report the molecular background of CLD via characterization of five distinct mutations in the coding region of the lactase (*LCT*) gene. Twenty-seven patients out of 32 (84%) were homozygous for a nonsense mutation, c.4170T→A (Y1390X), designated "Fin$_{major}$." Four rare mutations—two that result in a predicted frameshift and early truncation at S1666fsX1722 and S218fsX224 and two point mutations that result in substitutions Q268H and G1363S of the 1,927-aa polypeptide—confirmed the lactase mutations as causative for CLD. These findings facilitate genetic testing in clinical practice and enable genetic counseling for this severe disease. Further, our data demonstrate that, in contrast to common adult-type hypolactasia (lactose intolerance) caused by a variant of the regulatory element, the severe infancy form represents the outcome of mutations affecting the structure of the protein inactivating the enzyme.

Congenital lactase deficiency (CLD [MIM 223000]) is an autosomal, recessively inherited, severe gastrointestinal disorder of infants. Lactase activity in intestinal mucosa is decreased to 0–10 U/g of protein, leading to copious watery diarrhea shortly after breast-feeding or the introduction of lactose-containing formulas. However, duodenal morphology and the activities of maltase, sucrase, and isomaltase are normal. The severe osmotic diarrhea followed by dehydration, acidosis, and weight loss usually afflict sufferers during the first days of life. Despite the symptoms, CLD infants are vigorous and hungry. When they are on a lactose-free diet, the symptoms subside rapidly and the children have normal growth and psychomotor development (Launiala et al. 1966; Savilahti et al. 1983). CLD has an incidence of 1:60,000 and is one of the 36 rare monogenic disorders enriched in the Finnish population (Peltonen et al. 1999; Norio 2003*a*, 2003*b*). However, cases have also been reported elsewhere in the world (Holzel 1967).

In our linkage report, we defined the critical DNA region for CLD as from *D2S314* to *D2S2385*. These microsatellites shared haplotypes from both parents in 16 of 19 studied families and gave the highest linkage disequilibrium (LD) in disease alleles (Järvelä et al. 1998). However, sequence analyses of regional transcripts failed to reveal disease-causing mutations (data not shown). Importantly, the critical locus position excluded the *LCT* (MIM 603202) gene itself. Further, a published report of the sequence analysis of the *LCT* gene in a Finnish CLD patient failed to reveal disease-causing mutations in the coding region (Poggi and Sebastio 1991). However, since no mutations were found in transcripts on the linkage region, we wanted to confirm the position of the CLD locus with additional markers providing higher resolution. We genotyped 15 microsatellite markers covering 5.88 cM on 2q21–2q22 in 24 families with a total of 32 affected children. We were able to construct 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 disease chromosomes, representing the founder haplotype from which 15 of the remaining 20 different haplotypes have been derived (fig. 1). Five disease chromosomes, each of which carried a different haplotype, did not re-

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Mutation — Y1390X, S1666fsX1722, S218fsX224, G1363S, Q268H

Centromere	Y1390X																S1666fsX1722		S218fsX224	G1363S	Q268H
D2S1334	5	5	5	5	6	7	6	6	6	6	6	6	6	5	6	6	5	6	7	9	6
C/T$_{-13910}$	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	C	T	C	C	C
D2S2196	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	8	12	12	9	8	9
D2S442	5	5	5	5	5	5	5	5	5	5	5	5	6	5	5	5	6	6	6	7	6
D2S314	12	12	12	12	12	12	12	12	12	12	12	12	7	7	7	7	7	7	8	8	8
D2S2376	9	9	9	9	9	9	9	9	9	9	9	9	9	9	13	9	9	9	13	13	13
D2S3026	6	6	6	6	6	6	6	6	6	6	4	6	6	6	6	6	6	6	6	6	6
D2S3027	11	11	11	11	11	11	11	11	11	11	11	11	11	12	14	12	12	12	12	7	11
D2S3028	4	4	4	4	4	4	4	4	4	4	4	4	4	4	14	4	4	4	14	13	13
D2S3029	15	15	15	15	15	15	15	15	15	15	15	15	15	10	11	13	13	13	15	12	9
D2S2385	12	12	12	12	12	12	12	12	12	12	12	12	12	14	4	11	11	12	10	11	11
D2S2367	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	7	9
D2S2288	7	7	7	4	7	7	7	7	7	7	7	7	7	4	7	7	4	4	6	6	7
D2S255	5	5	5	6	5	5	5	5	5	8	5	5	5	5	7	5	8	8	5	7	6
D2S1326	4	12	5	5	4	4	7	5	7	8	4	4	4	5	9	12	5	5	6	6	5
D2S397	4	8	4	4	4	4	4	4	6	6	4	4	4	4	5	8	8	6	4	8	7
Telomere																					
No. of chr.	18	2	1	1	10	1	1	1	1	1	1	2	1*	1	1	1	1	1	1	1	1 (49)

Patients: 1-13, 15, 18-26, 28-31 (Y1390X); 17, 32 (S1666fsX1722); 16 (S218fsX224); 14 (G1363S); 27 (Q268H)

Figure 1   CLD haplotypes ordered according to each identified mutation. Patients 14, 16, 17, 27, and 32 are compound heterozygous for Y1390X and their respective rare mutations. Microsatellite mutation of *D2S314* in one patient increased the total number of CLD chromosomes to 49. Microsatellite markers were searched for and ordered on the basis of genetic maps of the Marshfield Clinic Research Foundation, as well as the physical map of the UCSC Genome Browser (December 2001 assembly). The Baylor College of Medicine (BCM) Search Launcher repeat-masker algorithm was used to identify novel microsatellites from clones between *D2S314* and *D2S2385* (Smith et al. 1996). Finally, 15 microsatellites and C/T$_{-13910}$, a SNP associated with adult-type hypolactasia, were analyzed, covering 5.88 cM of the CLD region. DNA was extracted by a standard protocol (Vandenplas et al. 1984) from the peripheral blood samples. PCR was performed and the genotypes were assigned as described elsewhere (Ylisaukko-oja et al. 2004). Haplotypes were constructed using the GENEHUNTER 2.1 program (Kruglyak et al. 1996). The CLD founder haplotype was generated with the assumption that there were a minimum number of recombinations.

late to the major haplotype. On the basis of information about shared haplotypes, we concluded that a critical region would contain the *LCT* gene, and we proceeded to perform sequence analysis of this gene. The transcript map of the critical DNA region for CLD is shown in figure 2.

We identified five distinct mutations in the *LCT* gene in 32 patients from 24 families. Three mutations were predicted to lead to a premature truncation of lactase, and two were missense mutations that resulted in amino acid substitutions (table 1 and figs. 3 and 4). All 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. Fin$_{major}$ was detected in 27 of 32 patients who were homozygous for the conserved founder haplotype or its modification. A total of 38 parents (5 did not participate) and 14 healthy siblings were found to be carriers of the Fin$_{major}$ mutation. Two patients had a deletion of four nucleotides, c.4998_5001delTGAG, in their paternal disease chromosome in exon 14, leading to a frameshift and a premature stop codon after 55 altered amino acids (S1666fsX1722). These patients were compound heterozygous for Fin$_{major}$ and S1666fsX1722. On the basis

of genealogical studies, both of these mutations are known to originate from central and northern Finland. 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. One patient carried this mutation in her paternal disease chromosome. 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. One patient carried this mutation in her maternal disease chromosome. The fifth mutation is a c.4087G→A transition resulting in a missense substitution of serine for an uncharged glycine, G1363S, at codon 1363 in exon 9. One patient carried this mutation in his maternal disease chromosome. A healthy sibling of the family also carried this rare mutation. These patients were compound heterozygous for the Fin$_{major}$ and their respective rare mutations. On the basis of the birthplaces of the great-grandparents of the patients, mutations three, four, and five would be assumed to originate from eastern Finland. The CLD mutations and the corresponding lactase activities measured from duodenal biopsy specimens are shown in table 1.

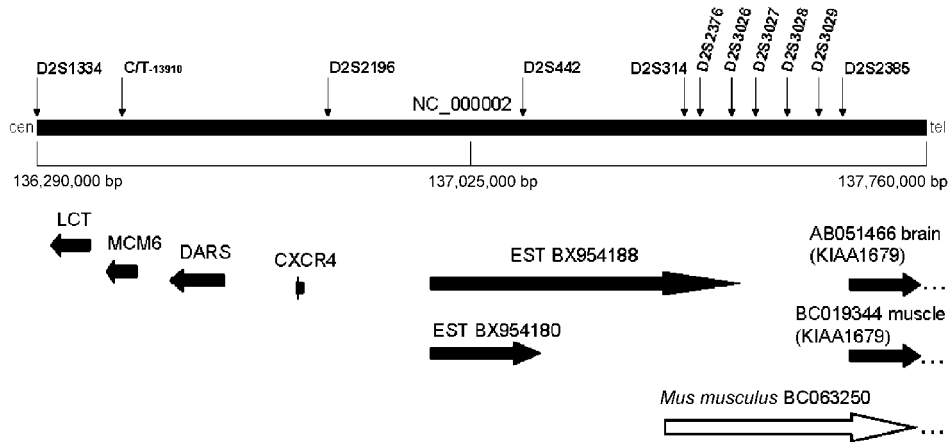Ninety percent (43/48) of the disease chromosomes

Figure 2 Transcript map of the critical DNA region for CLD. The physical map was constructed using public (International Human Genome Sequencing Consortium) Human Genome Project annotations. Evidence for transcripts and for conserved amino acids was searched with BLAST against different databases, including the dbEST and Refseq protein databases. Complete sequence annotations were performed by the NIX program (UK Human Genome Mapping Project Resource Centre) and the UCSC Genome Browser. *LCT,* minichromosome maintenance deficient 6 (*MCM6* [MIM 601806]), aspartyl-tRNA synthetase (*DARS* [MIM 603084]), chemokine receptor type 4 (*CXCR4* [MIM 162643]), and KIAA1679 are the known genes in the region (*horizontal arrows*). The mouse ortholog (BC063250) of human KIAA1679 suggests that ESTs BX954188 and BX954180 may be related to transcripts of KIAA1679. Microsatellite markers are indicated by vertical arrows. The centromere is on the left.

carried the Fin$_{major}$ mutation. The carrier frequency of Fin$_{major}$ was determined among 556 anonymous blood donors representing both the early and the later settlement regions of Finland. The highest carrier frequency of 1:35 (4/140) was seen in a little town, Nilsiä, in central Finland (fig. 5). This particular geographical region shows an enrichment of ancestors on genealogical studies (Järvelä et al. 1998). No carriers for other mutations (S218fsX224, S1666fsX1722, Q268H, and G1363S) were observed in any regional subpopulation screened.

The nascent lactase polypeptide contains four (I-IV) conserved structural and functional regions, but after posttranslational processing only two (III-IV) remain in the mature lactase enzyme (Mantei et al. 1988). The polypeptide has two catalytically active sites: phlorizin hydrolase activity is located in region III and lactase activity in region IV (Zecca et al. 1998; Arribas et al. 2000). Fin$_{major}$, Y1390X, truncates lactase polypeptide

in the beginning of region IV. We employed an allele-specific minisequencing method to characterize the potential impact of the mutation at the steady-state transcript level of the *LCT* allele carrying the nucleotide change resulting in Y1390X (Rasinperä et al. 2005). We used a duodenal biopsy sample of a patient who was heterozygous for the nonsense mutation resulting in Y1390X and heterozygous for C/T$_{-13910}$, the SNP associated with adult-type hypolactasia (MIM 223100) (Enattah et al. 2002; Rasinperä et al. 2005). We were able to determine that the transcript level of the allele carrying the premature stop codon was at the same level as the transcript of the C$_{-13910}$ allele, which typically represents ca 8% of the expressed *LCT* mRNA (Kuokkanen et al. 2003). We expect this to be caused by nonsense-mediated mRNA decay, a pathway which degrades mRNA induced by premature termination codons, eliminating the production of harmful truncated proteins

Table 1

CLD Mutations in the *LCT* Gene and Observed Lactase Activities of Duodenal Biopsy Specimens

DNA Mutation	Protein Mutation	Exon	Patient	Mean Lactase Protein Activity (U/g of Protein)	Genotype
c.4170T→A	Y1390X	9	1–13, 15, 18–26, 28–31	2.1 (range 0–7)[a]	Homozygote
c.4998_5001delTGAG	S1666fsX1722	14	17, 32	5 (range 3–7)	Compound heterozygote[b]
c.653_654delCT	S218fsX224	2	16	2	Compound heterozygote[b]
c.804G→C	Q268H	3	27	5	Compound heterozygote[b]
c.4087G→A	G1363S	9	14	3	Compound heterozygote[b]

[a] Lactase activities of patients 11, 18, 20, 26, and 29 were not available.
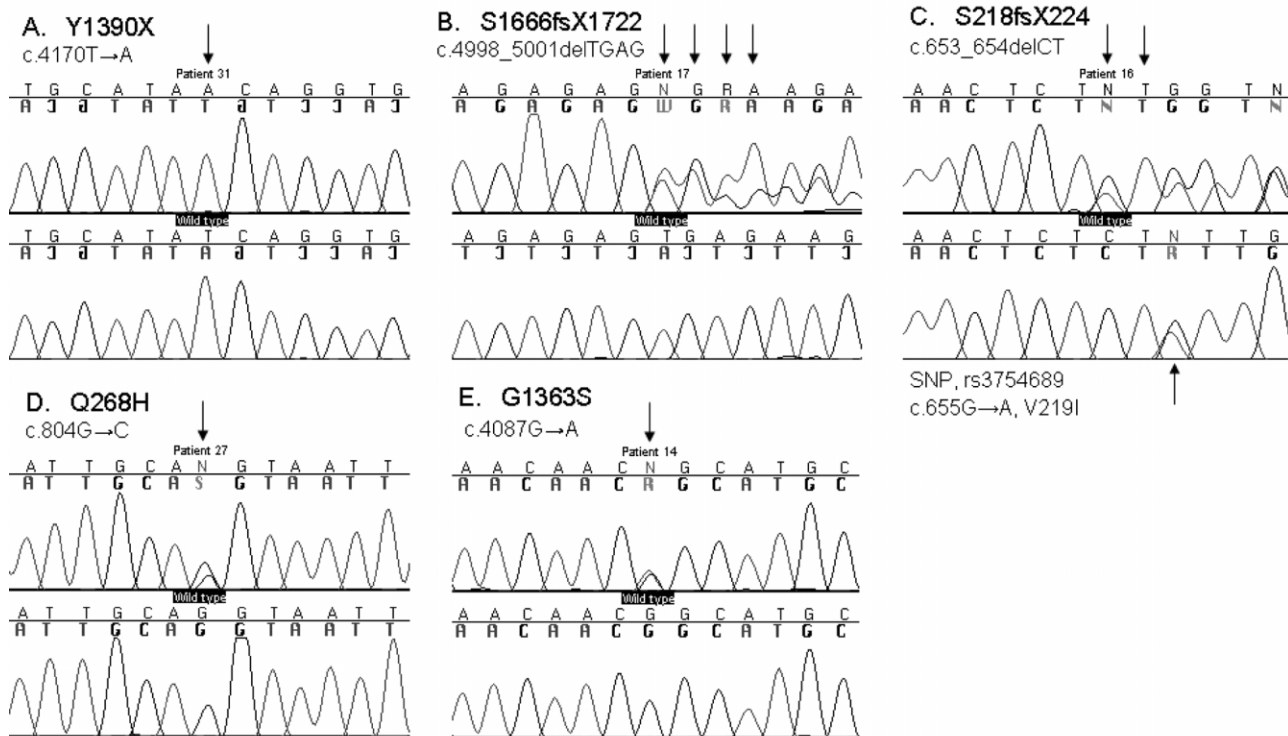
[b] Compound heterozygote for Y1390X.

Figure 3 CLD mutations in the *LCT* gene. *A–E*, DNA sequence chromatograms of five identified CLD mutations. One mutation, Y1390X, (*A*) is a homozygote, whereas the rest of the mutations are heterozygotes. The first row shows affected sequences and the second row wild-type sequences. In addition, c.655G→A, a SNP (rs3754689) leading to V219I, is shown in panel E (Boll et al. 1991). PCR was performed using the genomic DNA-amplifying promoter region, exons, flanking intron sequences, and 3′-UTRs of the *LCT* gene (primers are available on request). Sequencing was performed in both directions, and the sequenced products were electrophoresed on an ABI 3730 DNA analyzer (Applied Biosystems) in accordance with the manufacturer's instructions and were analyzed using ABI Sequencing Analysis 3.3 (Applied Biosystems) and Sequencher 4.1 (Gene Godes).

(Maquat 2005). This mechanism has been seen to modulate human disease phenotypes caused by disease-associated premature nonsense or frameshift mutations (Baserga and Benz 1988; Frischmeyer and Dietz 1999; Inoue et al. 2004). Furthermore, three of our CLD patients with the Y1390X mutation were included in an earlier study in which protein patterns of brush-border fragments were preliminarily examined (Freiburghaus et al. 1976). CLD patients had a complete or nearly complete absence of brush-border lactase in studied duodenal biopsies (Freiburghaus et al. 1976), which is in good agreement with our result that the mutant Y1390X *LCT* allele is down-regulated by nonsense-mediated decay. The second mutation, S1666fsX1722, truncates lactase in the middle of region IV, and the third mutation, S218fsX224, truncates polypeptide early, at region I. In theory, these mutant alleles may also be subjected to nonsense-mediated decay. The fourth mutation, Q268H, hits region I, substituting the basic histidine for an uncharged glutamine. Histidine has an imidazole ring that can be uncharged or positively charged, depending on its microenvironment. Although Q268H does not affect the mature enzyme, it may affect the structure of the lactase α-profragment, which has been demonstrated to act during lactase-folding processes as an intramolecular chaperone towards the mature enzyme (Naim et al. 1994; Jacob et al. 2002). The importance of the Q268 residue is also implied by cross-species conservation from human through chimpanzee, rabbit, cow, rat, and mouse. The fifth mutation, G1363S, replaces the smallest amino acid, glycine, with serine, which has a hydroxyl group that makes it polar and reactive. The replacement of the small glycine by the larger serine may alter the three-dimensional structure of the polypeptide. Since the G1363S mutation is located in the mature lactase at the end of region III, near the catalytically active sites, it may have serious functional consequences. Also, G1363 is conserved across species. The schematic structure of lactase polypeptide and the location of the five CLD mutations are illustrated in fig. 4.

Opposite to the rare congenital lactase deficiency, adult-type hypolactasia (MIM 223100) is the most com-
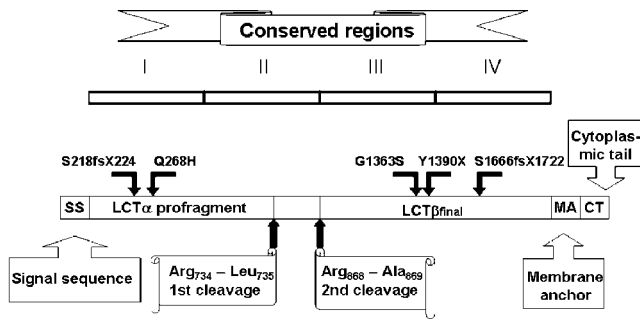
Figure 4 Structure of lactase and the location of the identified CLD mutations. The genomic size of the *LCT* gene is ~55 kb and is composed of 17 exons (Boll et al. 1991). The size of messenger RNA (mRNA) is 6,274 bases, and the primary translation product (prolactase) is 1,927 amino acids. The prolactase contains a cleavable signal sequence from Met_1 to Gly_{19} that guides the polypeptide to the endoplasmic reticulum (von Heijne 1986; Mantei et al. 1988). The region from Ser_{20} to Thr_{1882} consists of four homologous domains (I–IV). The prolactase is processed by two proteolytic cleavages. The first, an intracellular cleavage, occurs between Arg_{734} and Leu_{735}. The second cleavage in the intestinal lumen between Arg_{868} and Ala_{869} generates lactaseβ_{final}, the mature enzyme (Jacob et al. 1996; Wüthrich et al. 1996). The figure is modified from Naim (2001).

mon enzyme deficiency worldwide. It is caused by developmental down-regulation of lactase activity during childhood or early adulthood (Sahi 1994). The decline of lactase activity is a normal physiological phenomenon; however, the majority of northern Europeans have the ability to maintain lactase activity and digest lactose throughout life (lactase persistence). We previously identified C/T_{-13910}, a variant that is located 13.9 kb upstream of the *LCT* gene and is associated with the adult-type hypolactasia trait (Enattah et al. 2002). The down-regulation of lactase activity operates at the transcriptional level. *LCT* mRNA quantitated by minisequencing method showed that the T_{-13910} allele allows lactase gene expression and maintains lactase activity, whereas the C_{-13910} allele lets the down-regulation operate normally (Kuokkanen et al. 2003; Rasinperä et al. 2005). The lactase activity in Finns declines at 5–12 years of age (Rasinperä et al. 2004, 2005). It is of interest that the CLD Fin_{major} mutation, Y1390X in lactase, was systematically found here on the background of the lactase-persistent allele, T_{-13910}, in Finns. Thus, some CLD Fin_{major} carriers with the CT_{-13910} genotype may suffer from lactose-induced symptoms after down-regulation of the C_{-13910} allele.

In conclusion, this study confirms that both human lactase deficiencies are related to DNA variants affecting the *LCT* gene. Interestingly, the mutations resulting in the severe congenital form have direct consequences for the polypeptide leading to nonsense-mediated mRNA decay in at least 90% of the disease alleles, whereas the

critical nucleotide for the milder adult-type phenotype represents a distal enhancer regulating the transcript levels in intestinal cells (Kuokkanen et al. 2003; Olds and Sibley 2003; Troelsen et al. 2003; Rasinperä et al. 2005). Importantly, our study facilitates direct DNA-based diagnosis and carrier identification of CLD, which has so far been based on clinical symptoms supplemented by lactase activity assays from intestinal biopsy specimens.
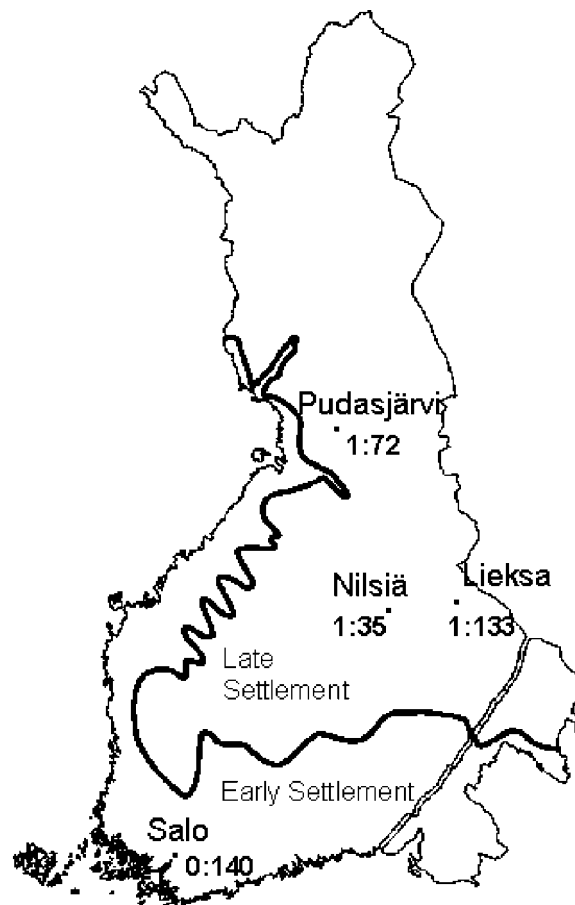
Acknowledgments

Figure 5 Carrier frequency of Y1390X in four subpopulations in the early and later settlement regions of Finland. The carrier frequencies of the CLD mutations were determined from 556 anonymous blood donors obtained from the Finnish Red Cross Blood Transfusion Service.

Web Resources

URLs for data presented herein are as follows:

Baylor College of Medicine (BCM) Search Launcher, http://searchlauncher.bcm.tmc.edu/

Marshfield Clinic Research Foundation Genetic Map, http://research.marshfieldclinic.org/

National Center for Biotechnology Information BLAST, http://www.ncbi.nlm.nih.gov/BLAST/

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim

UCSC Genome Browser, http://genome.ucsc.edu/

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