

# The Frequency of the $LCT^*-13910C>T$ Polymorphism Associated with Lactase Persistence Diverges among Euro-Descendant Groups from Brazil

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## Key Words

$LCT$  gene · Lactase persistence · Hypolactasia · Euro-Brazilians · Mennonites

## Abstract

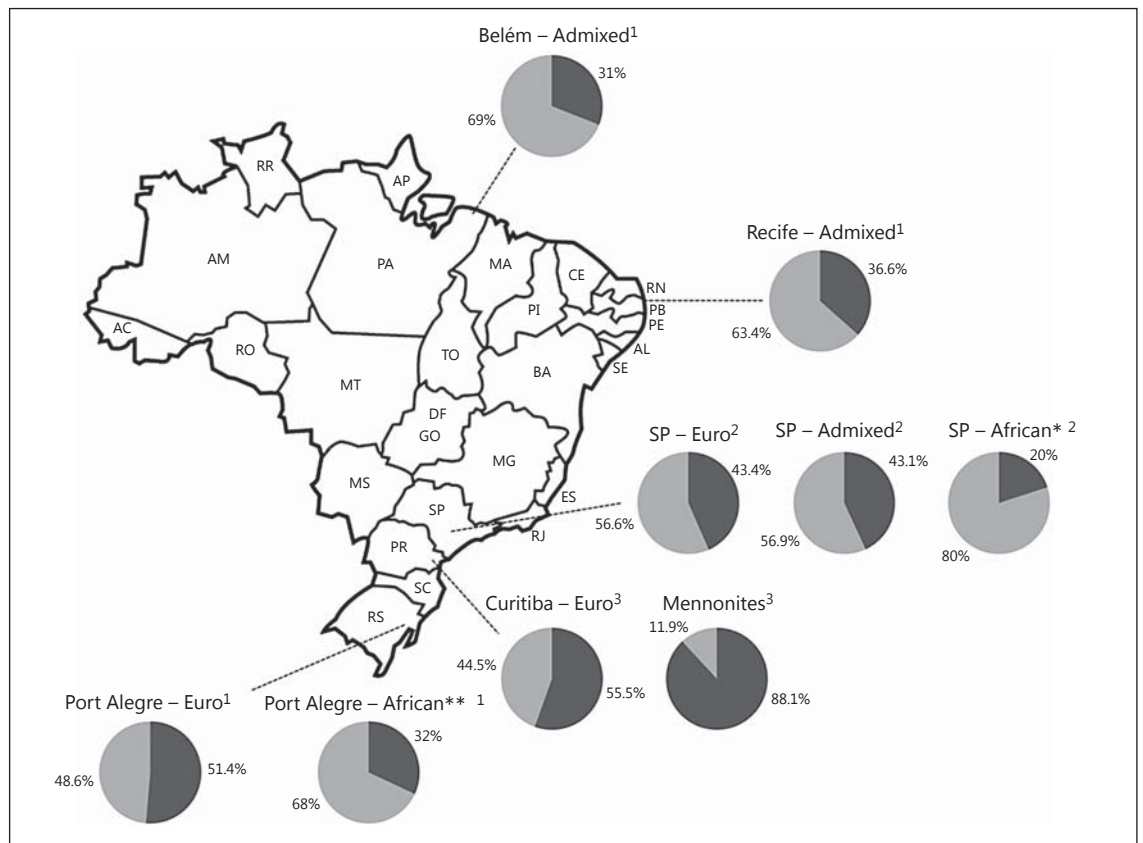
**Objective:** The aim of this study was to investigate the frequency of the  $LCT^*-13910C>T$  polymorphism associated with a high expression of lactase in the small intestine during adulthood, and to infer the lactase persistence and adult-type hypolactasia phenotypes among Euro-Brazilians and Mennonites from South Brazil. **Materials and Methods:** A sequence-specific PCR method to genotype the  $LCT^*-13910C>T$  polymorphism in 292 Euro-Brazilians and 151 Mennonites (a group with European ancestry and a long history of endogamy) was developed. Using an exact test of population differentiation, the genotype and allele frequency between these and other Brazilian populations were compared. **Results:** The frequency of  $-13910^*T$  was significantly higher among the Mennonites when compared to the Euro-Brazilian cohort (0.63 vs. 0.33,  $p < 0.000001$ ). Accordingly, Mennonites had a higher prevalence of the lactase persistence genotype (88.1 vs. 55.5%,  $p < 0.000001$ ). The distribution of  $-13910^*T$  differed between Mennonites and all other Brazilian groups ( $p < 0.0001$ ). The Euro-Brazilians from Cur-

tiba displayed differences when compared to all other Brazilian groups ( $p < 0.0001$ ), even to Euro-Brazilians from a different geographic region ( $p = 0.0003$ ), but were similar to those from Porto Alegre ( $p = 0.2$ ). **Conclusion:** Differences in the  $-13910^*T$ -associated lactase persistence distribution among Euro-Brazilian groups reflect the ancestry and admixture of each particular group and should be considered for adult-type hypolactasia screening.

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## Introduction

The lactase enzyme hydrolyzes lactose into galactose and glucose at the brush-border membrane of the small intestine. This enzyme is encoded by the lactase gene  $LCT$  located at chromosome 2q21, with 17 exons encoding an mRNA of 6,279 nucleotides [1, 2]. Although lactase expression and activity are high in newborns, they become downregulated after weaning in approximately 65% of individuals, causing adult-type hypolactasia or lactase non-persistence [3]. A lower capacity to hydrolyze lactose can result in gastrointestinal symptoms such as abdominal pain and distention, flatulence, diarrhea and vomiting, a condition known as lactose intolerance disorder [4]. A



**Fig. 1.** Distribution of the  $-13910C>T$ -associated LP (dark gray) and hypolactasia (light gray) among different Brazilian groups. \* Admixed people of African ancestry were not included; \*\* admixed people of African ancestry may have been included. <sup>1</sup> [8]; <sup>2</sup> [9]; <sup>3</sup> our study.

high prevalence of lactase persistence (LP) occurs in populations with a long history of pastoralism and milking, mainly in northern Europe and among nomads in Africa and the Middle East [3]. This phenotype is characterized by high levels of lactase during adulthood and a higher capacity to digest lactose. Enattah et al. [4] identified the major polymorphism responsible for this trait in Europeans, a  $C>T$  substitution in an enhancer region 13.9 kb upstream of the *LCT* gene ( $-13910C>T$ ), in intron 13 of the *MCM6* gene. Other causative single-nucleotide polymorphisms for LP occur in Africans and Middle-Eastern populations, all of them located in the same enhancer region [3]. The  $-13910*T$  allele is associated with enhanced mRNA expression of the *LCT* gene, causing LP in adulthood, even among heterozygotes, thus being inherited as a dominant trait [5].

Brazilian admixture has occurred since the Portuguese met different Amerindian tribes and Africans from Angola and Mozambique, brought in as slaves between the 16th and 19th century. After the abolishment of slavery

in 1850, the Brazilian government encouraged and supported the immigration of Europeans. Although European ancestry is predominant in Brazil, the range of admixture diverges across the geographic regions of the country [6]. The Mennonites are a pacifist group of German and Dutch ancestry, well-suited for genetic and epidemiological studies due to successive bottleneck effects caused by persecution in the 16th century, endogamy and successive migratory waves to the Soviet Union at the end of the 18th century and from there to North America, Paraguay and Brazil in the early 20th century [7].

Although  $LCT^*-13910C>T$  has been indicated as a good genetic marker for adult-type hypolactasia in Europe and in non-European countries [3, 8, 9], its frequency is unknown for Mennonites and is poorly estimated for Euro-Brazilians [8, 9]. Hence, the goal of this work was to estimate the prevalence of  $-13910C$ -associated adult-type hypolactasia among Mennonites and Euro-Brazilians from Curitiba (a major city of Paraná State) and to compare our results to those for other Brazilian groups.

## Materials and Methods

A total of 151 Mennonites (78 females and 73 males aged 18–89 years) and 292 Euro-Brazilians (150 females and 142 males aged 18–64 years) from South Brazil were included. The Euro-Brazilians were mainly blood donors from two blood banks of Curitiba. They were classified as ‘European-derived’ based on physical characteristics and self-reported ancestry. The project was approved by the Ethics Committee of the Hospital de Clínicas, Universidade Federal do Paraná. All participants were informed about the aims of the study and their written informed consent was obtained.

Peripheral blood samples were collected with EDTA and nucleic acid (DNA) extracted and amplified as previously published [10] using a touchdown strategy (10 cycles at 64°C, 10 at 62°C and 10 at 60°C). A fragment of 431 bp of the human growth hormone gene was coamplified in each reaction [10]. The *LCT\*–13910C>T* was identified using PCR sequence-specific (SSP) amplification of a 140-bp fragment with the forward primer *LCTf* (5'-GTT-TATAGAGTGCATAAAGACG-3') and the specific reverse primers *LCT–13910Cr* (5'-GTTCTTTGAGGCCAGGGG-3') or *LCT–13910Tr* (5'-GTTCTTTGAGGCCAGGGA-3'). Genotype and allele frequencies were obtained by direct counting and analyzed using the Arlequin 3.5 software and Fisher's exact test.

## Results

Both groups were in Hardy-Weinberg equilibrium. The allele frequency of *–13910\*T* was almost twice as high in Mennonites (0.65) as in Euro-Brazilians (0.33). The genotypes for *LCT\*–13910T*-associated LP, *–13910C/T* and *T/T*, were observed in 88% of Mennonites and in 55.5% of Euro-Brazilians ( $p < 0.000001$ ). Conversely, the genotype for *LCT\*–13910C*-associated adult-type hypolactasia (*–13910C/C*) was observed in 12% of Mennonites and in 44.5% of Euro-Brazilians ( $p < 0.000001$ ). In other words, Mennonites had an almost 6 times higher chance of presenting the *–13910T*-associated LP than Euro-Brazilians [OR 5.93 (95% CI 3.44–10.21)]. The distribution of *–13910\*T* differed significantly between Mennonites,

Euro-Brazilians and other Brazilian groups ( $p < 0.0001$ ). The Euro-Brazilians were similar to those from Porto Alegre ( $p = 0.2$ ), a city also located in South Brazil, but differed from all other Brazilian groups ( $p < 0.0001$ ), even from Euro-Brazilians from a different geographic region ( $p = 0.0003$ ; fig. 1).

## Discussion

According to our results, across all Brazilian groups investigated, Mennonites had the highest frequency of the *LCT\*–13910C>T* polymorphism and, accordingly, of the LP-associated genotypes, followed by Euro-Brazilians from South Brazil. The Mennonites differed from Euro-Brazilians who live in the same geographic area, which could be due to the founder effects suggested to have occurred among them [7]. The *–13910\*T* frequency was higher in Euro-Brazilians than in admixed and Afro-Brazilian groups. Thus, the allelic distribution of *–13910\*T* may reflect the ancestry and different admixture rates across various Brazilian geographical regions.

## Conclusion

Differences in the *–13910*-associated LP distribution across the various Brazilian groups are remarkable. They should thus be considered for adult-type hypolactasia screening as well as for dietary genetic counseling regarding milk consumption and digestive problems. Since there are other causative variants for LP, further studies are needed to investigate whether the distribution of the *–13910\*T* polymorphism is consistent with the distribution of the LP phenotype in Brazil.

## References

- 1 Boll W, Wagner P, Mantei N: Structure of the chromosomal gene and cDNAs coding for lactase-phlorizin hydrolase in humans with adult-type hypolactasia or persistence of lactase. *Am J Hum Genet* 1991;48:889–902.
- 2 Harvey CB, Fox MF, Jeggo PA, et al: Regional localization of the lactase-phlorizin hydrolase gene, LCT, to chromosome 2q21. *Ann Hum Genet* 1993;57:179–185.
- 3 Ingram CJE, Mulcare CA, Itan Y, et al: Lactose digestion and the evolutionary genetics of lactase persistence. *Hum Genet* 2009;124:579–591.
- 4 Enattah NS, Sahi T, Savilahti E, et al: Identification of a variant associated with adult-type hypolactasia. *Nat Genet* 2002;30:233–237.
- 5 Rasinperä H, Kuokkanen M, Kolho KL, et al: Transcriptional downregulation of the lactase (LCT) gene during childhood. *Gut* 2005;54:1660–1661.
- 6 Pena SD, di Pietro G, Fuchshuber-Moraes M, et al: The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 2011;6:e17063.
- 7 Jaworski MA, Slater JD, Severini A, et al: Unusual clustering of diseases in a Canadian Old Colony (Chortitza) Mennonite kindred and community. *CMAJ* 1988;138:1017–1025.
- 8 Mattar R, Monteiro MS, Villares CA, et al: Frequency of LCT-13910C>T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups. *Nutr J* 2009;8:46–49.
- 9 Friedrich DC, Santos SE, Ribeiro-dos-Santos ÂK, et al: Several different lactase persistence-associated alleles and high diversity of the lactase gene in the admixed Brazilian population. *PLoS One* 2012;7:e46520.
- 10 Boldt AB, Sanchez MI, Stahlke ER, et al: Susceptibility to leprosy is associated with M-ficolin polymorphisms. *J Clin Immunol* 2013;33:210–219.