BMJ open

The European lactase persistence genotype determines lactase persistence in the Hispanic and Amerindian Chilean population: relevance for prevalence of adult type hypolactasia and lactose intolerance in Latin American populations.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000125
Article Type:	Research
Date Submitted by the Author:	02-Apr-2011
Complete List of Authors:	Morales, Eugenia; Pontificia Universidad Catolica de Chile, Gastroenterology Azocar, Lorena; Pontificia Universidad Catolica de Chile, Gastroenterology Maul, Ximena; Pontificia Universidad Catolica de Chile, Medical student Perez, Claudio; Pontificia Universidad Catolica de Chile, Medical student Klassen, Julieta; Pontificia Universidad Catolica de Chile, Nutrition and Diabetes Nervi, Flavio; Pontificia Universidad Catolica de Chile, Gastroenterology Chianale, Jose; Pontificia Universidad Catolica de Chile, Gastroenterology Miquel, Juan; Pontificia Universidad Catolica de Chile, Gastroenterology
Subject Heading:	Gastroenterology
Keywords:	lactose intolerance, lactase persistence state, -13910T variant, IBS

The European lactase persistence genotype determines lactase persistence in the Hispanic and Amerindian Chilean population: relevance for prevalence of adult type hypolactasia and lactose intolerance in Latin American populations.

Eugenia Morales[1], Lorena Azocar[1], Ximena Maul[2], Claudio Perez[2], Julieta Klassen[3], Flavio Nervi[1], José Chianale[1], Juan Francisco Miquel[1*].

Department of Gastroenterology[1], Medical Students[2], and Department of Nutrition&Diabetes[3], Faculty of Medicine, Pontificia Universidad Católica de Chile.

Keywords: lactose intolerance, lactase persistence state, -13910T variant, IBS.

***Correspondence**:

Juan Francisco Miquel, MD

Marcoleta 367, Santiago

Telephone: 56 2 3543830

Fax: 56 2 6397780

e-mail: jfmiquel@med.puc.cl

Word count: 3,538

Abstract

Background: The lactase persistent (LP) or lactase non persistent (LNP) state in European adults is genetically determined by a single nucleotide polymorphism (SNP) located 13.9 kb upstream of the lactase (LCT) gene, known as LCT C>T_{-13910} (rs4988235). The LNP condition causes an inability to digest the milk sugar lactose leading to gastrointestinal symptoms and can affect nutrient and calcium intake in certain populations. **Objectives**: We studied a group of 51 Chilean patients to assess whether this SNP influences the LP/LNP state in this population, and determined the prevalence of LCT C>T_{-13910} genotypes in a representative sample of 216 Hispanics and 43 Amerindians with correlation to digestive symptoms. **Design**: Case-Control study done in Chilean patients with clinical suspicion of LNP that were assessed using clinical survey, hydrogen breath test (HBT), and SNP genotyping. The population sample of Hispanics and Amerindians was assessed by clinical survey and SNP genotyping. **Results**: Of the 51 patients with clinical suspicion of LNP, 29 were HBT positive. The CC genotype (LNP) was present in 89.7% of the patients with positive HBT and in only 4.7% of those with negative HBT. The prevalence of the CC genotype was 56.9% in the Hispanic population and 88.3% in Amerindians and was associated with a higher self reported clinical intolerance to dairies ingestion. **Conclusion**: The LP/LNP state is determined by the LCT C>T_{-13910} SNP in Chileans. This variant predicts digestive symptoms associated with the ingestion of lactose and is a good tool for the diagnosis of primary adult hypolactasia. The LCT T_{-13910} allele is rare in the Amerindian population and is suggestive of European ancestry in this contemporary population.

ARTICLE SUMMARY

Article focus: The aims of the present study were three questions; 1) If the European variant C/T-13910 determined lactose-persistent and lactose non-persistent state in adult Chilean Hispanic and Amerindian population; 2) If there is a correlation between the C/T-13910 genotyping and the hydrogen breath test (HBT) for diagnosis of LNP in our population; 3) To determine for the first time in an Latin American population the prevalence of the lactose non-persistence genotype (CC) and its correlation with digestive symptoms and ingestion of dairy products.

Significance of the study: The three key messages of the present study were: 1) We demonstrate for the first time that the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian population. 2) We demonstrate a strong correlation between the C-13910>T genotype with the hydrogen breath test and lactose tolerance in a group of patients with clinical suspicion of lactose intolerance. 3) In a representative sample of the Hispanic Chilean population (Mestizos) and in Amerindians, we observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between ethnic groups (56% and 88%, respectively). Interestingly, C-13910>T genotype was significantly associated with digestive symptoms and self reporting intolerance to dairy ingestion. This could explain the observed low consumption of dairy products (30 L milk/person/year) in our population.

Strengths and limitations of the study:

- Our strengths:

- This is the first study in Latin America that assesses if the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian general population.

- Our results may be of clinical and epidemiological relevance, not only for the Chilean population, but also for other Hispanic and Amerindian populations of the Americas with similar genetic background. The fact that the LNP state is an inherited frequent condition in Mestizo and Amerindians should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new and high quality lactose-free dairy products in these regions.

- We randomly selected a representative sample of the Hispanic Chilean population and Amerindians (n=216), and performed the genotyping of LCT C>T $_{-13910}$. We observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between both ethnic groups (56% and 88%, respectively), that correlates with digestive symptoms and self reporting intolerance to dairy ingestion.

- **Our limitations:**

  - Not randomized recruitment of the 51 patients with suspicion of lactose intolerance could induce selection bias.

  - A small number of patients with suspicion of lactose intolerance (n= 51).

- Semi-quantitative food frequency questionnaire with a 24 h dietary recall obtained in a subgroup of the Hispanics and performed years before the genetic analysis.

INTRODUCTION

Lactose, the main sugar in milk, must be hydrolyzed by lactase-phlorizin hydrolase into glucose and galactose for absorption. Intestinal activity of the lactase enzyme (LCT) (MIM 603202) decreases in all mammals after weaning, resulting in poor lactose absorption (1). In humans this condition is termed "lactase non-persistence" (LNP), "adult type hypolactasia", or "lactose intolerance" (MIM 223100) and is estimated to occur in approximately 65% of the contemporary world population (2, 3). LNP can generate intolerance to the ingestion of dairies with the development of abdominal and systemic symptoms that lead to medical consultations and evaluations. In contrast, lactase persistent (LP) individuals remain tolerant of lactose and maintain high levels of LCT expression throughout adult life (4). The prevalence of the LP phenotype, which is an autosomal dominant condition (MIM 223100) is reported to vary between 0 and 95% in different populations (3), but is unknown in contemporary Latin American populations (2, 5).

The recent discovery of single nucleotide polymorphisms (SNPs) that determine the LP state in humans, both in Europe (6) and among some pastoral populations in Africa, has generated growing interest in the study of this condition (2, 7). The appearance of these SNPs seems to have occurred on more than one occasion in human evolution between 1,500 and 12,000 y ago in northern Europe and among some nomadic populations of Asia and Africa (7). The SNP known as LCT C>T_{-13910} (rs4988235) is located 13.9 kb upstream of the LCT gene in the 13th intron of an adjacent gene (MCM6). The T allele of this SNP correlates with the LP state in European populations (6), and individuals with this allele have higher levels of LCT transcripts in enterocytes compared to individuals who carry the C allele (8). *In vitro* studies show that cells transfected with the LCT T_{-13910} variant show increased activity

of the LCT promoter (9). Another SNP known as LCT G>A$_{-22018}$, shows some association with the LP/LNP phenotype, and while it does not appear to be the causal variant, it is present in a block of linkage disequilibrium with little evidence of recombination in this region of the genome (5, 10).

In clinical practice, the LNP state is relevant because it can generate malabsorption and intolerance of lactose, with the development of clinical symptoms such as abdominal pain and distension, diarrhea, bloating, nausea, and vomiting (3). A significant percentage of patients do not relate these symptoms to dairies ingestion (11), and the recurring nature of the symptoms can lead to misdiagnosis of chronic diarrhea, irritable bowel syndrome, celiac illness, or other clinical conditions (12-16). The diagnosis of LNP is often based on clinical suspicion and the favorable response to a lactose restricted diet.

The most widely used diagnostic test of poor lactose absorption is the hydrogen breath test (HBT) (11, 17). Nevertheless, this test has practical limits and its sensitivity and specificity varies between 69 - 100% and 89 - 100%, respectively, compared to measurement of LCT activity in intestinal biopsies. Additionally, there is a false negative rate of 11 - 30%, and a false positive rate of up to 57% using this test (18-21). Using SNP genotyping in patients with suspected LNP may therefore aid in proper diagnosis (10, 18, 22-26). The only epidemiological study of adult hypolactasia in a Chilean population was performed more than three decades ago (27) and suggested that the prevalence of LNP was 56% and 75% for the pediatric and adult populations, respectively. Our objectives were to determine the influence of the LCT C>T$_{-13910}$ SNP on the LP/LNP state in the Chilean population and to determine the population prevalence of the SNP genotypes in a representative Hispanic and Amerindian

population with comparison of the genotypes to clinical symptoms related to dairies ingestion.

SUBJECTS AND METHODS

Patients with clinical suspicion of lactase non persistence (LNP).

In 2006, 51 patients were enrolled in a prospective study after being referred to the Gastroenterological Department of the Clinical Hospital at the Pontificia Universidad Catolica de Chile because of symptoms suggestive of LNP. A pre-coded survey was obtained from all patients to obtain demographic, anthropometric, familial, and clinical data, including any symptoms experienced during the diagnostic hydrogen breath test (HBT) such as abdominal pain, diarrhea, bloating, nausea, and vomiting. A 5 ml EDTA blood sample was obtained to extract DNA. Serum levels of immunoglobulin A and tissue anti-transglutaminase antibodies (hu tTG ELISA, IMMCO Diagnostics, Inc., Buffalo, NY) were quantified in all the patients to rule out the existence of celiac disease. This study was approved by the Ethics Committee of the Faculty of Medicine, PUC, and all patients signed a written informed consent form prior to the study.

Hydrogen breath test (HBT) for diagnosis of LNP.

Patients were instructed to maintain a low fiber diet without lactose for 48 h prior to the day of the examination. After 12 h of fasting, a baseline concentration of H_2 in exhaled breath was determined using a Quintron® microLyzer (QuinTron Instrument Company, Milwaukee, WI). Subjects then ingested 25 g lactose and H_2 concentration in breath was quantified every 20 m for a maximum of 4 h. Individuals were considered LNP with an increase in H_2 concentration 20 ppm above the base value (17, 28).

Population study.

A representative sample of 216 unrelated Hispanics and 43 unrelated Amerindians were randomly selected from an initial sample of 1581 Hispanics and 120 Amerindians (Mapuches, the Chilean native population) which were collected between 1993 and

2000 as described previously (29, 30). The sample size was selected by estimating an expected prevalence of the LCT-13910CC genotype to be at least 50% in the Hispanic population and 80% in the Amerindians, with an alpha error of 5% and a level of confidence of 80%. All study subjects provided a DNA sample and a medical survey that provided anthropometric and clinical data including information about diarrhea, abdominal pain, or bloating in response to ingestion of dairy products (29, 30). Furthermore, a subgroup of the Hispanic subjects provided a semi-quantitative food frequency questionnaire with a 24 h dietary recall. From this data, specific energy and nutrient ingestion from dairy products was estimated using the Datadiet program (29, 31). For all subjects, the occurrences of digestive symptoms in response to consumption of lactose were correlated to SNP genotyping results.

Genotyping of LCT C>T $_{-13910}$ and LCT G>A $_{-22018}$ SNPs

SNP genotyping was performed using PCR-RFLP methods (32) (GenBank reference sequence NM_005915.4). Briefly, genomic DNA was extracted from 300 µl of blood according to manufacturer's instructions (Wizard SV Genomic DNA Purification System, Promega, Madison, WI) and the regions surrounding the SNPs were amplified through PCR using primers (F) 5´-GGA TGC ACT GCT GTG ATG AG-3´ and (R) 5-CCC ACT GAC CTA TCC TCG TG-3´ for the LCT C>T $_{-13910}$ and (F) 5´-AAC AGG CAC GTG GAG GAG TT-3´ and (R) 5´- CCC ACC TCA GCC TCT TGA GT-3´ for LCT G>A $_{-22018}$. In both cases, the amplified products were of 448 bp. The PCR reactions and RFLP assays for the analysis of both genetic variants was carried out as described previously (32). Briefly, the amplified product for the LCT C>T $_{-13910}$ SNP was digested with *BsmFI*, generating two fragments of 351 and 97 bp in the presence of the CC genotype, and 3 fragments of 253, 98, and 97 bp in the presence of the TT genotype (**Figure 1**). For the LCT G>A $_{-22018}$ SNP, the PCR product was digested with

HhaI, generating two fragments of 284 and 184 bp in the presence of the GG genotype and one undigested fragment of 448 bp in the presence of the A/A genotype. The amplified and digested PCR products were separated by electrophoresis on 2% agar gels in 1X TAE buffer stained with ethidium bromide. Genotyping confirmation was carried out on 35 samples by direct sequencing of the 448 bp amplified fragments (models ABI-310 and ABI-3100 automated DNA sequencers, Applied Biosystems, Foster City, CA*)* (**Figure 1**). There was complete concordance between the PCR-RFLP analysis and the sequencing in all cases.

Statistical analysis

Mean and standard deviations were used to describe numeric variables. The variables of categorical types are given as a number and a percentage. Comparisons of continuous variables were performed using the student T test for independent samples. Comparisons of categorical variables were carried out using the chi-square test. Using the HBT breath test as diagnostic for LNP, an analysis was made of the sensitivity and specificity of the genetic test as an alternative diagnostic test. Differences were considered significant when $p<0.05$.

RESULTS

Hydrogen breath test and SNP genotyping

Fifty-one patients with clinical suspicion of LNP (44 women and 7 men, age 14 - 79 y) were included in the study. Twenty-nine patients (56.8%) had a positive HBT and 22 were negative (43.2%) (**Table 1**). There were no significant differences in the distribution by sex and age between the two groups. There was a positive correlation between the HBT results and digestive symptoms reported during the test: 82% of the patients with positive HBT had symptoms associated with the lactose load, such as bloating, abdominal pain, and diarrhea (79%, 58% and 20%, respectively). In contrast, only 27% of the patients with negative HBT presented symptoms after ingesting lactose ($p < 0.0001$). Additionally, 76% of patients with positive HBT indicated a personal and/or family history of functional digestive disorders, compared to 36% of those with negative HBT ($p < 0.0001$).

The LCTC>T_{-13910} SNP genotyping results for the 51 patients is shown in Table 1. Of the 29 patients with positive HBT, 26 (89.7%) had the LCT-13910CC genotype (the LNP genotype), and 3 (10.3%) had the LCT-13910CT genotype and were therefore phenotypically LNP but genotypically LP. However, two of these 3 patients experienced bloating and diarrhea during the HBT, which suggests malabsorption of lactose. Of the 22 patients who had a negative HBT, 19 were LCT-13910CT, two were LCT-13910TT, and a single 16-year-old patient had the LNP LCT-13910CC genotype (Table 1). However, this patient experienced abdominal pain during the HBT. Subsequent evaluation of this patient with a lactulose HBT (10 g load) revealed that this patient was not a non-producer of hydrogen. A single patient with LNP genotype and positive HBT had high levels of anti-tTG (27 μU/ml), which suggests the possibility of

coexistence of LNP and celiac disease. All the patients showed normal levels of serum IgA (data not shown).

There was complete genotypic correlation between the LCTC>T_{-13910} and the LCTG>A_{-22018} SNPs, such that any subject homozygous or heterozygous for one SNP was at the same time homozygous or heterozygous for the other SNP (data not shown). Furthermore, there was good correlation between the LCTC>T_{-13910} SNP findings and the HBT test results (**Table 2**). Genotyping results were confirmed by sequencing for 35 of the 51 samples (**Figure 2**). Interestingly, one of the three discordant patients with LNP phenotype but LP genotype was also heterozygous at a second location not previously described in the literature, LCTG>A_{-13937}.

Population study

We collected DNA samples and medical surveys from 216 unrelated Hispanic individuals and 43 unrelated Amerindians. The survey information is summarized in **Table 3**. The two groups were similar in age, with higher representation of females among the Amerindians. The Amerindian cohort reported a higher frequency of intolerance to ingesting dairy products and recurring diarrhea while both groups reported recurring abdominal bloating with equal frequency.

The genotype and allele frequencies for the LCTC>T_{-13910} SNP are shown in **Table 3**. As was found in the study group of 51 Chilean patients with suspected lactose intolerance, there was complete concordance between the genotypes of the LCTC>T_{-13910} and LCTG>A_{-22018} SNPs in each individual (data not shown). The cohort of Amerindian individuals was in Hardy-Weinberg equilibrium (**Figure 2**), but we observed a slightly higher than expected frequency of the T_{-13910} allele in the Hispanic population (deviation from HW equilibrium $p < 0.05$). The prevalence of the ancestral LCT-13910CC genotype (the LNP genotype) was 56.9%, significantly lower than the

prevalence of this genotype among the Amerindian individuals (88.3%). In turn, 41.7% of the Hispanics were LCT-13910CT genotype compared to only 11.7% of the Amerindians. The LCT-13910TT genotype was found in only 3 Hispanic subjects. Allele frequencies for the LCTC>T_{-13910} SNP were significantly different between the two groups, with the T_{-13910} allele being very infrequent in Amerindians.

For the 216 Hispanic individuals studied, we were able to gather clinical and genetic data (**Table 3**). Individuals with the LNP LCT-13910CC genotype were 3 times more likely to report intolerance to the ingestion of dairy products and recurring diarrhea than those with LP genotypes (LCT-13910CT or LCT-13910TT). For 112 of these individuals, we collected complete nutritional information. The consumption of dairy products was in general very low, equivalent to an average of 30 L of fresh milk per year per person. However, the group of individuals with an LP genotype had 38% higher consumption of dairy products than the group of individuals with the LNP genotype (38.4 L ± 67 L versus 27.7 L ± 40 L milk/year, respectively), although this difference was not statistically significant.

DISCUSSION

The normal physiologic decrease in lactase (LCT) production in the mammalian intestine following weaning is known as lactase non persistance (LNP), and results in the inability to digest the milk sugar, lactose, which can cause gastrointestinal symptoms after ingestion of dairies. The condition of lactase persistence (LP), found only in humans, allows the individual to continue to ingest and absorb lactose into adulthood. In European populations and some Afro-Arab pastoral groups, the LP condition has been shown to be due to a SNP (rs4988235) found 13.9 kb upstream of the LCT gene and actually located within the 13th intron of the MCM6 gene. This SNP, known in the literature by many names but herein called LCTC>T$_{-13910}$, appeared relatively recently in human history (approximately 10,000 y ago) and has spread progressively throughout the world through population migration and mixing, increasing the state of LP in different populations given its autosomal dominant property (2). The original populations of the Americas and Asia were likely LNP. There is no evidence of the existence of American variants that might have generated the LP state in the pre-Columbian era (2, 5). The T$_{-13910}$ allele, which leads to the LP state, was likely introduced to the Americas by the migration of European carriers of this variant approximately 500 years ago and spread rapidly in the Hispanic populations of contemporary America, as is shown in this study as well as others (5, 33).

Our study of a representative population of Chile consisting of Hispanic and Amerindian individuals showed the prevalence of the LNP state (LCT-13910CC genotype) to be 57% and 88%, respectively. It is interesting to note that this observed genotypic frequency is very similar to the Amerindian admixture index (AAI) estimated by us in these same populations based on the distribution of ABO blood groups (29).

This suggests that the analysis of the LCTC>T_{-13910} SNP in Latin American populations can be a good indicator of the degree Amerindian or Caucasian inheritance.

In all Chilean patients genotyped in this study, the LCTG>A_{-22018} SNP genotype correlated completely with the genotype found for the LCTC>T_{-13910} SNP, a finding reported in many studies. This phenomenon can be explained by the fact that both SNPs are located within a highly conserved block of linkage disequilibrium of at least 500 kb and likely co-segregate (2, 5).

In the group of 216 unrelated Hispanic individuals, we observed a higher than expected frequency of the T_{-13910} allele, which leads to the LP state. While this could be explained by selection bias or by a higher frequency of LP in the Spanish population that founded the Chilean colony, it could also be reflective of positive selection for LP allele carriers, as has been suggested for decades (34-36). Any evolutionary advantage conferred by the T_{-13910} allele may or may not be related to the consumption of dairy products. Indeed, it was recently reported that this allele confers protection not only against bone fractures (37, 38), but also colon cancer (39).

The validity of using LCTC>T_{-13910} SNP genotyping for diagnosis of the LNP or LP state was evaluated in the group of 51 Chilean patients with suspected LNP. Using HBT results as a reference, SNP genotyping showed a specificity of 95.5% and a sensitivity of 93% for the detection of LNP. These results are similar to those recently reported by other groups (10, 25, 32), and confirm the utility of the analysis of this SNP as a clinical test for the diagnosis of LP/LNP in the adult Chilean population. Only 3 patients (10.3%) with malabsorption of lactose shown by HBT, were genetically LP (LCT-13910CT genotype). It was not possible to recall these 3 patients to carry out complementary studies to rule out other causes of lactose malabsorption, such as intestinal parasitic infections like Giardiasis or seronegative celiac disease.

Interestingly, one of these patients was also heterozygous at an additional SNP not previously described, LCTG>A$_{-13937}$ (2). We do not know if this new variant can explain the phenotype-genotype discordance.

A recent study showed a high prevalence of celiac illness among patients with an initial diagnosis of LNP, suggesting that lactose intolerance can be the first manifestation of celiac illness; the authors recommend considering the existence of celiac illness in all patients with LNP before recommending a lactose free diet for life (40). In our study of 51 patients with clinical suspicion of lactose intolerance, only one with LNP confirmed by HBP and SNP genotyping had positive anti-tTG (2% of the total) suggesting the coexistence of both conditions.

One 16-year-old patient had negative HBT yet had the LNP genotype. It is possible that this patient has not yet manifested the LNP phenotype and still maintains sufficient levels of intestinal lactase to digest a 25 g load. The age at which the LNP state is initiated varies among different populations and ethnic groups, starting as early as 1 to 8 y in black and Asian populations, and much later (20 y) in northern European populations (10, 41-43). The age of initiation of the LNP state in Chilean individuals remains unknown.

Among the 51 Chilean patients with positive HBT and the LCT-13910CC genotype, significantly more report having relatives with functional digestive disorders than their HBT negative counterparts, suggesting that the condition of LNP could be in part responsible for digestive symptoms. In our complete clinical, nutritional, and genetic study of 112 Hispanic individuals, those with the LCT-13910CC genotype were significantly more likely to self-report lactose intolerance despite very low (30 L milk/person/year) consumption of dairy products. This level is well below the WHO recommended level of 240 L milk/person/year. Reasons for the low level of

consumption are likely due to multiple causes including cost, preference, and societal habits which are likely influenced by the existence of a high prevalence of LNP state within this population. It has been reported that the allele frequencies of the LCTC>T$_{-13910}$ SNP within a population correlates with the tendency of that population to consume dairy products and ingest calcium (44-46). Both this study and one other (47) show that the effective consumption of dairy products and calcium in Chile remains below international recommendations.

In summary, the LNP state continues to be predominant in the Chilean population. Epidemiologically, this may cause the development of digestive symptoms affecting quality of life and leading to medical consultations as well as lower tendency to ingest dairy products and calcium within the adult population of the contemporary Americas. The fact that the LNP state is an inherited condition should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new lactose-free dairy products.

Table 1. Clinical and genetic characteristics of 51 Chilean patients having hydrogen breath test (HBT) for suspicion of lactose non persistence (LNP).

	HBT Positive (n = 29)	**HBT Negative (n = 22)**	***p***
Female gender (%)	82.7	86.3	n.s.
Age (y)	36 ± 19	32 ± 12	n.s.
Number reporting gastrointestinal symptoms after lactose load	24 (82%)	6 (27%)	< 0.001
Number reporting family history of IBS	22 (76%)	8 (36%)	< 0.001
LCT-13910 CC genotype	26 (89.6%)	1 (4.5%)	< 0.001
LCT-13910 CT/TT genotype	3 (10.3%)	21(95.5%)	< 0.001

IBS, irritable bowel syndrome.

Table 2. Correlation of the LCT-13910CC genotype with positive HBT result in 51 Chilean patients, demonstrating sensitivity and specificity of genotyping for diagnosis of LNP.

Sensitivity	92.8%
Specificity	95.4%
Predictive value positive	96.2%
Predictive value negative	91.3%
LHR	20.4

LHR, likelihood ratio

Table 3. General characteristics, self reported gastrointestinal symptoms related to dairy ingestion and LCTC>T$_{-13910}$ SNP genotype and allele frequencies in a population of Hispanic and Amerindian individuals.

	Hispanics n = 216	**Amerindians** n = 43	*P*
Women (%)	46	65	0.02
Age (y)	50 ± 12	54 ± 15	n.s.
Number self reporting lactose intolerance	44 (20.4%)	19 (44.1%)	0.001
Number reporting bloating	77 (35.6%)	14 (32.5%)	n.s.
Number reporting diarrhea	15 (7%)	16 (37.2%)	0.06
LCT-13910 CC genotype	123 (56.9%)	38 (88.3%)	< 0.001
LCT-13910 CT genotype	90 (41.7%)	5 (11.7%)	< 0.001
LCT-13910 TT genotype	3 (1.4%)	0	n.s.
C allele frequency	77.7%	94.2%	< 0.001
T allele frequency	22.3%	5.8%	< 0.001

n.s., not significant

Author's contribution: Study concept and design (JFM); acquisition of data (JFM, EM, LA, JK, XM and CP); analysis and interpretation of data (JFM, EM, JCh); drafting of the manuscript (JFM, EM); critical revision of the manuscript for important intellectual content (JCh, FN); statistical analysis (JFM, EM); obtained funding (JFM, FN); final revision and edition (JFM, CP, XM).

Acknowledgments: The authors acknowledge the Alexander von Humboldt Foundation (Germany) for donating the NanoDrop ND-1000 Spectrophotometer used for DNA analysis and quantification. This study was partially supported grants of the National Commission of Scientific and Technological Research (CONICYT), FONDECYT N°1080325 to JFM, and from the Office of Research affairs of the Pontificia Universidad Catolica de Chile, School of Medicine to EM.

Competing Interest: We declare no conflicts of interest.

REFERENCES

1. Enattah N, Pekkarinen T, Valimaki MJ, Loyttyniemi E, Jarvela I. Genetically defined adult-type hypolactasia and self-reported lactose intolerance as risk factors of osteoporosis in Finnish postmenopausal women. Eur J Clin Nutr 2005;59:1105-1111.

2. Ingram CJ, Mulcare CA, Itan Y, Thomas MG, Swallow DM. Lactose digestion and the evolutionary genetics of lactase persistence. Hum Genet 2009;124:579-591.

3. Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice--myths and realities. Aliment Pharmacol Ther 2008;27:93-103.

4. Matthews SB, Waud JP, Roberts AG, Campbell AK. Systemic lactose intolerance: a new perspective on an old problem. Postgrad Med J 2005;81:167-173.

5. Bersaglieri T, Sabeti PC, Patterson N, Vanderploeg T, Schaffner SF, Drake JA, Rhodes M, et al. Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet 2004;74:1111-1120.

6. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat Genet 2002;30:233-237.

7. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, et al. Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 2007; 39:31-40.

8. Kuokkanen M, Enattah NS, Oksanen A, Savilahti E, Orpana A, Jarvela I. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. Gut 2003;52:647-652.

9. Lewinsky RH, Jensen TG, Moller J, Stensballe A, Olsen J, Troelsen JT. T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro. Hum Mol Genet 2005;14:3945-3953.

10. Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela I, et al. A genetic test which can be used to diagnose adult-type hypolactasia in children. Gut 2004;53:1571-1576.

11. Shaw AD, Davies GJ. Lactose intolerance: problems in diagnosis and treatment. J Clin Gastroenterol 1999;28:208-216.

12. Parker TJ, Woolner JT, Prevost AT, Tuffnell Q, Shorthouse M, Hunter JO. Irritable bowel syndrome: is the search for lactose intolerance justified? Eur J Gastroenterol Hepatol 2001;13:219-225.

13. Vesa TH, Seppo LM, Marteau PR, Sahi T, Korpela R. Role of irritable bowel syndrome in subjective lactose intolerance. Am J Clin Nutr 1998;67:710-715.

14. Bode S, Gudmand-Hoyer E. Incidence and clinical significance of lactose malabsorption in adult coeliac disease. Scand J Gastroenterol 1988;23:484-488.

15. Srinivasan U, Jones E, Weir DG, Feighery C. Lactase enzyme, detected immunohistochemically, is lost in active celiac disease, but unaffected by oats challenge. Am J Gastroenterol 1999;94:2936-2941.

16. Barr RG, Levine MD, Watkins JB. Recurrent abdominal pain of childhood due to lactose intolerance. N Engl J Med 1979;300:1449-1452.

17. Peuhkuri K, Poussa T, Korpela R. Comparison of a portable breath hydrogen analyser (Micro H2) with a Quintron MicroLyzer in measuring lactose maldigestion, and the evaluation of a Micro H2 for diagnosing hypolactasia. Scand J Clin Lab Invest 1998;58:217-224.

18. Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice - myths and realities. Aliment Pharmacol Ther 2007.

19. Rosado JL, Solomons NW. Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. Clin Chem 1983;29:545-548.

20. Barillas-Mury C, Solomons NW. Test-retest reproducibility of hydrogen breath test for lactose maldigestion in preschool children. J Pediatr Gastroenterol Nutr 1987;6:281-285.

21. Arola H, Koivula T, Jokela H, Jauhiainen M, Keyrilainen O, Ahola T, Uusitalo A, et al. Comparison of indirect diagnostic methods for hypolactasia. Scand J Gastroenterol 1988;23:351-357.

22. Jarvela IE. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). Scand J Clin Lab Invest 2005;65:535-539.

23. Bodlaj G, Stocher M, Hufnagl P, Hubmann R, Biesenbach G, Stekel H, Berg J. Genotyping of the lactase-phlorizin hydrolase -13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. Clin Chem 2006;52:148-151.

24. Enattah NS, Kuokkanen M, Forsblom C, Natah S, Oksanen A, Jarvela I, Peltonen L, et al. Correlation of intestinal disaccharidase activities with the C/T-13910 variant and age. World J Gastroenterol 2007;13:3508-3512.

25. Schirru E, Corona V, Usai-Satta P, Scarpa M, Oppia F, Loriga F, Cucca F, et al. Genetic testing improves the diagnosis of adult type hypolactasia in the Mediterranean population of Sardinia. Eur J Clin Nutr 2007;61:1220-1225.

26. Satta PU, Congia M, Schirru E, Scarpa M, Mura G. Genetic testing is ready to change the diagnostic scenario of lactose malabsorption. Gut 2008;57:137-138; author reply 138.

27. Lacassie Y, Weinberg R, Monckeberg F. Poor predictability of lactose malabsorption from clinical symptoms for Chilean populations. Am J Clin Nutr 1978;31:799-804.

28. Rana S, Bhasin DK, Gupta D, Mehta SK. Assessment of optimal dose of lactose for lactose hydrogen breath test in Indian adults. Indian J Gastroenterol 1995;14:13-14.

29. Miquel JF, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, et al. Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. Gastroenterology 1998;115:937-946.

30. Nervi F, Miquel JF, Alvarez M, Ferreccio C, Garcia-Zattera MJ, Gonzalez R, Perez-Ayuso RM, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. J Hepatol 2006;45:299-305.

31. Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F. Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr 2004;23:187-196.

32. Buning C, Genschel J, Jurga J, Fiedler T, Voderholzer W, Fiedler EM, Worm M, et al. Introducing genetic testing for adult-type hypolactasia. Digestion 2005;71:245-250.

33. Bulhoes AC, Goldani HA, Oliveira FS, Matte US, Mazzuca RB, Silveira TR. Correlation between lactose absorption and the C/T-13910 and G/A-22018 mutations of the lactase-phlorizin hydrolase (LCT) gene in adult-type hypolactasia. Braz J Med Biol Res 2007;40:1441-1446.

34. Cook GC, al-Torki MT. High intestinal lactase concentrations in adult Arbs in Saudi Arabia. Br Med J 1975;3:135-136.

35. Charney M, McCracken RD. Intestinal lactase deficiency in adult nonhuman primates: implications for selection pressures in man. Soc Biol 1971;18:416-421.

36. Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. Am J Dig Dis 1970;15:695-710.

37. Enattah NS, Sulkava R, Halonen P, Kontula K, Jarvela I. Genetic variant of lactase-persistent C/T-13910 is associated with bone fractures in very old age. J Am Geriatr Soc 2005;53:79-82.

38. Obermayer-Pietsch BM, Gugatschka M, Reitter S, Plank W, Strele A, Walter D, Bonelli C, et al. Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption? Osteoporos Int 2007;18:445-451.

39. Rasinpera H, Forsblom C, Enattah NS, Halonen P, Salo K, Victorzon M, Mecklin JP, et al. The C/C-13910 genotype of adult-type hypolactasia is associated with an increased risk of colorectal cancer in the Finnish population. Gut 2005;54:643-647.

40. Ojetti V, Nucera G, Migneco A, Gabrielli M, Lauritano C, Danese S, Zocco MA, et al. High prevalence of celiac disease in patients with lactose intolerance. Digestion 2005;71:106-110.

41. Sahi T, Isokoski M, Jussila J, Launiala K. Lactose malabsorption in Finnish children of school age. Acta Paediatr Scand 1972;61:11-16.

42. Simoons FJ. Age of onset of lactose malabsorption. Pediatrics 1980;66:646-648.

43. Wang Y, Harvey CB, Hollox EJ, Phillips AD, Poulter M, Clay P, Walker-Smith JA, et al. The genetically programmed down-regulation of lactase in children. Gastroenterology 1998;114:1230-1236.

44. Laaksonen MM, Mikkila V, Rasanen L, Rontu R, Lehtimaki TJ, Viikari JS, Raitakari OT. Genetic lactase non-persistence, consumption of milk products and intakes of milk nutrients in Finns from childhood to young adulthood. Br J Nutr 2009:1-10.

45. Torniainen S, Hedelin M, Autio V, Rasinpera H, Balter KA, Klint A, Bellocco R, et al. Lactase persistence, dietary intake of milk, and the risk for prostate cancer in Sweden and Finland. Cancer Epidemiol Biomarkers Prev 2007;16:956-961.

46. Lehtimaki T, Hemminki J, Rontu R, Mikkila V, Rasanen L, Laaksonen M, Hutri-Kahonen N, et al. The effects of adult-type hypolactasia on body height growth and dietary calcium intake from childhood into young adulthood: a 21-year follow-up study--the Cardiovascular Risk in Young Finns Study. Pediatrics 2006;118:1553-1559.

47. Castillo C, Atalah E, Benavides X, Urteaga C. [Food patterns among adults visiting primary care clinics in the metropolitan region]. Rev Med Chil 1997;125:283-289.

Figure 1. RFLP and sequence analysis of a 448 bp region of MCM6 intron 13 containing the LCTC>T_{-13910} SNP. Upper panel: RFLP analysis of the 448 bp amplicon digested with BsmFI. Left lane, 50-bp DNA ladder. Right lane, undigested PCR amplicon. Center lanes, digested products from representative individuals having the three different SNP genotypes. The ancestral LCT-13910CC genotype results in two bands of 350 bp and 110 bp; LCT-13910CT genotype results in three bands of 350 bp, 240 bp and 110 bp; LCT-13910TT genotype results in two bands of 240 bp and 110 bp. Lower panel: Representative sequence tracings showing confirmation of RFLP data. Direct sequence analysis was performed on 35 of the 51 samples.

Figure 2. De Finetti diagram for the LCTC>T_{-13910} SNP in Hispanic (black dotted line) and Amerindian (blue dotted line) populations. The diagram shows genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequencies of heterozygous genotypes are plotted on the vertical axis, and the allele frequencies are depicted by the interaction of the vertical dotted lines with the bottom perpendicular (allele 1 = C_{-13910}). The genotype frequencies plot on the parabola in the diagram, indicating that Amerindians are, but Hispanics are not, in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	9-10
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10

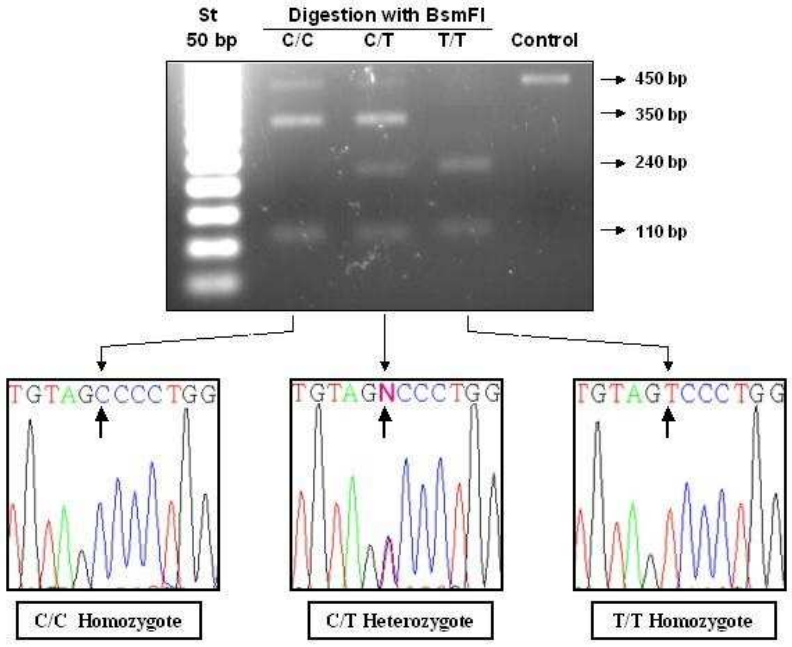
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	n.a.
		(*c*) Explain how missing data were addressed	n.a.
		(*d*) If applicable, explain how matching of cases and controls was addressed	n.a.
		(*e*) Describe any sensitivity analyses	n.a.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	n.a.
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12,26
		(b) Indicate number of participants with missing data for each variable of interest	n.a.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	13,28
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n.a.
		(*b*) Report category boundaries when continuous variables were categorized	n.a.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n.a.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n.a.
Discussion			
Key results	18	Summarize key results with reference to study objectives	16,17
Limitations	19	Discuss limitations of the study, taking into account sources of	16

		potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17,18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

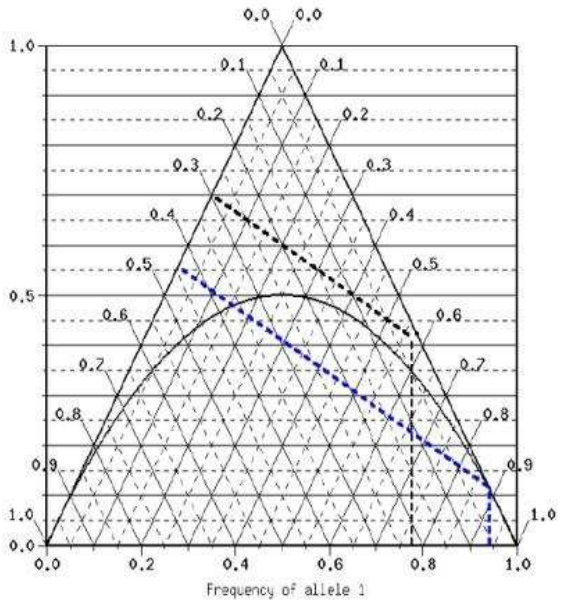
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Figure 1



254x190mm (72 x 72 DPI)

Figure 2



254x190mm (72 x 72 DPI)

BMJ open

The European lactase persistence genotype determines lactase persistence state and correlates with gastrointestinal symptoms in the Hispanic and Amerindian Chilean population.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000125.R1
Article Type:	Research
Date Submitted by the Author:	10-May-2011
Complete List of Authors:	Morales, Eugenia; Pontificia Universidad Catolica de Chile, Gastroenterology Azocar, Lorena; Pontificia Universidad Catolica de Chile, Gastroenterology Maul, Ximena; Pontificia Universidad Catolica de Chile, Medical student Perez, Claudio; Pontificia Universidad Catolica de Chile, Medical student Chianale, Jose; Pontificia Universidad Catolica de Chile, Gastroenterology Miquel, Juan; Pontificia Universidad Catolica de Chile, Gastroenterology
Subject Heading:	Gastroenterology
Keywords:	lactose intolerance, lactase persistence state, -13910T variant, IBS

The European lactase persistence genotype determines lactase persistence state and correlates with gastrointestinal symptoms in the Hispanic and Amerindian Chilean population.

Eugenia Morales[1], Lorena Azocar[1], Ximena Maul[2], Claudio Perez[2], José Chianale[1], Juan Francisco Miquel[1*].

Department of Gastroenterology[1], Medical Students[2], Faculty of Medicine, Pontificia Universidad Católica de Chile.

Keywords: lactose intolerance, lactase persistence state, -13910T variant, IBS.

***Correspondence**:

Juan Francisco Miquel, MD

Marcoleta 367, Santiago

Telephone: 56 2 3543830

Fax: 56 2 6397780

e-mail: jfmiquel@med.puc.cl

Word count: 3,359

List of abbreviations: LP, lactase persistent state; LNP, lactase non-persistent state; LCT, lactase-phlorizin hydrolase; HBT, hydrogen breath test; SNP, single nucleotide polymorphism.

Abstract

Background: The lactase persistent (LP) or lactase non persistent (LNP) state in European adults is genetically determined by a single nucleotide polymorphism (SNP) located 13.9 kb upstream of the lactase (LCT) gene, known as LCT C>T_{-13910} (rs4988235). The LNP condition causes an inability to digest the milk sugar lactose leading to gastrointestinal symptoms and can affect nutrient and calcium intake in certain populations. **Objectives**: We studied a group of 51 Chilean patients to assess whether this SNP influences the LP/LNP state in this population, and determined the prevalence of LCT C>T_{-13910} genotypes in a representative sample of 216 Hispanics and 43 Amerindians with correlation to digestive symptoms. **Design**: Case-Control study done in Chilean patients with clinical suspicion of LNP that were assessed using clinical survey, hydrogen breath test (HBT), and SNP genotyping. The population sample of Hispanics and Amerindians was assessed by clinical survey and SNP genotyping. **Results**: Of the 51 patients with clinical suspicion of LNP, 29 were HBT positive. The CC genotype (LNP) was present in 89.7% of the patients with positive HBT and in only 4.7% of those with negative HBT. The prevalence of the CC genotype was 56.9% in the Hispanic population and 88.3% in Amerindians and was associated with a higher self reported clinical intolerance to dairies ingestion. **Conclusion**: The LP/LNP state is determined by the LCT C>T_{-13910} variant in Chileans. This variant predicts digestive symptoms associated with the ingestion of lactose and is a good tool for the diagnosis of primary adult hypolactasia. The LCT T_{-13910} allele is rare in the Amerindian population and is suggestive of European ancestry in this contemporary population.

ARTICLE SUMMARY

Article focus: The aims of the present study were three questions; 1) If the European variant C/T-13910 determined lactose-persistent and lactose non-persistent state in adult Chilean Hispanic and Amerindian population; 2) If there is a correlation between the C/T-13910 genotyping and the hydrogen breath test (HBT) for diagnosis of LNP in our population; 3) To determine for the first time in an Latin American population the prevalence of the lactose non-persistence genotype (CC) and its correlation with digestive symptoms and ingestion of dairy products.

Significance of the study: The three key messages of the present study were: 1) We demonstrate for the first time that the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian population. 2) We demonstrate a strong correlation between the C-13910>T genotype with the hydrogen breath test and lactose tolerance in a group of patients with clinical suspicion of lactose intolerance. 3) In a representative sample of the Hispanic Chilean population (Mestizos) and in Amerindians, we observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between ethnic groups (56% and 88%, respectively). Interestingly, C-13910>T genotype was significantly associated with digestive symptoms and self reporting intolerance to dairy ingestion. This could explain the observed low consumption of dairy products (30 L milk/person/year) in our population.

Strengths and limitations of the study:

- Our strengths:

- This is the first study in Latin America that assesses if the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian general population.

- Our results may be of clinical and epidemiological relevance, not only for the Chilean population, but also for other Hispanic and Amerindian populations of the Americas with similar genetic background. The fact that the LNP state is an inherited frequent condition in Mestizo and Amerindians should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new and high quality lactose-free dairy products in these regions.

- We randomly selected a representative sample of the Hispanic Chilean population (n=216) and Amerindians (n=43), and performed the genotyping of LCT C>T $_{-13910}$. We observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between both ethnic groups (56% and 88%, respectively), that correlates with digestive symptoms and self reporting intolerance to dairy ingestion.

- **Our limitations:**

  - Non randomized recruitment of the 51 patients with suspicion of lactose intolerance could induce selection bias.

  - A small number of patients with suspicion of lactose intolerance (n= 51).

- Semi-quantitative food frequency questionnaire with a 24 h dietary recall obtained in a subgroup of the Hispanics and performed years before the genetic analysis.

INTRODUCTION

Lactose, the main sugar in milk, must be hydrolyzed by lactase-phlorizin hydrolase into glucose and galactose for absorption. Intestinal activity of the lactase enzyme (LCT) (MIM 603202) decreases in all mammals after weaning, resulting in poor lactose absorption (1). In humans this condition is termed "lactase non-persistence" (LNP), "adult type hypolactasia" (MIM 223100) and is estimated to occur in approximately 65% of the contemporary world population (2, 3). LNP can generate intolerance to the ingestion of dairies with the development of abdominal and systemic symptoms that lead to medical consultations and evaluations. In contrast, lactase persistent (LP) individuals remain tolerant of lactose and maintain high levels of LCT expression throughout adult life (4). The prevalence of the LP phenotype, which is an autosomal dominant condition (MIM 223100) is reported to vary between 0 and 95% in different populations (3), but is unknown in contemporary Latin American populations (2, 5).

The recent discovery of single nucleotide polymorphisms (SNPs) that determine the LP state in humans, both in Europe (6) and among some pastoral populations in Africa, has generated growing interest in the study of this condition (2, 7). The appearance of these SNPs seems to have occurred on more than one occasion in human evolution between 1,500 and 12,000 y ago in northern Europe and among some nomadic populations of Asia and Africa (7). The SNP known as LCT C>T_{-13910} (rs4988235) is located 13.9 kb upstream of the LCT gene in the 13th intron of an adjacent gene (MCM6). The T allele of this SNP correlates with the LP state in European populations (6), and individuals with this allele have higher levels of LCT transcripts in enterocytes compared to individuals who carry the C allele (8). *In vitro* studies show that cells transfected with the LCT T_{-13910} variant show increased activity

of the LCT promoter (9). Another SNP known as LCT G>A$_{-22018}$, shows some association with the LP/LNP phenotype, and while it does not appear to be the causal variant, it is present in a block of linkage disequilibrium with little evidence of recombination in this region of the genome (5, 10).

In clinical practice, the LNP state is relevant because it can generate malabsorption and intolerance of lactose, with the development of clinical symptoms such as abdominal pain and distension, diarrhea, bloating, nausea, and vomiting (3). A significant percentage of patients do not relate these symptoms to dairies ingestion (11), and the recurring nature of the symptoms can lead to misdiagnosis of chronic diarrhea, irritable bowel syndrome, celiac illness, or other clinical conditions (12-16). The diagnosis of LNP is often based on clinical suspicion and the favorable response to a lactose restricted diet.

The most widely used diagnostic test of poor lactose absorption is the hydrogen breath test (HBT) (11, 17). Nevertheless, this test has practical limits and its sensitivity and specificity varies between 69 - 100% and 89 - 100%, respectively, compared to measurement of LCT activity in intestinal biopsies. Additionally, there is a false negative rate of 11 - 30%, and a false positive rate of up to 57% using this test (18-20). Using SNP genotyping in patients with suspected LNP may therefore aid in proper diagnosis (10, 21-25). The only epidemiological study of adult hypolactasia in a Chilean population was performed more than three decades ago (26) and suggested that the prevalence of LNP was 56% and 75% for the pediatric and adult populations, respectively. Our objectives were to determine the influence of the LCT C>T$_{-13910}$ SNP on the LP/LNP state in the Chilean population and to determine the population prevalence of the SNP genotypes in a representative Hispanic and Amerindian

population with comparison of the genotypes to clinical symptoms related to dairies ingestion.

SUBJECTS AND METHODS

Patients with clinical suspicion of lactase non persistence (LNP).

In 2006, 51 patients were enrolled in a prospective study after being referred to the Gastroenterological Department of the Clinical Hospital at the Pontificia Universidad Catolica de Chile because of symptoms suggestive of LNP. A pre-coded survey was obtained from all patients to obtain demographic, anthropometric, familial, and clinical data, including any symptoms experienced during the diagnostic hydrogen breath test (HBT) such as abdominal pain, diarrhea, bloating, nausea, and vomiting. A 5 ml EDTA blood sample was obtained to extract DNA. Serum levels of immunoglobulin A and tissue anti-transglutaminase antibodies (hu tTG ELISA, IMMCO Diagnostics, Inc., Buffalo, NY) were quantified in all the patients to rule out the existence of celiac disease. This study was approved by the Ethics Committee of the Faculty of Medicine, PUC, and all patients signed a written informed consent form prior to the study.

Hydrogen breath test (HBT) for diagnosis of LNP.

Patients were instructed to maintain a low fiber diet without lactose for 48 h prior to the day of the examination. After 12 h of fasting, a baseline concentration of H_2 in exhaled breath was determined using a Quintron® microLyzer (QuinTron Instrument Company, Milwaukee, WI). Subjects then ingested 25 g lactose and H_2 concentration in breath was quantified every 20 m for a maximum of 4 h. Individuals were considered LNP with an increase in H_2 concentration 20 ppm above the base value (17, 27).

Population study.

A representative sample of 216 unrelated Hispanics and 43 unrelated Amerindians were randomly selected from an initial sample of 1581 Hispanics and 120 Amerindians (Mapuches, the Chilean native population) which were collected between 1993 and

2000 as described previously (28, 29). By using an Amerindian Admixture Index based on ABO blood group distribution, we have previously demonstrated in these cohorts a 40% and 80% Amerindian ancestry in the Hispanic and Mapuche population, respectively. Furthermore, 88% of Hispanics and 100% of Mapuches shared ancestral Amerindian mtDNA polymorphism (29). These results were in accordance with the biparental founder origin of the mixed Chilean population, which could be similar to other Hispanic populations from America (30-32).

The sample size was selected by estimating an expected prevalence of the LCT-13910CC genotype to be at least 50% in the Hispanic population and 80% in the Amerindians, with an alpha error of 5% and a level of confidence of 80%. All study subjects provided a DNA sample and a medical survey that provided anthropometric and clinical data including information about diarrhea, abdominal pain, or bloating in response to ingestion of dairy products (28, 29). Furthermore, a subgroup of the Hispanic subjects provided a semi-quantitative food frequency questionnaire with a 24 h dietary recall. From this data, specific energy and nutrient ingestion from dairy products was estimated using the Datadiet program (28, 33). For all subjects, the occurrences of digestive symptoms in response to consumption of lactose were correlated to SNP genotyping results.

Genotyping of LCT C>T $_{-13910}$ and LCT G>A $_{-22018}$ SNPs

SNP genotyping was performed using PCR-RFLP methods (34) (GenBank reference sequence NM_005915.4). Briefly, genomic DNA was extracted from 300 µl of blood according to manufacturer's instructions (Wizard SV Genomic DNA Purification System, Promega, Madison, WI) and the regions surrounding the SNPs were amplified through PCR using primers (F) 5´-GGA TGC ACT GCT GTG ATG AG-3´ and (R) 5-CCC ACT GAC CTA TCC TCG TG-3´ for the LCT C>T $_{-13910}$ and (F) 5´-AAC AGG

CAC GTG GAG GAG TT-3´ and (R) 5´- CCC ACC TCA GCC TCT TGA GT-3´ for LCT G>A $_{-22018}$. In both cases, the amplified products were of 448 bp. The PCR reactions and RFLP assays for the analysis of both genetic variants was carried out as described previously (34). Briefly, the amplified product for the LCT C>T $_{-13910}$ SNP was digested with *BsmFI*, generating two fragments of 351 and 97 bp in the presence of the CC genotype, and 3 fragments of 253, 98, and 97 bp in the presence of the TT genotype (**Figure 1**). For the LCT G>A $_{-22018}$ SNP, the PCR product was digested with *HhaI*, generating two fragments of 284 and 184 bp in the presence of the GG genotype and one undigested fragment of 448 bp in the presence of the A/A genotype. The amplified and digested PCR products were separated by electrophoresis on 2% agar gels in 1X TAE buffer stained with ethidium bromide. Genotyping confirmation was carried out on 35 samples by direct sequencing of the 448 bp amplified fragments (models ABI-310 and ABI-3100 automated DNA sequencers, Applied Biosystems, Foster City, CA*)* (**Figure 1**). There was complete concordance between the PCR-RFLP analysis and the sequencing in all cases.

Statistical analysis

Mean and standard deviations were used to describe numeric variables. The variables of categorical types are given as a number and a percentage. Comparisons of continuous variables were performed using the student T test for independent samples. Comparisons of categorical variables were carried out using the chi-square test. Using the HBT breath test as diagnostic for LNP, an analysis was made of the sensitivity and specificity of the genetic test as an alternative diagnostic test. Differences were considered significant when $p<0.05$.

RESULTS

Hydrogen breath test and SNP genotyping

Fifty-one patients with clinical suspicion of LNP (44 women and 7 men, age 14 - 79 y) were included in the study. Twenty-nine patients (56.8%) had a positive HBT and 22 were negative (43.2%) (**Table 1**). There were no significant differences in the distribution by sex and age between the two groups. There was a positive correlation between the HBT results and digestive symptoms reported during the test: 82% of the patients with positive HBT had symptoms associated with the lactose load, such as bloating, abdominal pain, and diarrhea (79%, 58% and 20%, respectively). In contrast, only 27% of the patients with negative HBT presented symptoms after ingesting lactose ($p < 0.001$). Additionally, 76% of patients with positive HBT indicated a personal and/or family history of functional digestive disorders, compared to 36% of those with negative HBT ($p < 0.001$).

The LCTC>T_{-13910} SNP genotyping results for the 51 patients is shown in Table 1. Of the 29 patients with positive HBT, 26 (89.7%) had the LCT-13910CC genotype (the LNP genotype), and 3 (10.3%) had the LCT-13910CT genotype and were therefore phenotypically LNP but genotypically LP. However, two of these 3 patients experienced bloating and diarrhea during the HBT, which suggests malabsorption of lactose. Of the 22 patients who had a negative HBT, 19 were LCT-13910CT, two were LCT-13910TT, and a single 16-year-old patient had the LNP LCT-13910CC genotype (Table 1). However, this patient experienced abdominal pain during the HBT. Subsequent evaluation of this patient with a lactulose HBT (10 g load) revealed that this patient was not a non-producer of hydrogen. A single patient with LNP genotype and positive HBT had high levels of anti-tTG (27 µU/ml), which suggests the possibility of

coexistence of LNP and celiac disease. All the patients showed normal levels of serum IgA (data not shown).

There was complete genotypic correlation between the LCTC>T$_{-13910}$ and the LCTG>A$_{-22018}$ SNPs, such that any subject homozygous or heterozygous for one SNP was at the same time homozygous or heterozygous for the other SNP (data not shown). Furthermore, there was good correlation between the LCTC>T$_{-13910}$ SNP findings and the HBT test results (**Table 2**). Genotyping results were confirmed by sequencing for 35 of the 51 samples (**Figure 2**). Interestingly, one of the three discordant patients with LNP phenotype but LP genotype was also heterozygous at a second location not previously described in the literature, LCTG>A$_{-13937}$.

Population study

We collected DNA samples and medical surveys from 216 unrelated Hispanic individuals and 43 unrelated Amerindians. The survey information is summarized in **Table 3**. The two groups were similar in age, with higher representation of females among the Amerindians. The Amerindian cohort reported a higher frequency of intolerance to ingesting dairy products and recurring diarrhea while both groups reported recurring abdominal bloating with equal frequency.

The genotype and allele frequencies for the LCTC>T $_{-13910}$ SNP are shown in **Table 3**. As was found in the study group of 51 Chilean patients with suspected lactose intolerance, there was complete concordance between the genotypes of the LCTC>T$_{-13910}$ and LCTG>A$_{-22018}$ SNPs in each individual (data not shown). The cohort of Amerindian individuals was in Hardy-Weinberg equilibrium (**Figure 2**), but we observed a slightly higher than expected frequency of the T$_{-13910}$ allele in the Hispanic population (deviation from HW equilibrium $p < 0.05$).). Interestingly, this deviation from HW equilibrium has also been observed in other mixed ethnicity groups (3, 35).

The prevalence of the ancestral LCT-13910CC genotype (the LNP genotype) was 56.9%, significantly lower than the prevalence of this genotype among the Amerindian individuals (88.3%). In turn, 41.7% of the Hispanics were LCT-13910CT genotype compared to only 11.7% of the Amerindians. The LCT-13910TT genotype was found in only 3 Hispanic subjects. Allele frequencies for the LCTC>T_{-13910} SNP were significantly different between the two groups, with the T_{-13910} allele being very infrequent in Amerindians.

For the 216 Hispanic individuals studied, we were able to gather clinical and genetic data (**Table 3**). Individuals with the LNP LCT-13910CC genotype were 3 times more likely to report intolerance to the ingestion of dairy products and recurring diarrhea than those with LP genotypes (LCT-13910CT or LCT-13910TT). For 112 of these individuals, we collected complete nutritional information. The consumption of dairy products was in general very low, equivalent to an average of 30 L of fresh milk per year per person. However, the group of individuals with an LP genotype had 38% higher consumption of dairy products than the group of individuals with the LNP genotype (38.4 L ± 67 L versus 27.7 L ± 40 L milk/year, respectively), although this difference was not statistically significant.

DISCUSSION

The normal physiologic decrease in lactase (LCT) production in the mammalian intestine following weaning is known as lactase non persistance (LNP), and results in the inability to digest the milk sugar, lactose, which can cause gastrointestinal symptoms after ingestion of dairies. The condition of lactase persistence (LP), found only in humans, allows the individual to continue to ingest and absorb lactose into adulthood. In European populations and some Afro-Arab pastoral groups, the LP condition has been shown to be due to SNP's found 13.9 kb upstream of the LCT gene and actually located within the 13th intron of the MCM6 gene. One of these SNP's, LCTC>T$_{-13910}$ (rs4988235), appeared approximately 10,000y ago in the human history and, has spread progressively from Northen Europe through the world due to population migration and mixing, increasing the state of LP in different populations (2). The original populations of the Americas and Asia were likely LNP. There is no evidence of the existence of American variants that might have generated the LP state in the pre-Columbian era (2, 5). The T$_{-13910}$ allele, which leads to the LP state, was likely introduced to the Americas by the migration of European carriers of this variant approximately 500 years ago and spread rapidly in the descending admixed Hispanic (Mestizo) populations of contemporary America, as is shown in this study as well as others (5, 36).

Our study of a representative population of Chile consisting of Hispanic and Amerindian individuals showed the prevalence of the LNP state (LCT-13910CC genotype) to be 57% and 88%, respectively. It is interesting to note that this observed genotypic frequency is very similar to the Amerindian admixture index (AAI) estimated by us in these same populations based on the distribution of ABO blood groups (28).

This suggests that the analysis of the LCTC>T_{-13910} SNP in Latin American populations can be a good indicator of the degree of Amerindian or Caucasian inheritance.

In all Chilean patients genotyped in this study, the LCTG>A_{-22018} SNP genotype correlated completely with the genotype found for the LCTC>T_{-13910} SNP, a finding reported in many studies. This phenomenon can be explained by the fact that both SNPs are located within a highly conserved block of linkage disequilibrium of at least 500 kb and likely co-segregate (2, 5).

According to HW equilibrium, in the group of 216 unrelated Hispanic individuals we observed a higher than expected frequency of the T_{-13910} allele. While this could be explained by selection bias or by a higher frequency of LP in the Spanish population that founded the Chilean colony, it could also be reflective of positive selection for LP allele carriers, as has been suggested for decades (37-39). Any evolutionary advantage conferred by the T_{-13910} allele may or may not be related to the consumption of dairy products. Indeed, it was reported that this allele confers protection not only against bone fractures (40, 41), but also colon cancer (42).

The validity of using LCTC>T_{-13910} SNP genotyping for diagnosis of the LNP or LP state was evaluated in the group of 51 Chilean patients with suspected LNP. The HBT showed a high positive and negative predictive value, sensitivity and specificity compared to genetic testing (Table 2). These results are similar to those recently reported by other groups (10, 24, 34, 43) and confirm the utility of the analysis of this SNP as a clinical test for the diagnosis of LP/LNP in the adult Chilean population. Only 3 patients (10.3%) with malabsorption of lactose shown by HBT, were genetically LP (LCT-13910CT genotype). It was not possible to recall these 3 patients to carry out complementary studies to rule out other causes of lactose malabsorption, such as intestinal parasitic infections like Giardiasis, seronegative celiac disease or other

conditions as has been shown in other recent studies (43). Interestingly, one of these patients was also heterozygous at an additional SNP not previously described, LCTG>A$_{-13937}$ (2). We do not know if this new variant can explain the phenotype-genotype discordance.

A recent study showed a high prevalence of celiac illness among patients with an initial diagnosis of LNP, suggesting that lactose intolerance can be the first manifestation of celiac illness; the authors recommend considering the existence of celiac illness in all patients with LNP before recommending a lactose free diet for life (44). In our study of 51 patients with clinical suspicion of lactose intolerance, only one with LNP confirmed by HBP and SNP genotyping had positive anti-tTG (2% of the total) suggesting the coexistence of both conditions.

One 16-year-old patient had negative HBT yet had the LNP genotype. It is possible that this patient has not yet manifested the LNP phenotype and still maintains sufficient levels of intestinal lactase to digest a 25 g load. The age at which the LNP state is initiated varies among different populations and ethnic groups, starting as early as 1 to 8 y in black and Asian populations, and much later (20 y) in northern European populations (10, 45-47). The age of initiation of the LNP state in Chilean individuals remains unknown.

Among the 51 Chilean patients with positive HBT and the LCT-13910CC genotype, significantly more report having relatives with functional digestive disorders than their HBT negative counterparts, suggesting that the condition of LNP could be in part responsible for digestive symptoms. In our complete clinical, nutritional, and genetic study of 112 Hispanic individuals, those with the LCT-13910CC genotype were significantly more likely to self-report lactose intolerance despite very low (30 L milk/person/year) consumption of dairy products. This level is well below the WHO

recommended level of 240 L milk/person/year. Reasons for the low level of consumption are likely due to multiple causes including cost, preference, and societal habits which are likely influenced by the existence of a high prevalence of LNP and lactose intolerant state within this population. It has been reported that the allele frequencies of the LCTC>T_{-13910} SNP within a population correlates with the tendency of that population to consume dairy products and ingest calcium (48-50). Both this study and one other (51) show that the effective consumption of dairy products and calcium in Chile remains below international recommendations.

In summary, the LNP state continues to be predominant in the Chilean population. Epidemiologically, this may cause the development of digestive symptoms affecting quality of life and leading to medical consultations as well as lower tendency to ingest dairy products and calcium within the adult population of the contemporary Americas. The fact that the LNP state is an inherited condition should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new lactose-free dairy products.

Table 1. Clinical and genetic characteristics of 51 Chilean patients having hydrogen breath test (HBT) for suspicion of lactose non persistence (LNP).

	HBT Positive (n = 29)	**HBT Negative (n = 22)**	***p***
Female gender (%)	82.7	86.3	n.s.
Age (y)	36 ± 19	32 ± 12	n.s.
Number reporting gastrointestinal symptoms after lactose load	24 (82%)	6 (27%)	< 0.001
Number reporting family history of IBS	22 (76%)	8 (36%)	< 0.001
LCT-13910 CC genotype	26 (89.6%)	1 (4.5%)	< 0.001
LCT-13910 CT/TT genotype	3 (10.3%)	21(95.5%)	< 0.001

IBS, irritable bowel syndrome.

Table 2. Correlation of the HBT in comparison to LCT C>T$_{-13910}$ genotype in 51 Chilean patients.

Sensitivity	96.3%
Specificity	87.5%
Predictive value positive	89.7%
Predictive value negative	95.5%
LHR positive	7.7
LHR negative	0.04

LHR, likelihood ratio

Table 3. General characteristics, self reported gastrointestinal symptoms related to dairy ingestion and LCTC>T$_{-13910}$ SNP genotype and allele frequencies in a population of Hispanic and Amerindian individuals.

	Hispanics n = 216	**Amerindians** n = 43	*P*
Women (%)	46	65	0.02
Age (y)	50 ± 12	54 ± 15	n.s.
Number self reporting lactose intolerance	44 (20.4%)	19 (44.1%)	0.001
Number reporting diarrhea	15 (7%)	16 (37.2%)	<0.001
Number reporting bloating	77 (35.6%)	14 (32.5%)	n.s.
LCT-13910 CC genotype	123 (56.9%)	38 (88.3%)	< 0.001
LCT-13910 CT genotype	90 (41.7%)	5 (11.7%)	< 0.001
LCT-13910 TT genotype	3 (1.4%)	0	n.s.
C allele frequency	77.7%	94.2%	< 0.001
T allele frequency	22.3%	5.8%	< 0.001

n.s., not significant

Author's contribution: Study concept and design (JFM); acquisition of data (JFM, EM, LA, XM and CP); analysis and interpretation of data (JFM, EM, JCh); drafting of the manuscript (JFM, EM, XM, CP); critical revision of the manuscript for important intellectual content (JCh); statistical analysis (JFM, EM, XM, CP); obtained funding (JFM, JCh); final revision and edition (JFM, CP, XM).

Acknowledgments: The authors acknowledge the Alexander von Humboldt Foundation (Germany) for donating the NanoDrop ND-1000 Spectrophotometer used for DNA analysis and quantification. The authors thank Dr. Flavio Nervi for providing access to the database and DNA bank of the Hispanic and Mapuche populations, and Dr. Julieta Klassen for collaboration in the analysis of dairy product ingestion. This study was partially supported grants of the National Commission of Scientific and Technological Research (CONICYT), FONDECYT N°1080325 to JFM, and from the Office of Research affairs of the Pontificia Universidad Catolica de Chile, School of Medicine to EM.

Competing Interest: We declare no conflicts of interest.

REFERENCES

1. Enattah N, Pekkarinen T, Valimaki MJ, Loyttyniemi E, Jarvela I. Genetically defined adult-type hypolactasia and self-reported lactose intolerance as risk factors of osteoporosis in Finnish postmenopausal women. Eur J Clin Nutr 2005;59:1105-1111.

2. Ingram CJ, Mulcare CA, Itan Y, Thomas MG, Swallow DM. Lactose digestion and the evolutionary genetics of lactase persistence. Hum Genet 2009;124:579-591.

3. Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice--myths and realities. Aliment Pharmacol Ther 2008;27:93-103.

4. Matthews SB, Waud JP, Roberts AG, Campbell AK. Systemic lactose intolerance: a new perspective on an old problem. Postgrad Med J 2005;81:167-173.

5. Bersaglieri T, Sabeti PC, Patterson N, Vanderploeg T, Schaffner SF, Drake JA, Rhodes M, et al. Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet 2004;74:1111-1120.

6. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat Genet 2002;30:233-237.

7. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, et al. Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 2007;39:31-40.

8. Kuokkanen M, Enattah NS, Oksanen A, Savilahti E, Orpana A, Jarvela I. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. Gut 2003;52:647-652.

9. Lewinsky RH, Jensen TG, Moller J, Stensballe A, Olsen J, Troelsen JT. T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro. Hum Mol Genet 2005;14:3945-3953.

10. Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela I, et al. A genetic test which can be used to diagnose adult-type hypolactasia in children. Gut 2004;53:1571-1576.

11. Shaw AD, Davies GJ. Lactose intolerance: problems in diagnosis and treatment. J Clin Gastroenterol 1999;28:208-216.

12. Parker TJ, Woolner JT, Prevost AT, Tuffnell Q, Shorthouse M, Hunter JO. Irritable bowel syndrome: is the search for lactose intolerance justified? Eur J Gastroenterol Hepatol 2001;13:219-225.

13. Vesa TH, Seppo LM, Marteau PR, Sahi T, Korpela R. Role of irritable bowel syndrome in subjective lactose intolerance. Am J Clin Nutr 1998;67:710-715.

14. Bode S, Gudmand-Hoyer E. Incidence and clinical significance of lactose malabsorption in adult coeliac disease. Scand J Gastroenterol 1988;23:484-488.

15. Srinivasan U, Jones E, Weir DG, Feighery C. Lactase enzyme, detected immunohistochemically, is lost in active celiac disease, but unaffected by oats challenge. Am J Gastroenterol 1999;94:2936-2941.

16. Barr RG, Levine MD, Watkins JB. Recurrent abdominal pain of childhood due to lactose intolerance. N Engl J Med 1979;300:1449-1452.

17. Peuhkuri K, Poussa T, Korpela R. Comparison of a portable breath hydrogen analyser (Micro H2) with a Quintron MicroLyzer in measuring lactose maldigestion, and the evaluation of a Micro H2 for diagnosing hypolactasia. Scand J Clin Lab Invest 1998;58:217-224.

18. Rosado JL, Solomons NW. Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. Clin Chem 1983;29:545-548.

19. Barillas-Mury C, Solomons NW. Test-retest reproducibility of hydrogen breath test for lactose maldigestion in preschool children. J Pediatr Gastroenterol Nutr 1987;6:281-285.

20. Arola H, Koivula T, Jokela H, Jauhiainen M, Keyrilainen O, Ahola T, Uusitalo A, et al. Comparison of indirect diagnostic methods for hypolactasia. Scand J Gastroenterol 1988;23:351-357.

21. Jarvela IE. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). Scand J Clin Lab Invest 2005;65:535-539.

22. Bodlaj G, Stocher M, Hufnagl P, Hubmann R, Biesenbach G, Stekel H, Berg J. Genotyping of the lactase-phlorizin hydrolase -13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. Clin Chem 2006;52:148-151.

23. Enattah NS, Kuokkanen M, Forsblom C, Natah S, Oksanen A, Jarvela I, Peltonen L, et al. Correlation of intestinal disaccharidase activities with the C/T-13910 variant and age. World J Gastroenterol 2007;13:3508-3512.

24. Schirru E, Corona V, Usai-Satta P, Scarpa M, Oppia F, Loriga F, Cucca F, et al. Genetic testing improves the diagnosis of adult type hypolactasia in the Mediterranean population of Sardinia. Eur J Clin Nutr 2007;61:1220-1225.

25. Satta PU, Congia M, Schirru E, Scarpa M, Mura G. Genetic testing is ready to change the diagnostic scenario of lactose malabsorption. Gut 2008;57:137-138; author reply 138.

26. Lacassie Y, Weinberg R, Monckeberg F. Poor predictability of lactose malabsorption from clinical symptoms for Chilean populations. Am J Clin Nutr 1978;31:799-804.

27. Rana S, Bhasin DK, Gupta D, Mehta SK. Assessment of optimal dose of lactose for lactose hydrogen breath test in Indian adults. Indian J Gastroenterol 1995;14:13-14.

28. Miquel JF, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, et al. Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. Gastroenterology 1998;115:937-946.

29. Nervi F, Miquel JF, Alvarez M, Ferreccio C, Garcia-Zattera MJ, Gonzalez R, Perez-Ayuso RM, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. J Hepatol 2006;45:299-305.

30. Merriwether DA, Rothhammer F, Ferrell RE. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. Am J Phys Anthropol 1995;98:411-430.

31. Moraga ML, Rocco P, Miquel JF, Nervi F, Llop E, Chakraborty R, Rothhammer F, et al. Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the southern cone of the continent. Am J Phys Anthropol 2000;113:19-29.

32. Rocco P, Morales C, Moraga M, Miquel JF, Nervi F, Llop E, Carvallo P, et al. [Genetic composition of the Chilean population. Analysis of mitochondrial DNA polymorphism]. Rev Med Chil 2002;130:125-131.

33. Cuevas A, Miquel JF, Reyes MS, Zanlungo S, Nervi F. Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr 2004;23:187-196.

34. Buning C, Genschel J, Jurga J, Fiedler T, Voderholzer W, Fiedler EM, Worm M, et al. Introducing genetic testing for adult-type hypolactasia. Digestion 2005;71:245-250.

35. Almon R, Engfeldt P, Tysk C, Sjostrom M, Nilsson TK. Prevalence and trends in adult-type hypolactasia in different age cohorts in Central Sweden diagnosed by genotyping for the adult-type hypolactasia-linked LCT -13910C > T mutation. Scand J Gastroenterol 2007;42:165-170.

36. Bulhoes AC, Goldani HA, Oliveira FS, Matte US, Mazzuca RB, Silveira TR. Correlation between lactose absorption and the C/T-13910 and G/A-22018 mutations of the lactase-phlorizin hydrolase (LCT) gene in adult-type hypolactasia. Braz J Med Biol Res 2007;40:1441-1446.

37. Cook GC, al-Torki MT. High intestinal lactase concentrations in adult Arbs in Saudi Arabia. Br Med J 1975;3:135-136.

38. Charney M, McCracken RD. Intestinal lactase deficiency in adult nonhuman primates: implications for selection pressures in man. Soc Biol 1971;18:416-421.

39. Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. Am J Dig Dis 1970;15:695-710.

40. Enattah NS, Sulkava R, Halonen P, Kontula K, Jarvela I. Genetic variant of lactase-persistent C/T-13910 is associated with bone fractures in very old age. J Am Geriatr Soc 2005;53:79-82.

41. Obermayer-Pietsch BM, Gugatschka M, Reitter S, Plank W, Strele A, Walter D, Bonelli C, et al. Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption? Osteoporos Int 2007;18:445-451.

42. Rasinpera H, Forsblom C, Enattah NS, Halonen P, Salo K, Victorzon M, Mecklin JP, et al. The C/C-13910 genotype of adult-type hypolactasia is associated with an increased risk of colorectal cancer in the Finnish population. Gut 2005;54:643-647.

43. Krawczyk M, Wolska M, Schwartz S, Gruenhage F, Terjung B, Portincasa P, Sauerbruch T, et al. Concordance of genetic and breath tests for lactose intolerance in a tertiary referral centre. J Gastrointestin Liver Dis 2008;17:135-139.

44. Ojetti V, Nucera G, Migneco A, Gabrielli M, Lauritano C, Danese S, Zocco MA, et al. High prevalence of celiac disease in patients with lactose intolerance. Digestion 2005;71:106-110.

45. Sahi T, Isokoski M, Jussila J, Launiala K. Lactose malabsorption in Finnish children of school age. Acta Paediatr Scand 1972;61:11-16.

46. Simoons FJ. Age of onset of lactose malabsorption. Pediatrics 1980;66:646-648.

47. Wang Y, Harvey CB, Hollox EJ, Phillips AD, Poulter M, Clay P, Walker-Smith JA, et al. The genetically programmed down-regulation of lactase in children. Gastroenterology 1998;114:1230-1236.

48. Laaksonen MM, Mikkila V, Rasanen L, Rontu R, Lehtimaki TJ, Viikari JS, Raitakari OT. Genetic lactase non-persistence, consumption of milk products and intakes of milk nutrients in Finns from childhood to young adulthood. Br J Nutr 2009:1-10.

49. Torniainen S, Hedelin M, Autio V, Rasinpera H, Balter KA, Klint A, Bellocco R, et al. Lactase persistence, dietary intake of milk, and the risk for prostate cancer in Sweden and Finland. Cancer Epidemiol Biomarkers Prev 2007;16:956-961.

50. Lehtimaki T, Hemminki J, Rontu R, Mikkila V, Rasanen L, Laaksonen M, Hutri-Kahonen N, et al. The effects of adult-type hypolactasia on body height growth

and dietary calcium intake from childhood into young adulthood: a 21-year follow-up study--the Cardiovascular Risk in Young Finns Study. Pediatrics 2006;118:1553-1559.

51. Castillo C, Atalah E, Benavides X, Urteaga C. [Food patterns among adults visiting primary care clinics in the metropolitan region]. Rev Med Chil 1997;125:283-289.

FIGURE LEGENDS

Figure 1. RFLP and sequence analysis of a 448 bp region of MCM6 intron 13 containing the LCTC>T$_{-13910}$ SNP. Upper panel: RFLP analysis of the 448 bp amplicon digested with BsmFI. Left lane, 50-bp DNA ladder. Right lane, undigested PCR amplicon. Center lanes, digested products from representative individuals having the three different SNP genotypes. The ancestral LCT-13910CC genotype results in two bands of 350 bp and 110 bp; LCT-13910CT genotype results in three bands of 350 bp, 240 bp and 110 bp; LCT-13910TT genotype results in two bands of 240 bp and 110 bp. Lower panel: Representative sequence tracings showing confirmation of RFLP data. Direct sequence analysis was performed on 35 of the 51 samples.

Figure 2. De Finetti diagram for the LCTC>T$_{-13910}$ SNP in Hispanic (black dotted line) and Amerindian (blue dotted line) populations. The diagram shows genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequencies of heterozygous genotypes are plotted on the vertical axis, and the allele frequencies are depicted by the interaction of the vertical dotted lines with the bottom perpendicular (allele 1 = C$_{-13910}$). The genotype frequencies plot on the parabola in the diagram, indicating that Amerindians are, but Hispanics are not, in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	9-10
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	n.a.
		(*c*) Explain how missing data were addressed	n.a.
		(*d*) If applicable, explain how matching of cases and controls was addressed	n.a.
		(*e*) Describe any sensitivity analyses	n.a.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	n.a.
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12,26
		(b) Indicate number of participants with missing data for each variable of interest	n.a.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	13,28
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n.a.
		(*b*) Report category boundaries when continuous variables were categorized	n.a.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n.a.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n.a.
Discussion			
Key results	18	Summarize key results with reference to study objectives	16,17
Limitations	19	Discuss limitations of the study, taking into account sources of	16

		potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17,18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Figure 1

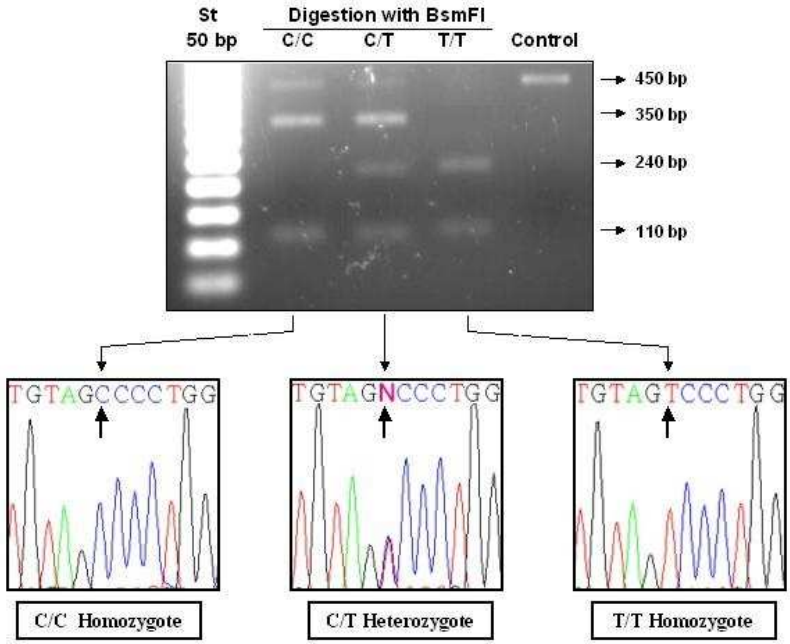


Figure 1. RFLP and sequence analysis of a 448 bp region of MCM6 intron 13 containing the LCTC>T-13910 SNP. Upper panel: RFLP analysis of the 448 bp amplicon digested with BsmFI. Left lane, 50-bp DNA ladder. Right lane, undigested PCR amplicon. Center lanes, digested products from representative individuals having the three different SNP genotypes. The ancestral LCT-13910CC genotype results in two bands of 350 bp and 110 bp; LCT-13910CT genotype results in three bands of 350 bp, 240 bp and 110 bp; LCT-13910TT genotype results in two bands of 240 bp and 110 bp. Lower panel: Representative sequence tracings showing confirmation of RFLP data. Direct sequence analysis was performed on 35 of the 51 samples.
254x190mm (72 x 72 DPI)

Figure 2

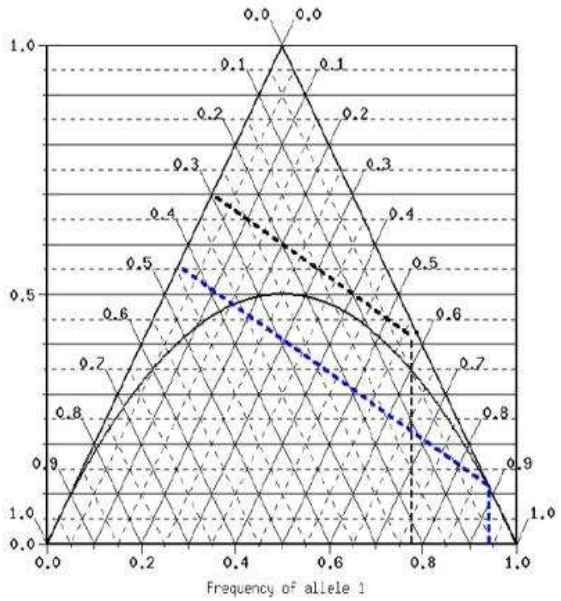


Figure 2. De Finetti diagram for the LCTC>T-13910 SNP in Hispanic (black dotted line) and Amerindian (blue dotted line) populations. The diagram shows genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequencies of heterozygous genotypes are plotted on the vertical axis, and the allele frequencies are depicted by the interaction of the vertical dotted lines with the bottom perpendicular (allele 1 = C-13910). The genotype frequencies plot on the parabola in the diagram, indicating that Amerindians are, but Hispanics are not, in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).
254x190mm (72 x 72 DPI)

BMJ open

The European lactase persistence genotype determines lactase persistence state and correlates with gastrointestinal symptoms in the Hispanic and Amerindian Chilean population: a case-control and population-based study.

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2011-000125.R2
Article Type:	Research
Date Submitted by the Author:	26-May-2011
Complete List of Authors:	Morales, Eugenia; Pontificia Universidad Catolica de Chile, Gastroenterology Azocar, Lorena; Pontificia Universidad Catolica de Chile, Gastroenterology Maul, Ximena; Pontificia Universidad Catolica de Chile, Medical student Perez, Claudio; Pontificia Universidad Catolica de Chile, Medical student Chianale, Jose; Pontificia Universidad Catolica de Chile, Gastroenterology Miquel, Juan; Pontificia Universidad Catolica de Chile, Gastroenterology
Subject Heading:	Gastroenterology
Keywords:	lactose intolerance, lactase persistence state, -13910T variant, IBS

The European lactase persistence genotype determines lactase persistence state and correlates with gastrointestinal symptoms in the Hispanic and Amerindian Chilean population: a case-control and population-based study.

Eugenia Morales[1], Lorena Azocar[1], Ximena Maul[2], Claudio Perez[2], José Chianale[1], Juan Francisco Miquel[1*].

Department of Gastroenterology[1], Medical Students[2], Faculty of Medicine, Pontificia Universidad Católica de Chile.

Keywords: lactose intolerance, lactase persistence state, -13910T variant, IBS.

***Correspondence**:

Juan Francisco Miquel, MD

Marcoleta 367, Santiago

Telephone: 56 2 3543830

Fax: 56 2 6397780

e-mail: jfmiquel@med.puc.cl

Word count: 3,359

List of abbreviations: LP, lactase persistent state; LNP, lactase non-persistent state; LCT, lactase-phlorizin hydrolase; HBT, hydrogen breath test; SNP, single nucleotide polymorphism.

Abstract

Background: The lactase persistent (LP) or lactase non persistent (LNP) state in European adults is genetically determined by a single nucleotide polymorphism (SNP) located 13.9 kb upstream of the lactase (LCT) gene, known as LCT C>T_{-13910} (rs4988235). The LNP condition causes an inability to digest the milk sugar lactose leading to gastrointestinal symptoms and can affect nutrient and calcium intake in certain populations. **Objectives**: We studied a group of 51 Chilean patients to assess whether this SNP influences the LP/LNP state in this population, and determined the prevalence of LCT C>T_{-13910} genotypes in a representative sample of 216 Hispanics and 43 Amerindians with correlation to digestive symptoms. **Design**: Case-Control study done in Chilean patients with clinical suspicion of LNP that were assessed using clinical survey, hydrogen breath test (HBT), and SNP genotyping. The population sample of Hispanics and Amerindians was assessed by clinical survey and SNP genotyping. **Results**: Of the 51 patients with clinical suspicion of LNP, 29 were HBT positive. The CC genotype (LNP) was present in 89.7% of the patients with positive HBT and in only 4.7% of those with negative HBT. The prevalence of the CC genotype was 56.9% in the Hispanic population and 88.3% in Amerindians and was associated with a higher self reported clinical intolerance to dairies ingestion. **Conclusion**: The LP/LNP state is determined by the LCT C>T_{-13910} variant in Chileans. This variant predicts digestive symptoms associated with the ingestion of lactose and is a good tool for the diagnosis of primary adult hypolactasia. The LCT T_{-13910} allele is rare in the Amerindian population and is suggestive of European ancestry in this contemporary population.

ARTICLE SUMMARY

Article focus: The aims of the present study were three questions; 1) If the European variant C/T-13910 determined lactose-persistent and lactose non-persistent state in adult Chilean Hispanic and Amerindian population; 2) If there is a correlation between the C/T-13910 genotyping and the hydrogen breath test (HBT) for diagnosis of LNP in our population; 3) To determine for the first time in an Latin American population the prevalence of the lactose non-persistence genotype (CC) and its correlation with digestive symptoms and ingestion of dairy products.

Significance of the study: The three key messages of the present study were: 1) We demonstrate for the first time that the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian population. 2) We demonstrate a strong correlation between the C-13910>T genotype with the hydrogen breath test and lactose tolerance in a group of patients with clinical suspicion of lactose intolerance. 3) In a representative sample of the Hispanic Chilean population (Mestizos) and in Amerindians, we observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between ethnic groups (56% and 88%, respectively). Interestingly, C-13910>T genotype was significantly associated with digestive symptoms and self reporting intolerance to dairy ingestion. This could explain the observed low consumption of dairy products (30 L milk/person/year) in our population.

Strengths and limitations of the study:

- Our strengths:

- This is the first study in Latin America that assesses if the European lactase persistence -13910T variant determines lactase persistence in the Chilean Hispanic and Amerindian general population.

- Our results may be of clinical and epidemiological relevance, not only for the Chilean population, but also for other Hispanic and Amerindian populations of the Americas with similar genetic background. The fact that the LNP state is an inherited frequent condition in Mestizo and Amerindians should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new and high quality lactose-free dairy products in these regions.

- We randomly selected a representative sample of the Hispanic Chilean population (n=216) and Amerindians (n=43), and performed the genotyping of LCT C>T $_{-13910}$. We observed a high prevalence of the lactose non-persistence genotype (CC) with a clear gradient between both ethnic groups (56% and 88%, respectively), that correlates with digestive symptoms and self reporting intolerance to dairy ingestion.

- **Our limitations:**

  - Non randomized recruitment of the 51 patients with suspicion of lactose intolerance could induce selection bias.

  - A small number of patients with suspicion of lactose intolerance (n= 51).

- Semi-quantitative food frequency questionnaire with a 24 h dietary recall obtained in a subgroup of the Hispanics and performed years before the genetic analysis.

INTRODUCTION

Lactose, the main sugar in milk, must be hydrolyzed by lactase-phlorizin hydrolase into glucose and galactose for absorption. Intestinal activity of the lactase enzyme (LCT) (MIM 603202) decreases in all mammals after weaning, resulting in poor lactose absorption[1]. In humans this condition is termed "lactase non-persistence" (LNP), "adult type hypolactasia" (MIM 223100) and is estimated to occur in approximately 65% of the contemporary world population[2, 3]. LNP can generate intolerance to the ingestion of dairies with the development of abdominal and systemic symptoms that lead to medical consultations and evaluations. In contrast, lactase persistent (LP) individuals remain tolerant of lactose and maintain high levels of LCT expression throughout adult life[4]. The prevalence of the LP phenotype, which is an autosomal dominant condition (MIM 223100) is reported to vary between 0 and 95% in different populations[3], but is unknown in contemporary Latin American populations [2, 5].

The recent discovery of single nucleotide polymorphisms (SNPs) that determine the LP state in humans, both in Europe[6] and among some pastoral populations in Africa, has generated growing interest in the study of this condition[2, 7]. The appearance of these SNPs seems to have occurred on more than one occasion in human evolution between 1,500 and 12,000 y ago in northern Europe and among some nomadic populations of Asia and Africa[7]. The SNP known as LCT C>T_{-13910} (rs4988235) is located 13.9 kb upstream of the LCT gene in the 13th intron of an adjacent gene (MCM6). The T allele of this SNP correlates with the LP state in European populations[6], and individuals with this allele have higher levels of LCT transcripts in enterocytes compared to individuals who carry the C allele[8]. *In vitro* studies show that cells transfected with the LCT T_{-13910} variant show increased activity

of the LCT promoter[9]. Another SNP known as LCT G>A$_{-22018}$, shows some association with the LP/LNP phenotype, and while it does not appear to be the causal variant, it is present in a block of linkage disequilibrium with little evidence of recombination in this region of the genome[5, 10].

In clinical practice, the LNP state is relevant because it can generate malabsorption and intolerance of lactose, with the development of clinical symptoms such as abdominal pain and distension, diarrhea, bloating, nausea, and vomiting[3]. A significant percentage of patients do not relate these symptoms to dairies ingestion[11], and the recurring nature of the symptoms can lead to misdiagnosis of chronic diarrhea, irritable bowel syndrome, celiac illness, or other clinical conditions[12, 13, 14, 15, 16]. The diagnosis of LNP is often based on clinical suspicion and the favorable response to a lactose restricted diet.

The most widely used diagnostic test of poor lactose absorption is the hydrogen breath test (HBT)[11, 17]. Nevertheless, this test has practical limits and its sensitivity and specificity varies between 69 - 100% and 89 - 100%, respectively, compared to measurement of LCT activity in intestinal biopsies. Additionally, there is a false negative rate of 11 - 30%, and a false positive rate of up to 57% using this test[18, 19, 20]. Using SNP genotyping in patients with suspected LNP may therefore aid in proper diagnosis[10, 21, 22, 23, 24, 25]. The only epidemiological study of adult hypolactasia in a Chilean population was performed more than three decades ago[26] and suggested that the prevalence of LNP was 56% and 75% for the pediatric and adult populations, respectively. Our objectives were to determine the influence of the LCT C>T$_{-13910}$ SNP on the LP/LNP state in the Chilean population and to determine the population prevalence of the SNP genotypes in a representative Hispanic and Amerindian

population with comparison of the genotypes to clinical symptoms related to dairies ingestion.

SUBJECTS AND METHODS

Patients with clinical suspicion of lactase non persistence (LNP).

In 2006, 51 patients were enrolled in a prospective study after being referred to the Gastroenterological Department of the Clinical Hospital at the Pontificia Universidad Catolica de Chile because of symptoms suggestive of LNP. A pre-coded survey was obtained from all patients to obtain demographic, anthropometric, familial, and clinical data, including any symptoms experienced during the diagnostic hydrogen breath test (HBT) such as abdominal pain, diarrhea, bloating, nausea, and vomiting. A 5 ml EDTA blood sample was obtained to extract DNA. Serum levels of immunoglobulin A and tissue anti-transglutaminase antibodies (hu tTG ELISA, IMMCO Diagnostics, Inc., Buffalo, NY) were quantified in all the patients to rule out the existence of celiac disease. This study was approved by the Ethics Committee of the Faculty of Medicine, PUC, and all patients signed a written informed consent form prior to the study.

Hydrogen breath test (HBT) for diagnosis of LNP.

Patients were instructed to maintain a low fiber diet without lactose for 48 h prior to the day of the examination. After 12 h of fasting, a baseline concentration of H_2 in exhaled breath was determined using a Quintron® microLyzer (QuinTron Instrument Company, Milwaukee, WI). Subjects then ingested 25 g lactose and H_2 concentration in breath was quantified every 20 m for a maximum of 4 h. Individuals were considered LNP with an increase in H_2 concentration 20 ppm above the base value[17, 27].

Population study.

A representative sample of 216 unrelated Hispanics and 43 unrelated Amerindians were randomly selected from an initial sample of 1581 Hispanics and 120 Amerindians (Mapuches, the Chilean native population) which were collected between 1993 and

2000 as described previously[28, 29]. By using an Amerindian Admixture Index based on ABO blood group distribution, we have previously demonstrated in these cohorts a 40% and 80% Amerindian ancestry in the Hispanic and Mapuche population, respectively. Furthermore, 88% of Hispanics and 100% of Mapuches shared ancestral Amerindian mtDNA polymorphism[28, 29]. These results were in accordance with the biparental founder origin of the mixed Chilean population, which could be similar to other Hispanic populations from America[30, 31, 32].

The sample size was selected by estimating an expected prevalence of the LCT-13910CC genotype to be at least 50% in the Hispanic population and 80% in the Amerindians, with an alpha error of 5% and a level of confidence of 80%. All study subjects provided a DNA sample and a medical survey that provided anthropometric and clinical data including information about diarrhea, abdominal pain, or bloating in response to ingestion of dairy products[28, 29]. Furthermore, a subgroup of the Hispanic subjects provided a semi-quantitative food frequency questionnaire with a 24 h dietary recall. From this data, specific energy and nutrient ingestion from dairy products was estimated using the Datadiet program[28, 33]. For all subjects, the occurrences of digestive symptoms in response to consumption of lactose were correlated to SNP genotyping results.

Genotyping of LCT C>T $_{-13910}$ and LCT G>A $_{-22018}$ SNPs

SNP genotyping was performed using PCR-RFLP methods[34] (GenBank reference sequence NM_005915.4). Briefly, genomic DNA was extracted from 300 µl of blood according to manufacturer's instructions (Wizard SV Genomic DNA Purification System, Promega, Madison, WI) and the regions surrounding the SNPs were amplified through PCR using primers (F) 5´-GGA TGC ACT GCT GTG ATG AG-3´ and (R) 5-CCC ACT GAC CTA TCC TCG TG-3´ for the LCT C>T $_{-13910}$ and (F) 5´-AAC AGG

CAC GTG GAG GAG TT-3´ and (R) 5´- CCC ACC TCA GCC TCT TGA GT-3´ for LCT G>A $_{-22018}$. In both cases, the amplified products were of 448 bp. The PCR reactions and RFLP assays for the analysis of both genetic variants was carried out as described previously [34]. Briefly, the amplified product for the LCT C>T $_{-13910}$ SNP was digested with *BsmFI*, generating two fragments of 351 and 97 bp in the presence of the CC genotype, and 3 fragments of 253, 98, and 97 bp in the presence of the TT genotype (**Figure 1**). For the LCT G>A $_{-22018}$ SNP, the PCR product was digested with *HhaI*, generating two fragments of 284 and 184 bp in the presence of the GG genotype and one undigested fragment of 448 bp in the presence of the A/A genotype. The amplified and digested PCR products were separated by electrophoresis on 2% agar gels in 1X TAE buffer stained with ethidium bromide. Genotyping confirmation was carried out on 35 samples by direct sequencing of the 448 bp amplified fragments (models ABI-310 and ABI-3100 automated DNA sequencers, Applied Biosystems, Foster City, CA*)* (**Figure 1**). There was complete concordance between the PCR-RFLP analysis and the sequencing in all cases.

Statistical analysis

Mean and standard deviations were used to describe numeric variables. The variables of categorical types are given as a number and a percentage. Comparisons of continuous variables were performed using the student T test for independent samples. Comparisons of categorical variables were carried out using the chi-square test. Using the HBT breath test as diagnostic for LNP, an analysis was made of the sensitivity and specificity of the genetic test as an alternative diagnostic test. Differences were considered significant when $p<0.05$.

RESULTS

Hydrogen breath test and SNP genotyping

Fifty-one patients with clinical suspicion of LNP (44 women and 7 men, age 14 - 79 y) were included in the study. Twenty-nine patients (56.8%) had a positive HBT and 22 were negative (43.2%) (**Table 1**). There were no significant differences in the distribution by sex and age between the two groups. There was a positive correlation between the HBT results and digestive symptoms reported during the test: 82% of the patients with positive HBT had symptoms associated with the lactose load, such as bloating, abdominal pain, and diarrhea (79%, 58% and 20%, respectively). In contrast, only 27% of the patients with negative HBT presented symptoms after ingesting lactose ($p < 0.001$). Additionally, 76% of patients with positive HBT indicated a personal and/or family history of functional digestive disorders, compared to 36% of those with negative HBT ($p < 0.001$).

The LCTC>T_{-13910} SNP genotyping results for the 51 patients is shown in Table 1. Of the 29 patients with positive HBT, 26 (89.7%) had the LCT-13910CC genotype (the LNP genotype), and 3 (10.3%) had the LCT-13910CT genotype and were therefore phenotypically LNP but genotypically LP. However, two of these 3 patients experienced bloating and diarrhea during the HBT, which suggests malabsorption of lactose. Of the 22 patients who had a negative HBT, 19 were LCT-13910CT, two were LCT-13910TT, and a single 16-year-old patient had the LNP LCT-13910CC genotype (Table 1). However, this patient experienced abdominal pain during the HBT. Subsequent evaluation of this patient with a lactulose HBT (10 g load) revealed that this patient was not a non-producer of hydrogen. A single patient with LNP genotype and positive HBT had high levels of anti-tTG (27 μU/ml), which suggests the possibility of

coexistence of LNP and celiac disease. All the patients showed normal levels of serum IgA (data not shown).

There was complete genotypic correlation between the LCTC>T_{-13910} and the LCTG>A_{-22018} SNPs, such that any subject homozygous or heterozygous for one SNP was at the same time homozygous or heterozygous for the other SNP (data not shown). Furthermore, there was good correlation between the LCTC>T_{-13910} SNP findings and the HBT test results (**Table 2**). Genotyping results were confirmed by sequencing for 35 of the 51 samples (**Figure 2**). Interestingly, one of the three discordant patients with LNP phenotype but LP genotype was also heterozygous at a second location not previously described in the literature, LCTG>A_{-13937}.

Population study

We collected DNA samples and medical surveys from 216 unrelated Hispanic individuals and 43 unrelated Amerindians. The survey information is summarized in **Table 3**. The two groups were similar in age, with higher representation of females among the Amerindians. The Amerindian cohort reported a higher frequency of intolerance to ingesting dairy products and recurring diarrhea while both groups reported recurring abdominal bloating with equal frequency.

The genotype and allele frequencies for the LCTC>T_{-13910} SNP are shown in **Table 3**. As was found in the study group of 51 Chilean patients with suspected lactose intolerance, there was complete concordance between the genotypes of the LCTC>T_{-13910} and LCTG>A_{-22018} SNPs in each individual (data not shown). The cohort of Amerindian individuals was in Hardy-Weinberg equilibrium (**Figure 2**), but we observed a slightly higher than expected frequency of the T_{-13910} allele in the Hispanic population (deviation from HW equilibrium $p < 0.05$).). Interestingly, this deviation from HW equilibrium has also been observed in other mixed ethnicity groups[3, 35].

The prevalence of the ancestral LCT-13910CC genotype (the LNP genotype) was 56.9%, significantly lower than the prevalence of this genotype among the Amerindian individuals (88.3%). In turn, 41.7% of the Hispanics were LCT-13910CT genotype compared to only 11.7% of the Amerindians. The LCT-13910TT genotype was found in only 3 Hispanic subjects. Allele frequencies for the LCTC>T_{-13910} SNP were significantly different between the two groups, with the T_{-13910} allele being very infrequent in Amerindians.

For the 216 Hispanic individuals studied, we were able to gather clinical and genetic data (**Table 3**). Individuals with the LNP LCT-13910CC genotype were 3 times more likely to report intolerance to the ingestion of dairy products and recurring diarrhea than those with LP genotypes (LCT-13910CT or LCT-13910TT). For 112 of these individuals, we collected complete nutritional information. The consumption of dairy products was in general very low, equivalent to an average of 30 L of fresh milk per year per person. However, the group of individuals with an LP genotype had 38% higher consumption of dairy products than the group of individuals with the LNP genotype (38.4 L ± 67 L versus 27.7 L ± 40 L milk/year, respectively), although this difference was not statistically significant.

DISCUSSION

The normal physiologic decrease in lactase (LCT) production in the mammalian intestine following weaning is known as lactase non persistance (LNP), and results in the inability to digest the milk sugar, lactose, which can cause gastrointestinal symptoms after ingestion of dairies. The condition of lactase persistence (LP), found only in humans, allows the individual to continue to ingest and absorb lactose into adulthood. In European populations and some Afro-Arab pastoral groups, the LP condition has been shown to be due to SNP's found 13.9 kb upstream of the LCT gene and actually located within the 13th intron of the MCM6 gene. One of these SNP's, LCTC>T$_{-13910}$ (rs4988235), appeared approximately 10,000y ago in the human history and, has spread progressively from Northen Europe through the world due to population migration and mixing, increasing the state of LP in different populations[2]. The original populations of the Americas and Asia were likely LNP. There is no evidence of the existence of American variants that might have generated the LP state in the pre-Columbian era[2, 5]. The T$_{-13910}$ allele, which leads to the LP state, was likely introduced to the Americas by the migration of European carriers of this variant approximately 500 years ago and spread rapidly in the descending admixed Hispanic (Mestizo) populations of contemporary America, as is shown in this study as well as others[5, 36].

Our study of a representative population of Chile consisting of Hispanic and Amerindian individuals showed the prevalence of the LNP state (LCT-13910CC genotype) to be 57% and 88%, respectively. It is interesting to note that this observed genotypic frequency is very similar to the Amerindian admixture index (AAI) estimated by us in these same populations based on the distribution of ABO blood groups [28].

This suggests that the analysis of the LCTC>T_{-13910} SNP in Latin American populations can be a good indicator of the degree of Amerindian or Caucasian inheritance.

In all Chilean patients genotyped in this study, the LCTG>A_{-22018} SNP genotype correlated completely with the genotype found for the LCTC>T_{-13910} SNP, a finding reported in many studies. This phenomenon can be explained by the fact that both SNPs are located within a highly conserved block of linkage disequilibrium of at least 500 kb and likely co-segregate[2, 5].

According to HW equilibrium, in the group of 216 unrelated Hispanic individuals we observed a higher than expected frequency of the T_{-13910} allele. While this could be explained by selection bias or by a higher frequency of LP in the Spanish population that founded the Chilean colony, it could also be reflective of positive selection for LP allele carriers, as has been suggested for decades[37, 38, 39]. Any evolutionary advantage conferred by the T_{-13910} allele may or may not be related to the consumption of dairy products. Indeed, it was reported that this allele confers protection not only against bone fractures[40, 41], but also colon cancer[42].

The validity of using LCTC>T_{-13910} SNP genotyping for diagnosis of the LNP or LP state was evaluated in the group of 51 Chilean patients with suspected LNP. The HBT showed a high positive and negative predictive value, sensitivity and specificity compared to genetic testing (Table 2). These results are similar to those recently reported by other groups[10, 24, 34, 43] and confirm the utility of the analysis of this SNP as a clinical test for the diagnosis of LP/LNP in the adult Chilean population. Only 3 patients (10.3%) with malabsorption of lactose shown by HBT, were genetically LP (LCT-13910CT genotype). It was not possible to recall these 3 patients to carry out complementary studies to rule out other causes of lactose malabsorption, such as intestinal parasitic infections like Giardiasis, seronegative celiac disease or other

conditions as has been shown in other recent studies[43]. Interestingly, one of these patients was also heterozygous at an additional SNP not previously described, LCTG>A$_{-13937}$[2]. We do not know if this new variant can explain the phenotype-genotype discordance.

A recent study showed a high prevalence of celiac illness among patients with an initial diagnosis of LNP, suggesting that lactose intolerance can be the first manifestation of celiac illness; the authors recommend considering the existence of celiac illness in all patients with LNP before recommending a lactose free diet for life[44]. In our study of 51 patients with clinical suspicion of lactose intolerance, only one with LNP confirmed by HBP and SNP genotyping had positive anti-tTG (2% of the total) suggesting the coexistence of both conditions.

One 16-year-old patient had negative HBT yet had the LNP genotype. It is possible that this patient has not yet manifested the LNP phenotype and still maintains sufficient levels of intestinal lactase to digest a 25 g load. The age at which the LNP state is initiated varies among different populations and ethnic groups, starting as early as 1 to 8 y in black and Asian populations, and much later (20 y) in northern European populations[10, 45, 46, 47]. The age of initiation of the LNP state in Chilean individuals remains unknown.

Among the 51 Chilean patients with positive HBT and the LCT-13910CC genotype, significantly more report having relatives with functional digestive disorders than their HBT negative counterparts, suggesting that the condition of LNP could be in part responsible for digestive symptoms. In our complete clinical, nutritional, and genetic study of 112 Hispanic individuals, those with the LCT-13910CC genotype were significantly more likely to self-report lactose intolerance despite very low (30 L milk/person/year) consumption of dairy products. This level is well below the WHO

recommended level of 240 L milk/person/year. Reasons for the low level of consumption are likely due to multiple causes including cost, preference, and societal habits which are likely influenced by the existence of a high prevalence of LNP and lactose intolerant state within this population. It has been reported that the allele frequencies of the LCTC>T_{-13910} SNP within a population correlates with the tendency of that population to consume dairy products and ingest calcium[48, 49, 50]. Both this study and one other[51] show that the effective consumption of dairy products and calcium in Chile remains below international recommendations.

In summary, the LNP state continues to be predominant in the Chilean population. Epidemiologically, this may cause the development of digestive symptoms affecting quality of life and leading to medical consultations as well as lower tendency to ingest dairy products and calcium within the adult population of the contemporary Americas. The fact that the LNP state is an inherited condition should be considered when developing public programs that encourage consumption of dairy products, and should be a stimulus for the food industry to develop new lactose-free dairy products.

Table 1. Clinical and genetic characteristics of 51 Chilean patients having hydrogen breath test (HBT) for suspicion of lactose non persistence (LNP).

	HBT Positive (n = 29)	**HBT Negative (n = 22)**	***p***
Female gender (%)	82.7	86.3	n.s.
Age (y)	36 ± 19	32 ± 12	n.s.
Number reporting gastrointestinal symptoms after lactose load	24 (82%)	6 (27%)	< 0.001
Number reporting family history of IBS	22 (76%)	8 (36%)	< 0.001
LCT-13910 CC genotype	26 (89.6%)	1 (4.5%)	< 0.001
LCT-13910 CT/TT genotype	3 (10.3%)	21(95.5%)	< 0.001

IBS, irritable bowel syndrome.

Table 2. Correlation of the HBT in comparison to LCT C>T$_{-13910}$ genotype in 51 Chilean patients.

Sensitivity	96.3%
Specificity	87.5%
Predictive value positive	89.7%
Predictive value negative	95.5%
LHR positive	7.7
LHR negative	0.04

LHR, likelihood ratio

Table 3. General characteristics, self reported gastrointestinal symptoms related to dairy ingestion and LCTC>T$_{-13910}$ SNP genotype and allele frequencies in a population of Hispanic and Amerindian individuals.

	Hispanics n = 216	**Amerindians** n = 43	*P*
Women (%)	46	65	0.02
Age (y)	50 ± 12	54 ± 15	n.s.
Number self reporting lactose intolerance	44 (20.4%)	19 (44.1%)	0.001
Number reporting diarrhea	15 (7%)	16 (37.2%)	<0.001
Number reporting bloating	77 (35.6%)	14 (32.5%)	n.s.
LCT-13910 CC genotype	123 (56.9%)	38 (88.3%)	< 0.001
LCT-13910 CT genotype	90 (41.7%)	5 (11.7%)	< 0.001
LCT-13910 TT genotype	3 (1.4%)	0	n.s.
C allele frequency	77.7%	94.2%	< 0.001
T allele frequency	22.3%	5.8%	< 0.001

n.s., not significant

Author's contribution: Study concept and design (JFM); acquisition of data (JFM, EM, LA, XM and CP); analysis and interpretation of data (JFM, EM, JCh); drafting of the manuscript (JFM, EM, XM, CP, LA); critical revision of the manuscript for important intellectual content (JCh); statistical analysis (JFM, EM, XM, CP); obtained funding (JFM, JCh); final revision and edition (JFM, CP, XM, EM, LA, JCh).

Acknowledgments: The authors acknowledge the Alexander von Humboldt Foundation (Germany) for donating the NanoDrop ND-1000 Spectrophotometer used for DNA analysis and quantification. The authors thank Dr. Flavio Nervi for providing access to the database and DNA bank of the Hispanic and Mapuche populations, and Dr. Julieta Klassen for collaboration in the analysis of dairy product ingestion.

Funding: This study was supported by grants of the National Commission of Scientific and Technological Research (FONDECYT N°1080325 to JFM), and of the Office of Research affairs of the Pontificia Universidad Catolica de Chile, School of Medicine to EM and JFM (PG-42/06).

Competing Interest: We declare no conflicts of interest.

License for Publication:

REFERENCES

1 Enattah N, Pekkarinen T, Valimaki MJ, *et al.* Genetically defined adult-type hypolactasia and self-reported lactose intolerance as risk factors of osteoporosis in Finnish postmenopausal women. Eur J Clin Nutr 2005;**59**:1105-11.

2 Ingram CJ, Mulcare CA, Itan Y, *et al.* Lactose digestion and the evolutionary genetics of lactase persistence. Hum Genet 2009;**124**:579-91.

3 Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice--myths and realities. Aliment Pharmacol Ther 2008;**27**:93-103.

4 Matthews SB, Waud JP, Roberts AG, *et al.* Systemic lactose intolerance: a new perspective on an old problem. Postgrad Med J 2005;**81**:167-73.

5 Bersaglieri T, Sabeti PC, Patterson N, *et al.* Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet 2004;**74**:1111-20.

6 Enattah NS, Sahi T, Savilahti E, *et al.* Identification of a variant associated with adult-type hypolactasia. Nat Genet 2002;**30**:233-7.

7 Tishkoff SA, Reed FA, Ranciaro A, *et al.* Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 2007;**39**:31-40.

8 Kuokkanen M, Enattah NS, Oksanen A, *et al.* Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. Gut 2003;**52**:647-52.

9 Lewinsky RH, Jensen TG, Moller J, *et al.* T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro. Hum Mol Genet 2005;**14**:3945-53.

10 Rasinpera H, Savilahti E, Enattah NS, *et al.* A genetic test which can be used to diagnose adult-type hypolactasia in children. Gut 2004;**53**:1571-6.

11 Shaw AD, Davies GJ. Lactose intolerance: problems in diagnosis and treatment. J Clin Gastroenterol 1999;**28**:208-16.

12 Parker TJ, Woolner JT, Prevost AT, *et al.* Irritable bowel syndrome: is the search for lactose intolerance justified? Eur J Gastroenterol Hepatol 2001;**13**:219-25.

13 Vesa TH, Seppo LM, Marteau PR, *et al.* Role of irritable bowel syndrome in subjective lactose intolerance. Am J Clin Nutr 1998;**67**:710-5.

14 Bode S, Gudmand-Hoyer E. Incidence and clinical significance of lactose malabsorption in adult coeliac disease. Scand J Gastroenterol 1988;**23**:484-8.

15 Srinivasan U, Jones E, Weir DG, *et al.* Lactase enzyme, detected immunohistochemically, is lost in active celiac disease, but unaffected by oats challenge. Am J Gastroenterol 1999;**94**:2936-41.

16 Barr RG, Levine MD, Watkins JB. Recurrent abdominal pain of childhood due to lactose intolerance. N Engl J Med 1979;**300**:1449-52.

17 Peuhkuri K, Poussa T, Korpela R. Comparison of a portable breath hydrogen analyser (Micro H2) with a Quintron MicroLyzer in measuring lactose maldigestion, and the evaluation of a Micro H2 for diagnosing hypolactasia. Scand J Clin Lab Invest 1998;**58**:217-24.

18 Rosado JL, Solomons NW. Sensitivity and specificity of the hydrogen breath-analysis test for detecting malabsorption of physiological doses of lactose. Clin Chem 1983;**29**:545-8.

19 Barillas-Mury C, Solomons NW. Test-retest reproducibility of hydrogen breath test for lactose maldigestion in preschool children. J Pediatr Gastroenterol Nutr 1987;**6**:281-5.

20 Arola H, Koivula T, Jokela H, *et al.* Comparison of indirect diagnostic methods for hypolactasia. Scand J Gastroenterol 1988;**23**:351-7.

21 Jarvela IE. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). Scand J Clin Lab Invest 2005;**65**:535-9.

22 Bodlaj G, Stocher M, Hufnagl P, *et al.* Genotyping of the lactase-phlorizin hydrolase -13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. Clin Chem 2006;**52**:148-51.

23 Enattah NS, Kuokkanen M, Forsblom C, *et al.* Correlation of intestinal disaccharidase activities with the C/T-13910 variant and age. World J Gastroenterol 2007;**13**:3508-12.

24 Schirru E, Corona V, Usai-Satta P, *et al.* Genetic testing improves the diagnosis of adult type hypolactasia in the Mediterranean population of Sardinia. Eur J Clin Nutr 2007;**61**:1220-5.

25 Satta PU, Congia M, Schirru E, Scarpa M, Mura G. Genetic testing is ready to change the diagnostic scenario of lactose malabsorption. Gut 2008;**57**:137-8; author reply 8.

26 Lacassie Y, Weinberg R, Monckeberg F. Poor predictability of lactose malabsorption from clinical symptoms for Chilean populations. Am J Clin Nutr 1978;**31**:799-804.

27 Rana S, Bhasin DK, Gupta D, *et al.* Assessment of optimal dose of lactose for lactose hydrogen breath test in Indian adults. Indian J Gastroenterol 1995;**14**:13-4.

28 Miquel JF, Covarrubias C, Villaroel L, *et al.* Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. Gastroenterology 1998;**115**:937-46.

29 Nervi F, Miquel JF, Alvarez M, *et al.* Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. J Hepatol 2006;**45**:299-305.

30 Merriwether DA, Rothhammer F, Ferrell RE. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. Am J Phys Anthropol 1995;**98**:411-30.

31 Moraga ML, Rocco P, Miquel JF, *et al.* Mitochondrial DNA polymorphisms in Chilean aboriginal populations: implications for the peopling of the southern cone of the continent. Am J Phys Anthropol 2000;**113**:19-29.

32 Rocco P, Morales C, Moraga M, *et al.* [Genetic composition of the Chilean population. Analysis of mitochondrial DNA polymorphism]. Rev Med Chil 2002;**130**:125-31.

33 Cuevas A, Miquel JF, Reyes MS, *et al.* Diet as a risk factor for cholesterol gallstone disease. J Am Coll Nutr 2004;**23**:187-96.

34 Buning C, Genschel J, Jurga J, *et al.* Introducing genetic testing for adult-type hypolactasia. Digestion 2005;**71**:245-50.

35 Almon R, Engfeldt P, Tysk C, *et al.* Prevalence and trends in adult-type hypolactasia in different age cohorts in Central Sweden diagnosed by genotyping for the adult-type hypolactasia-linked LCT -13910C > T mutation. Scand J Gastroenterol 2007;**42**:165-70.

36 Bulhoes AC, Goldani HA, Oliveira FS, *et al.* Correlation between lactose absorption and the C/T-13910 and G/A-22018 mutations of the lactase-phlorizin hydrolase (LCT) gene in adult-type hypolactasia. Braz J Med Biol Res 2007;**40**:1441-6.

37 Cook GC, al-Torki MT. High intestinal lactase concentrations in adult Arbs in Saudi Arabia. Br Med J 1975;**3**:135-6.

38 Charney M, McCracken RD. Intestinal lactase deficiency in adult nonhuman primates: implications for selection pressures in man. Soc Biol 1971;**18**:416-21.

39 Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. Am J Dig Dis 1970;**15**:695-710.

40 Enattah NS, Sulkava R, Halonen P, *et al.* Genetic variant of lactase-persistent C/T-13910 is associated with bone fractures in very old age. J Am Geriatr Soc 2005;**53**:79-82.

41 Obermayer-Pietsch BM, Gugatschka M, Reitter S, *et al.* Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption? Osteoporos Int 2007;**18**:445-51.

42 Rasinpera H, Forsblom C, Enattah NS, *et al.* The C/C-13910 genotype of adult-type hypolactasia is associated with an increased risk of colorectal cancer in the Finnish population. Gut 2005;**54**:643-7.

43 Krawczyk M, Wolska M, Schwartz S, *et al.* Concordance of genetic and breath tests for lactose intolerance in a tertiary referral centre. J Gastrointestin Liver Dis 2008;**17**:135-9.

44 Ojetti V, Nucera G, Migneco A, *et al.* High prevalence of celiac disease in patients with lactose intolerance. Digestion 2005;**71**:106-10.

45 Sahi T, Isokoski M, Jussila J, *et al.* Lactose malabsorption in Finnish children of school age. Acta Paediatr Scand 1972;**61**:11-6.

46 Simoons FJ. Age of onset of lactose malabsorption. Pediatrics 1980;**66**:646-8.

47 Wang Y, Harvey CB, Hollox EJ, *et al.* The genetically programmed down-regulation of lactase in children. Gastroenterology 1998;**114**:1230-6.

48 Laaksonen MM, Mikkila V, Rasanen L, *et al.* Genetic lactase non-persistence, consumption of milk products and intakes of milk nutrients in Finns from childhood to young adulthood. Br J Nutr 2009:1-10.

49 Torniainen S, Hedelin M, Autio V, *et al.* Lactase persistence, dietary intake of milk, and the risk for prostate cancer in Sweden and Finland. Cancer Epidemiol Biomarkers Prev 2007;**16**:956-61.

50 Lehtimaki T, Hemminki J, Rontu R, *et al.* The effects of adult-type hypolactasia on body height growth and dietary calcium intake from childhood into young adulthood: a 21-year follow-up study--the Cardiovascular Risk in Young Finns Study. Pediatrics 2006;**118**:1553-9.

51 Castillo C, Atalah E, Benavides X, Urteaga C. [Food patterns among adults visiting primary care clinics in the metropolitan region]. Rev Med Chil 1997;**125**:283-9.

FIGURE LEGENDS

Figure 1. RFLP and sequence analysis of a 448 bp region of MCM6 intron 13 containing the LCTC>T$_{-13910}$ SNP. Upper panel: RFLP analysis of the 448 bp amplicon digested with BsmFI. Left lane, 50-bp DNA ladder. Right lane, undigested PCR amplicon. Center lanes, digested products from representative individuals having the three different SNP genotypes. The ancestral LCT-13910CC genotype results in two bands of 350 bp and 110 bp; LCT-13910CT genotype results in three bands of 350 bp, 240 bp and 110 bp; LCT-13910TT genotype results in two bands of 240 bp and 110 bp. Lower panel: Representative sequence tracings showing confirmation of RFLP data. Direct sequence analysis was performed on 35 of the 51 samples.

Figure 2. De Finetti diagram for the LCTC>T$_{-13910}$ SNP in Hispanic (black dotted line) and Amerindian (blue dotted line) populations. The diagram shows genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequencies of heterozygous genotypes are plotted on the vertical axis, and the allele frequencies are depicted by the interaction of the vertical dotted lines with the bottom perpendicular (allele 1 = C$_{-13910}$). The genotype frequencies plot on the parabola in the diagram, indicating that Amerindians are, but Hispanics are not, in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).

STROBE Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	9
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	9-10
		(*b*) For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	9-10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	n.a.
		(*c*) Explain how missing data were addressed	n.a.
		(*d*) If applicable, explain how matching of cases and controls was addressed	n.a.
		(*e*) Describe any sensitivity analyses	n.a.
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	n.a.
		(b) Give reasons for non-participation at each stage	n.a.
		(c) Consider use of a flow diagram	n.a.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	12,26
		(b) Indicate number of participants with missing data for each variable of interest	n.a.
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	13,28
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n.a.
		(*b*) Report category boundaries when continuous variables were categorized	n.a.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n.a.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	n.a.
Discussion			
Key results	18	Summarize key results with reference to study objectives	16,17
Limitations	19	Discuss limitations of the study, taking into account sources of	16

		potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	17,18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Figure 1

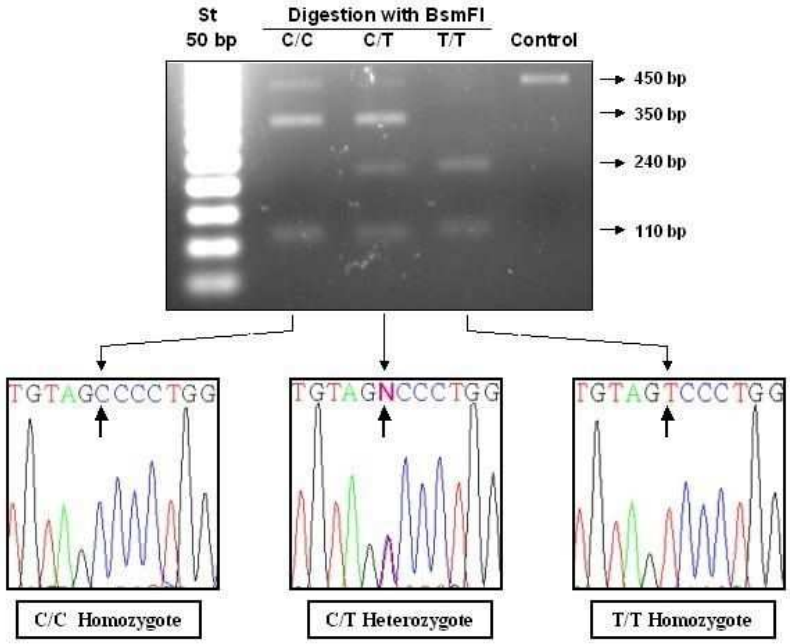


Figure 1. RFLP and sequence analysis of a 448 bp region of MCM6 intron 13 containing the LCTC>T-13910 SNP. Upper panel: RFLP analysis of the 448 bp amplicon digested with BsmFI. Left lane, 50-bp DNA ladder. Right lane, undigested PCR amplicon. Center lanes, digested products from representative individuals having the three different SNP genotypes. The ancestral LCT-13910CC genotype results in two bands of 350 bp and 110 bp; LCT-13910CT genotype results in three bands of 350 bp, 240 bp and 110 bp; LCT-13910TT genotype results in two bands of 240 bp and 110 bp. Lower panel: Representative sequence tracings showing confirmation of RFLP data. Direct sequence analysis was performed on 35 of the 51 samples.
60x45mm (300 x 300 DPI)

Figure 2

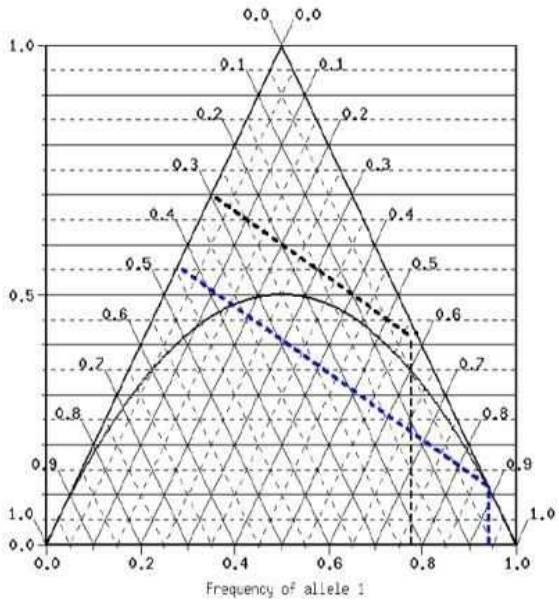


Figure 2. De Finetti diagram for the LCTC>T-13910 SNP in Hispanic (black dotted line) and Amerindian (blue dotted line) populations. The diagram shows genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequencies of heterozygous genotypes are plotted on the vertical axis, and the allele frequencies are depicted by the interaction of the vertical dotted lines with the bottom perpendicular (allele 1 = C-13910). The genotype frequencies plot on the parabola in the diagram, indicating that Amerindians are, but Hispanics are not, in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwal.pl).
60x45mm (300 x 300 DPI)