Accuracy of a Genetic Test for the Diagnosis of Hypolactasia in Chilean Children: Comparison With the Breath Test

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ABSTRACT

Background: Lactase nonpersistence (LNP) in humans is a genetically determined trait. This age-dependent decrease of lactase expression is most frequently caused by single nucleotide polymorphisms in the regulatory region of the lactase (*LCT*) gene. The homozygous LCT-13,910C/C genotype (rs 4988235) predominates in Caucasian adults with LNP, and is useful for its diagnosis in this population. The accuracy of this genetic test (GT) has not been completely established in children or in a Latin-American population.

Objectives: The aim of the study was to determine diagnostic accuracy of GT for LNP in Chilean children using the lactose breath test (BT) as a reference, and to compare diagnostic yield in preschool- (<6 years) and in school-age (≥6 years) children.

Methods: Children referred for BT for diagnosis of lactose malabsorption to the Gastroenterology Laboratory at Clínica Alemana, Santiago, from October 2011 to March 2012 were invited to participate. After informed consent, symptom questionnaires, both historic and post lactose ingestion were completed. H_2 and CH_4 in expired air and −13,910 C>T single nucleotide polymorphism by polymerase chain reaction, restriction enzyme analysis, and/or Sanger sequencing were determined. GT accuracy was calculated compared to BT as reference method. Diagnostic yield of GT in preschool- and school-age children was compared.

Results: Lactose malabsorption was detected by BT in 42 of 60 children (70%). Genotype −13,910C/C was identified in 41 of 60 patients (68%). GT showed 80% sensitivity, 63% specificity, and 74% accuracy for LNP in the preschool population. In school-age children values were higher, 85%, 80%, and 84%, respectively.

Conclusions: GT results were significantly concordant with BT results for hypolactasia detection in Chilean children, particularly in those of age 6 years and older.

Key Words: adult type, lactase, lactase nonpersistence, lactose intolerance

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What Is Known

- Lactase nonpersistence is a genetically determined trait with age-dependant phenotypic manifestation.
- The −13,910C/C genotype in the lactase gene regulatory region is the predominant single nucleotide polymorphism in Caucasian adults with lactose malabsorption.
- The detection of −13,910C/C single nucleotide polymorphism is useful for the diagnosis of hypolactasia in the Caucasian adult population; however, it has not been completely validated in children.

What Is New

- Our results suggest that −13,910C/C genotype is an accurate predictor of lactose malabsorption in Chilean children since 6 years of age.

Lactose malabsorption is a condition due to relative or absolute intestinal lactase deficiency (hypolactasia). Nonabsorbed lactose reaches the colon where it is fermented by bacteria, leading to production of short-chain fatty acids and volatile gas (CO_2, CH_4, H_2). In susceptible individuals, the presence of these metabolites in the intestinal lumen induce clinical manifestations such as bloating, abdominal pain, and diarrhea, a syndrome known as lactose intolerance (1,2).

Three forms of lactose malabsorption have been described: congenital lactose malabsorption, adult-type hypolactasia or lactase nonpersistence (LNP), and secondary lactose malabsorption, associated to intestinal villous atrophy (1). LNP is the predominant phenotype worldwide. The prevalence of this condition increases with age and depends also on ethnicity (1,3). In adults varies widely from 3% to 100%, with the lowest rates in Northwestern Europeans, whereas Asian individuals have the highest prevalence (4–6). During the 1970s, an epidemiologic study done in Chile detected lactose malabsorption in 61% of children and 75% of adults (7). More recently, 43% prevalence of lactose malabsorption has been described by breath test (BT) in Chilean school-age children (8).

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LNP is a genetically determined trait associated with the presence of specific single nucleotide polymorphisms (SNPs) in a regulatory region upstream from the gene that codifies lactase enzyme, *LCT* (MIM 603202). This noncoding region, included within the *MCM6* gene (MIM 601806), contains the *LCT* promoter and other elements that regulate *LCT* transcription. The predominant SNP in Caucasian population that determines *LCT* expression is in the −13,910 position upstream of the *LCT* gene, where the genotype cytosine/cytosine (C/C) is associated with the age-dependent reduction in *LCT* expression. In contrast, genotypes C/T and T/T are associated with persistence of lactase activity (4,5,9). LNP genotype −13,910C/C has been described in 57% of Chilean adults of Hispanic origin and 88% of those of Native American (10). This genotype has been detected in up to 90% of BT-positive cases in Chilean adults with suspected lactose malabsorption (10,11). Other variants in this region have been described in African and Asian populations (12–14) but have not been found in Chilean individuals (10,11). There is scarce information pertaining to children.

Although *LCT* activity determination in intestinal biopsies is the criterion standard for hypolactasia diagnosis (9), BT after lactose ingestion is currently most widely used, because it is a noninvasive, simple and relatively low-cost test (1,15,16). BT, however, has limitations related to preparation with restricted diet, fasting, a 2- to 3-hour period for sample collection, and reproduction of uncomfortable symptoms that must be considered especially in children (2). In addition, its sensitivity and specificity vary between 69% and 100% and 89% and 100%, respectively, compared with determination of *LCT* activity in intestinal biopsies and false positive and negative results have been reported (10,15,17,18).

More recently, the detection of SNPs in the LCT regulatory region associated with the LNP phenotype, by molecular biology, has raised as a new diagnostic tool for hypolactasia in adults (9,11,19,20). This genetic test (GT) has the advantage to be more reproducible and less annoying for the patient. In children, there is reasonable doubt about the validity of this GT compared to BT, because the phenotypic expression of the LNP variant may not manifest before adulthood (21).

The aim of the present study was to determine the diagnostic accuracy of the GT (detection of −13910C/C) compared to BT for the diagnosis of hypolactasia in Chilean children and to compare the yield in preschool- and school-age children.

METHODS

Setting and Patients

Ambulatory patients younger than 18 years of referred by their physicians to the Digestive Physiology Unit of Clínica Alemana de Santiago, for BT to rule out lactose malabsorption, were invited to participate, from October 2011 to March 2012. After informed consent and assent whenever appropriate, children were recruited and a symptom questionnaire (SQ), BT, and a blood sample were obtained for GT. Exclusion criteria were antibiotics, laxatives, prokinetics, or antispasmodics use in the last 2 weeks; previous known intestinal surgery; or chronic gastrointestinal disease. The present study was approved by the Institutional Ethical Committee.

Clinical Information

Before and after the BT each patient or their parents completed a previously validated SQ to detect both historic and post lactose ingestion symptoms (22). In brief, this questionnaire evaluated 5 items: diarrhea, abdominal cramping, vomiting, audible bowel sounds, and flatulence or gas. Symptom severity was rated on a 10-cm visual analogue scale ranging from 0 (no symptom) to 10 (maximum intensity of the symptom). Total score was obtained by the sum of the individual results of the 5 visual analogue scales. Thus, the total score of the SQ ranged from 0 to 50. A score ≥7 was considered positive for lactose intolerance (22).

Breath Test

H_2 and CH_4 were measured in expired air by a QuinTron SC MicroLyzer (QT00130-M; Milwaukee, WI) according to the manufacturer?s instructions. Patients older than 2 years were on a lactose-free diet for 24 hours before BT, whereas in children younger than this age a lactose-free diet was not required. Fasting period required according to age was the following: 4 hours in children younger than 2 years of age, 6 hours in toddlers 2 to 5 years old, and 12 hours in older children. After determination of basal H_2 and CH_4 concentration in exhaled air, patients received 1 dose of powdered lactose (1 g/kg of weight with a maximum dose of 25 g) dissolved in 200 to 250 mL of water. Subsequently, gas measurements were performed every 30 minutes until completing an observation period of 180 minutes. In children younger than 3 years of age samples were obtained using a bag-mask (KidSampler, Quintron, Milwaukee, USA) and in older children a standard mouth adapted breath sampling device was used (AlveoSampler, Quintron). The CO_2, H_2, and CH_4 observed values were interpreted according to international standards, considering malabsorption if there was at least a 20 ppm elevation of H_2 compared to the lowest preceding value and/or if there was at least a 12 ppm elevation of CH_4 compared with basal value (15). All the BT were performed in outpatient setting by the same Medical Technician.

Genetic Test

A blood sample obtained by finger prick was collected on FTA Mini Card (Whatman, GE Healthcare, PA). Three discs were taken from the FTA card with a 2.0 mm Harris Micro-Punch (Whatman, GE Healthcare) and genomic DNA was isolated by FTA purification reagents according to manufacturer's instructions. MCM6 −13,910 polymorphism was assayed by polymerase chain reaction, followed by enzyme digestion and analysis of restriction fragment length polymorphisms (polymerase chain reaction-restriction fragment length polymorphism) as previously described (13). Primers and enzymes were the following: primer forward: GCTGGCAATACAGATAAGATAATGGA, Primer reverse: CTGC TTTGGTTGAAGCGAAGAT, restriction enzyme: HinfI (New England Biolabs, MA). The genotype was determined according to the size of the obtained fragments analyzed in acrylamide gel. Sanger sequencing was used to validate results.

Statistical Analysis

Categorical variables were described by percentages and compared between groups by Chi square test (or Fisher exact whenever appropriate). Continuous variables were described by median and interquartile range, and compared by nonparametric test. EPIDAT 3.1 (Pan American Health Association, Colombia) was used to analyze data. Because the size of the sample studied did not allow us to determine the yield of GT for each year of age, we decided to compare 2 arbitrarily defined groups, younger than 6 and 6 years of age and older. It was a practical decision because this is the age in which Chilean education and health care system categorize children.

RESULTS

Between October 2011 and March 2012, 60 Hispanic children were recruited, from whom 35 (58%) were girls, with a median

TABLE 1. Demographic, clinical, and genetic characteristics of 60 Chilean children subjected to breath test for evaluation of presumptive lactose malabsorption

	Positive Breath Test (n = 42)	Negative Breath Test (n = 18)	*P*
Female, n (%)	25 (60)	9 (50)	NS
Median age (IQ range; years)	7 (5–12)	7 (5–12)	NS
Patients with family history of lactose intolerance, n (%)	14 (33)	7 (39)	NS
Patients with positive historic SQ score for lactose intolerance, n (%)	38 (90)	14 (78)	NS
Median historic symptom score (IQ range)	16 (11–23)	16 (12–23)	NS
Patients with positive post-lactose ingestion score for lactose intolerance, n (%)	5 (12)	7 (39)	NS
Median post-lactose ingestion symptom score (IQ range)	4 (2–11)	4 (2–11)	NS
−13910 CC genotype, n (%)	36 (86%)	5 (28%)	0.00002
−13910 CT/TT genotypes, n (%)	6 (14%)	13 (72%)	

IQ = interquartile; SQ = symptom questionnaire.

age of 7 years (interquartile range 5–12 years old). Forty-two of the patients (70%) showed lactose malabsorption by significant increase in H_2 and/or CH_4 after lactose ingestion in BT. There were no significant differences in demographic characteristics and family history of lactose intolerance between patients with positive and negative BT (Table 1). Presence and severity of both historic and post-lactose ingestion symptoms suggestive of lactose intolerance were similar in children with positive and negative BT (Table 1). As reference laboratory, we did not have access to definitive diagnosis of these patients.

The *LCT* genotype −13,910 C/C was detected in 68% of children and was significantly more frequent in patients with positive BT (86% and 28% in patients with positive and negative BT, respectively; $P < 0.01$; Table 1).

Comparing the results of BT and GT, there was 82% concordance (49/60 cases). Six of the nonconcordant cases were positive only by BT and 5 were positive only by GT. Four of 6 patients positive only by BT did have only increase in CH_4 and not in H_2 slope and post-lactose ingestion symptom score under the cut-off for lactose intolerance. Three of 5 children positive only by GT were younger than 6 years of age.

Table 2 represents diagnostic yield of GT compared to BT in the whole group of patients and comparing preschool- and school-age children. The diagnostic accuracy of GT was higher in patients 6 years of age or older (Table 2).

DISCUSSION

The frequency of lactose malabsorption by BT in this selected children population was 70%. Currently, BT is accepted as the most appropriate method for the diagnosis of lactose malabsorption, due to its reliability and noninvasiveness. This technique is, however, not universally standardized, there are differences in lactose dose, methane assay, test duration, needs preparation with special diet, may reproduce uncomfortable symptoms, and false positive and negative results have been described (10,15). On the contrary, GT is simpler, cost effective, and easier to interpret. It only requires a blood or saliva sample, with no previous preparation (4,10,20). The main disadvantage is that genotype may not coincide with phenotype, a relevant problem in the pediatric age group, because lactase enzyme *switch-off* usually presents at different times from the age of 3 years onward depending on ethnicity (5,9,23). Younger age at presentation has been described in children of African origin compared with Caucasians (9).

In this series, the LNP haplotype −13,910C/C was detected in almost 90% of children with positive BT, which is concordant with previous reports in Chilean adults (10,11). This finding also agrees with the predominant SNP previously identified in other series including Caucasian and African origin hypolactasic individuals (9,24).

The protocol did not inquire about ethnic origins, and ancestry informative markers were not analyzed; thus, no association could be made between ancestry, genotype, and LNP. Studies by our group and others have shown that Chilean population is the result of European and Amerindian admixture, with a minor African component (23). Prior ancestry characterization of individuals seen at Clínica Alemana showed a higher proportion of European ancestry compared with other groups in the country (24).

Six patients with positive BT and negative GT were identified. This observation may be explained by a secondary-type lactose malabsorption, heterozygous individuals with low lactase expression, presence of other LNP-associated SNP, or a false positive BT (25–27). Five patients were positive for hypolactasia only by GT, from which 3 were younger than 6 years. These cases may be explained, as mentioned previously, by the age-dependent decrease of lactase activity expression (5,9,23). We did not have access to definitive diagnosis of these patients, because we are a

TABLE 2. Diagnostic yield of genetic test compared to breath test in Chilean children with clinical presentation suggestive of lactose malabsorption

	All patients (n = 60)	Preschool age, <6 years old (n = 23)	School age, ≥6 years old (n = 37)
Sensitivity (CI 95%)	83% (71%–96%)	80% (56%–100%)	85% (70%–100%)
Specificity (CI 95%)	72% (49%–96%)	63% (23%–100%)	80% (50%–100%)
PPV (CI 95%)	88% (76%–99%)	80% (56%–100%)	92% (79%–100%)
NPV (CI 95%)	65% (42%–88%)	63% (23%–100%)	67% (36%–98%)
LR (+) (CI 95%)	3.0 (1.4–6.4)	2.1 (0.8–5.4)	4.3 (1.2–14.9)
LR (−) (CI 95%)	0.2 (0.1–0.5)	0.3 (0.1–1.0)	0.2 (0.1–0.5)
Accuracy	80% (69%–91%)	74% (54%–94%)	84% (71–97%)

CI = confidence interval; LR (+) = (+) likelihood ratio; LR (−) = (−) Likelihood ratio; NPV = (−) predictive value; PPV = (+) predictive value.

reference laboratory for BT, which is a limitation of the study design.

The present study shows that in children 6 years of age or older, the presence of C/C genotype at position −13,910 *LCT*, is a good predictor of lactose malabsorption defined by a positive BT result. The diagnostic yield of GT is clearly lower when analyzed in children younger than 6 years of age or the total study population. That makes sense, because coincidence of the 2 tests is expected as children age and the LNP phenotype expresses itself (9). These findings agree with those described by Rasinpera et al in Finnish and African children. They found a 94% sensitivity, 81% specificity, 64% positive predictive value, and 97% negative predictive value in children 6 to 11 years old, and even a better accuracy in children older than 11 years of age, compared to lactase activity in intestinal biopsies (9). The slightly better yield of GT in the study performed by Rasinpera et al may be explained because they compare against lactase activity, which is the criterion standard for the diagnosis of hypolactasia and their patients (or participants) have a different genetic background.

In conclusion, genotyping for the diagnosis of lactose malabsorption proved to be a reliable method in Chilean children ages 6 years and older, when compared to BT. It is a simple and consistent diagnostic test and we recommend its use in this group.

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