Frequency of lactose malabsorption among healthy southern and northern Indian populations by genetic analysis and lactose hydrogen breath and tolerance tests[1–3]

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ABSTRACT

Background: Lactose malabsorption (LM), the inability to break down lactose into glucose and galactose, is due to a deficiency in the small intestinal lactase phlorizin hydrolase enzyme. Ethnic and geographic variations of LM are known.

Objective: The aim of this study was to compare the frequency of LM in healthy southern and northern Indian populations.

Design: A total of 153 healthy volunteers (76 from southern and 77 from northern India) were evaluated for LM by using a lactose tolerance test (LTT), a lactose hydrogen breath test (lactose HBT), and polymerase chain reaction–restriction fragment length polymorphism to identify the lactase gene *C/T*-13910 polymorphism (confirmed by sequencing).

Results: Volunteers from southern and northern India were comparable in age and sex. The LTT result was abnormal in 88.2% of southern Indians and in 66.2% of northern Indians ($P = 0.001$). The lactose HBT result was abnormal in 78.9% of southern Indians and in 57.1% of northern Indians ($P = 0.003$). The *CC* genotype was present in 86.8% and 67.5% ($P = 0.002$), the *CT* genotype was present in 13.2% and 26.0% ($P = 0.036$), and the *TT* genotype was present in 0% and 6.5% ($P = 0.03$) of southern and northern Indians, respectively. The frequency of symptoms after the lactose load (47.4% compared with 15.6%; $P < 0.001$) and peak concentrations of breath hydrogen (88.5 $\pm$ 71.9 compared with 55.4 $\pm$ 61.9 ppm; $P = 0.003$), both of which might indicate the degree of lactase deficiency, were higher in southern than in northern Indians.

Conclusion: The frequency and degree of LM is higher in southern than in northern Indian healthy populations because of genetic differences in these populations. *Am J Clin Nutr* 2010;91:140–6.

INTRODUCTION

Lactose malabsorption (LM), the inability to break down lactose because of reduced concentrations of an enzyme called lactase phlorizin hydrolase (lactase, or LPH) in the small intestine is fairly common in adults (1, 2). Normally, lactose is broken down in the small intestine by lactase to glucose and galactose (3); the lactase concentration is high in infants (2). The persistence of high lactase concentrations in adults is known as lactase persistence (LP) (4). In subjects with LM, undigested lactose gets fermented by colonic flora causing diarrhea, abdominal pain, and flatulence (symptoms of lactase nonpersistence; LNP) (5–7). The severity of symptoms depends on the degree of lactase deficiency (8), age (9, 10), ethnicity (11), digestion rate (12), and gastrointestinal transit time (8). Of these, ethnic and genetic variations are of major importance. LM is common in regions that traditionally consume low amounts of milk, such as Asia (13) and South America and Africa (14, 15). The LP trait is believed to have spread throughout the dairying communities of Europe because of its selective advantage (14, 16). Indo-Aryan migration brought the LP trait into northern India, which was spread by intermixing with the native population; the migrants also established the dairying culture. There was less intermixing of migrants with the southern Indian population. The difference between northern and southern Indian populations can be observed in traits such as height, skin color, diet, and language. Thus, it is of interest to study whether the distribution of the genetic marker responsible for the LP trait varies between northern and southern Indian populations.

Methods used to evaluate LM include the lactose hydrogen breath test (HBT) (17–20), the lactose tolerance test (LTT) (21, 22), estimation of lactase concentrations in intestinal biopsy samples, and genetic tests (23). Recent studies have shown that the single nucleotide polymorphism (SNP) at position 13910 nucleotide upstream to the gene coding for lactase on chromosome-2 (LCT locus) determines the LP/LNP status (23). This SNP has been used as the diagnostic tool to determine LM in our study (24). LTT and lactose HBT have been popular because of ease of performance and wide availability. In one study conducted in India using LTT alone, it was shown that the frequency of LM in southern India to be 66% and that in northern India to be 27.4% (25). In 2 other studies from northern India, frequency of LM has been shown to be 50% with the lactose HBT (26) and 77% with both the LTT and lactose HBT (21). All studies from India that estimated LM in southern and northern Indian populations have used either the LTT (25) or lactose HBT (26, 27) or

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both (21). In the present study, for the first time, we used lactose HBT, LTT, and genetic tests. The genetic test identifies lactase gene C/T-13910 polymorphism by using polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) and sequencing the relevant region (23, 24).

SUBJECTS AND METHODS

Subjects

Between January 2008 and June 2008, 153 volunteers [76 from southern India (Bangalore) and 77 from northern India (Lucknow)] were evaluated for LM by LTT and lactose HBT by using a 25-g lactose load; genotyping for *C/T* at 13910 nucleotide upstream of the LCT locus by PCR-RFLP and sequencing was carried out in all subjects. Volunteers were interviewed for any gastrointestinal symptoms; if present, Rome II criteria (28) were applied to diagnose irritable bowel syndrome (IBS). The Institutional Ethics Committee approved the study protocol, and consent was obtained from each subject.

Lactose HBT

Lactose HBT was performed by using a breath gas analyzer (Gastrolyzer Breath H_2 Monitor; Bedfont Scientific Ltd, Rochester, United Kingdom) (21). Basal breath specimens were obtained after an overnight fast; the subjects avoided eating slowly absorbed carbohydrates (bread, potato, and corn) and fiber the previous evening to avoid delayed exhalation of hydrogen in breath. Cigarette smoking and physical exercise were not permitted for 2 h before and during the test to prevent hyperventilation and consequent changes in breath hydrogen content. The subjects then brushed their teeth and rinsed their mouth with an antiseptic wash, followed by tap water, to eliminate an early hydrogen peak due to action of oral bacteria on lactose. An average of 4 values was taken as the basal breath hydrogen concentration. The test was repeated on the next day with proper preparation if basal breath hydrogen on first day was >20 ppm. The subjects ingested 25 g lactose dissolved in 100 mL water. Thereafter, breath hydrogen was estimated every 15 min for 3 h. An increase in exhaled hydrogen (ppm) after lactose administration was calculated by subtracting the fasting value from the highest value of hydrogen exhaled. A persistent rise in breath hydrogen by 20 ppm above the basal concentration (at least on 2 consecutive readings) after lactose administration was considered an abnormal lactose HBT result. A subjective increase or development of new symptoms such as diarrhea, bloating sensation, abdominal pain, or flatulence during the test was also noted.

LTT

Each subject also underwent an LTT; blood sugar was estimated with a glucometer (Optium Xceed; MediSense, Abbott Diabetes Care Inc, Alameda, CA), by using a compatible glucostix, in a fasting state and 30 min after ingestion of lactose. The failure of blood sugar to rise by >20 mg/dL from the fasting blood sugar concentration 30 min after lactose ingestion was considered an abnormal LTT result (21).

Genotyping

DNA was isolated from EDTA-blood by using a QiaAmp blood DNA Extraction kit (Qiagen, Hilden, Germany). In brief, 200 μL EDTA-blood was treated with protease for 15 min at 56° C followed by addition of AL (lysis) buffer and ethanol. The mixture was passed through a spin column and washed as per the manufacturer's instructions. DNA was eluted with 100 μL AE buffer and quantified on agarose gel by using lambda DNA as the standard.

The DNA fragment spanning C/T-13910 variants were amplified by using forward primer (5′-GGA TGC ACT GCT GTG ATG AG-3′) and reverse primer (5′-CCC ACT GAC CTA TCC TCG TG-3′) to also include positions −13915, −13907, and −14010 variants, which are believed to have an effect on LP/LNP status in parts of Africa and the Arabian peninsula (29). The amplification was done in a 20-μL reaction mixture containing ≈100 ng genomic DNA; 5 pmol each of forward and reverse primers; 200 μmol/L each of dATP, dCTP, and dGTP; 100 μmol dTTP/L; 1 mmol dUTP/L; 0.3 units of uracil DNA glycosylase (UDG) enzyme; and 0.5 units of Hotstart Taq DNA polymerase in a standard buffer (Bangalore Genei, Bangalore, India) containing 1.5 mmol $MgCl_2$/L and supplemented with an additional 1 mmol $MgCl_2$/L. A dUTP/UDG protocol was used in the PCR reaction mix to avoid carryover contamination (30). The cycle conditions were as follows: an initial dUTP/UDG treatment at 22°C for 10 min; an initial denaturation at 95°C for 10 min followed by 34 cycles at 94°C for 15 s, 58°C for 15 s, and 72°C for 40 s; and a final extension at 72°C for 2 min. A portion (3 μL) of the PCR product was run on agarose gel to confirm the integrity of the PCR product. Both sequencing and RFLP were carried out by using this PCR product, which was done without knowledge of the clinical data and the results of the LTT and lactose HBT.

Sequencing

The PCR product was sequenced by using an automated DNA Sequencer (ABI3100; Applied Biosystems, Ipswich, MA) with forward primer to read ≈400 base pairs (bps) in one direction. When necessary, the result was reconfirmed by sequencing the other strand with the reverse primer. All sequence data could be read with a high confidence level from −13830 to −14190 bps (ie, 361 bps spanning the C/T13910 upstream of the LCT locus). MCM6 reference gene sequence (GenBank reference sequence) was used because this SNP lies within the MCM6 locus, which is the neighboring gene upstream of the LCT locus.

Restriction fragment length polymorphism

The PCR product (≈300 ng) was digested with 1–2 units of BsmFI restriction enzyme (New England Biolabs, Foster City, CA) and 1× reaction buffer B in a 30-μl reaction volume. The reaction mixture was incubated at 65°C for 4 h and then electrophoresed on a 2.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light (302 nm). On the basis of the sequence information around the 13910 upstream position of the LCT locus, the expected band pattern was as follows: *C/C* variant: 342 and 106 bps (2 bands); *C/T* variant: 342, 224, 118, and 106 bps (seen as 3 bands on the gel because the 106 bps and 118 bps were usually not well resolved); and the

TABLE 1
Comparison of all symptoms (Rome II criteria is a group of symptoms) present in southern and northern Indian populations[1]

Symptoms	Southern India (n = 76)	Northern India (n = 77)
	n (%)	*n (%)*
Abdominal pain	7 (9.2)	5 (6.5)
Abdominal distension/bloating	15 (19.7)	13 (16.9)
Relief of pain with bowel movement	7 (9.2)	5 (6.5)
More frequent stools at onset of pain	5 (6.6)	5 (6.5)
Loose stools at onset of pain	5 (6.6)	3 (3.9)
Feeling of incomplete evacuation	21 (27.6)	17 (22.1)
IBS according to Rome II criteria	7 (9.2)	5 (6.5)

[1] IBS, irritable bowel syndrome. There were no significant differences between groups.

T/T variant: 224, 118, and 106 bps (seen as 2 bands, for the same reason); the size of the uncut PCR product was 448 bps (one band).

Statistical analysis

Data were analyzed by using SPSS (version 15.0; The Predictive Analytics Company, SPSS Inc, Chicago, IL) and Epi Info (version 6.0, a database and statistics program for public health professionals; Centers for Disease Control and Prevention, Atlanta, GA), and the continuous and categorical variables were compared by using an unpaired t test and chi-square test, respectively. The time-by-group interaction for the set of panel data was analyzed by using repeated-measures of analysis of variance. P values <0.05 were considered significant.

RESULTS

Subjects from southern and northern India were comparable in age (41.3 ± 15.0 compared with 37.8 ± 17.7 y; $P = 0.19$) and sex (male 37/76 compared with 48/77; $P > 0.05$), respectively. IBS was diagnosed by Rome II criteria (28) (**Table 1**) in 9.2% (7/76) of southern Indian and in 6.5% (5/77) of northern Indian populations (NS). The frequency of LM among IBS is given in **Table 2**.

LTT results

Southern and northern Indian populations had 88.2% (67/76) and 66.2% (51/77) LM by LTT ($P = 0.001$). In southern Indians, the difference in blood sugar [30 min after ingestion of lactose and fasting (mean of difference of blood sugar: 4.99 ± 13.8 mg/dL)] was lower than in northern Indians (mean of difference of blood sugar: 14.2 ± 18.9 mg/dL) ($P = 0.001$) (**Figure 1**, A and C).This might indicate lower absorption of lactose by southern Indians than by northern Indians (31).

Lactose HBT results

On the basis of the lactose HBT results, 78.9% (60/76) and 57.1% (44/77) of southern and northern Indian populations had LM, respectively ($P = 0.003$). The amount of basal breath hydrogen exhaled (5.9 ± 5.9 ppm compared with 6.1 ± 6.8 ppm; NS) was comparable between these 2 groups (Figure 1, B and D). Time at peak hydrogen >20 ppm above basal (67.9 ± 46.6 min compared with 43± 47.1 min; $P = 0.001$) was higher in southern than in northern Indians. After lactose ingestion, development of symptoms was more common among southern Indians (47.4%; 36/76) than among northern Indians (15.6%; 12/77) ($P < 0.001$). Southern Indians exhaled higher amounts of hydrogen in breath than did northern Indians (mean of peak hydrogen: 88.5 ± 71.9 ppm compared with 55.4 ± 61.9 ppm; $P = 0.003$) (Figure 1, B and D); both of which values might indicate some degree of lactase deficiency.

Sequencing and genotype results

Sequencing data for all 153 samples for 13830–14190 positions upstream of the LCT locus showed no polymorphism at positions −13915, −13907, and −14010. Furthermore, in 152 of 153 samples, other than the −13910 SNP, the sequence data conformed exactly to the reference sequence for this entire region; in the one nonconforming sample it was *G/A* in position −13879 as against *G* in the reference sequence. Thus, the sequencing data showed that only the *C/T* polymorphism at 13910 upstream of the LCT locus is the relevant polymorphism for LP/LNP status for the Indian population. Furthermore, the sequence information confirmed the RFLP data on *C/T* polymorphism in all the samples.

LNP existed for 86.8% (66/76) of the southern Indian population and for 67.5% (52/77) of the northern Indian population, based on *CC* genotype (at the −13910 position) ($P = 0.002$). LP based on the *CT* genotype was observed in 13.2% (10/76) and 26.0% (20/77) ($P = 0.036$) and LP based on the *TT* genotype was observed in 0% (0/76) and 6.5% (5/77) ($P = 0.03$) of the southern and northern Indian populations, respectively. *CC* is the

TABLE 2
Distribution of lactase nonpersistence (LNP) and lactase persistence (LP) among healthy volunteers who were positive or negative for irritable bowel syndrome (IBS)[1]

	LTT		Lactose HBT		Genotype	
Groups	LM	LP	LM	LP	LNP (*CC*)	LP (*CT*, *TT*)
	n (%)		*n (%)*		*n (%)*	
Subjects with IBS by Rome II criteria (n = 12)	8 (66.7)	4 (33.3)	7 (58.3)	5 (41.7)	7 (58.3)	5 (41.7)
Subjects without IBS (n = 141)	110 (78)	31 (22)	97 (68.8)	44 (31.2)	111 (78.7)	30 (21.3)

[1] LTT, lactose tolerance test; HBT, hydrogen breath test; LM, lactose malabsorption. There were no significant differences between LM and LP (by Fisher's exact test).

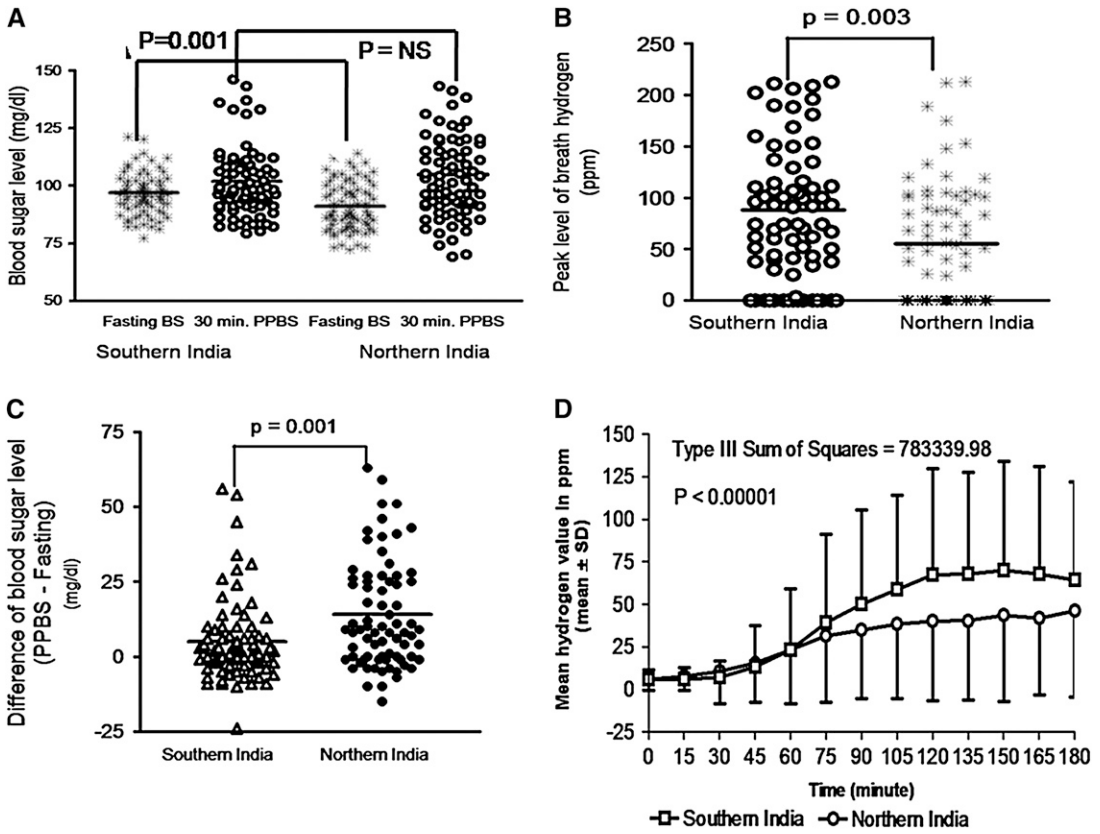

FIGURE 1. Comparison of blood sugar (BS) concentrations after fasting and 30 min after the ingestion of lactose (A), comparison of peak concentrations of breath hydrogen between southern and northern Indian populations (B), differences in BS concentrations after fasting and 30 min after ingestion of lactose (C), and mean (±SD) concentrations of breath hydrogen after oral administration of lactose in healthy southern and northern Indian populations (D). The time-by-group interaction was tested by using repeated-measures ANOVA ($P < 0.00001$), and the type 3 sum of squares was 783339.98. The fasting BS concentration was higher in the southern than in the northern Indian populations (A; $P = 0.001$), but there was no difference in 30-min postprandial BS (PPBS) concentrations (NS). The maximum concentration of hydrogen exhaled in breath was higher in the southern than in the northern Indian populations (B; $P = 0.003$). The differences in BS concentrations were lower in the southern than in the northern Indian populations (C; $P = 0.001$).

wild-type genotype, which corresponds to LNP, whereas the *CT* and *TT* genotypes correspond to LP (29, 32).

Correlation between genotype and LTT and lactose HBT observations

Of the 153 volunteers, 77% ($n = 118$) had LM based on the LTT; of these volunteers, 96.6% ($n = 114$), 3.4% ($n = 4$), and 0% ($n = 0$) had the *CC*, *CT*, and *TT* genotypes, respectively (**Table 3**). Of the 153 volunteers, 68% ($n = 104$) had LM based on the lactose HBT; of these volunteers, 99% ($n = 103$), 1% ($n = 1$), and 0% ($n = 0$) had the *CC*, *CT*, and *TT* genotypes, respectively (Table 3).

Of these 153 volunteers, 100 had LM based on both the LTT and lactose HBT; all 100 of these volunteers had the *CC* genotype (**Table 4**). Of the 153 volunteers, 31 had LP based on both the LTT and lactose HBT; of these 31 volunteers, 3.2% ($n = 1$), 80.7% ($n = 25$), and 16.1% ($n = 5$) had the *CC*, *CT*, and *TT* genotypes, respectively (Table 4). In other words, 96.8% (30/31) had LP based on the genetic criteria.

Sensitivity and specificity of the LTT and lactose HBT

The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for the LTT, considering the lactase gene C/T-13910 polymorphism as the gold standard, were 96.6%, 88.6%, 96.6%, 88.6%, and 94.8%, respectively. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for the lactose HBT, considering the *C/T* polymorphism as the gold standard, were 87.2%, 97.0%, 99.0%, 69.4%, and 89.5%, respectively.

DISCUSSION

The present study showed that the frequency of LM is higher among healthy populations from southern India than from northern India. The southern Indian population more often developed symptoms after lactose ingestion. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of the LTT and lactose HBT using a 25-g lactose load was high, considering the genotype as the gold standard.

LTT and lactose HBT are the tests currently used to detect LM in a pathophysiological manner at the level of the target organ (the intestine). However, the sensitivity and specificity of these tests, although good, are not 100%. Falsely abnormal LTT or lactose HBT results may result from secondary damage to the villi due to repeated gastrointestinal infection, infestation, and small intestinal bacterial overgrowth (33), which are common in tropical countries including India (27). Falsely normal lactose

TABLE 3
Correlation of lactose tolerance test (LTT) and lactose hydrogen breath test (HBT) results with genotype[1]

Test and genotype	Subjects
	n (%)
LTT result	
LM (*n* = 118)	
CC	114 (96.6)[2]
CT	4 (3.4)
TT	0 (0)
LP (*n* = 35)	
CC	4 (11.4)
CT	26 (74.3)
TT	5 (14.3)
Lactose HBT result	
LM (*n* = 104)	
CC	103 (99)[2]
CT	1 (1)
TT	0 (0)
LP (*n* = 49)	
CC	15 (30.6)
CT	29 (59.2)
TT	5 (10.2)

[1] LM, lactose malabsorption; LP, lactase persistence.
[2] Significantly different from *CC* genotype for LP, $P < 0.00001$ (chi-square test).

HBT results may result from the presence of predominantly methanogenic flora (found in ≈15% of the population) if methane is not measured in the breath (34). Another method used to assess LM is the estimation of lactase concentrations in intestinal biopsy samples. However, because the current study was conducted in a healthy population, an invasive test such as intestinal biopsy was neither ethical nor feasible. In the context of the above limitations of the available tests for identifying lactase deficiency, we considered the genetic test to be reasonably acceptable for assessing lactase deficiency. Discordant results between the genetic test, LTT, and lactose HBT in a small proportion of subjects might be related to the falsely abnormal or falsely normal results of the LTT and lactose HBT. Also, the expression of genotype at the target organ concentration may be confounded by gene-gene interaction, gene-environment interaction, and varying expressivity.

The glucometer is the standard and popular method used to estimate blood sugar in clinical practice; the values obtained by glucometer vary by only 10% from that obtained by estimation at the laboratory (35, 36). Because, we used the same glucometer to estimate blood glucose before and after the lactose load, even this minor variation was not expected to influence our results.

The finding of a higher frequency of LM among southern than among northern Indian populations agrees with previous observations (9, 25, 26). Furthermore, in the present study, we also aimed to evaluate whether the higher frequency of LM among the southern Indian population was related to genetic factors. We found, probably for the first time, that this was indeed the case. This finding was expected because a larger proportion of the northern than of the southern Indian population carries European traits, including the LP trait (25). Frequent gastrointestinal infections in developing countries, including India, can cause temporary LM. Furthermore, this may be compounded by the higher frequency of mild malabsorption syndrome, such as tropical sprue, in the southern than in the northern Indian population (37–39). Small intestinal bacterial overgrowth (SIBO) may be another factor contributing to LM, which complicates the situation (33). Because, symptoms of LM closely resemble those of SIBO, it remains to be studied in a larger sample size whether SIBO is more common in the southern Indian population, whether LM in SIBO is more common in southern India than in northern India, and whether eradication of SIBO would improve symptoms of LM.

The higher frequency of LM among the southern Indian population may have clinical significance (40, 41). Milk is the major source of calcium in the diet (42). Therefore, if milk is restricted in the diet because of LM, a deficiency of calcium and consequent osteoporosis may develop (42, 43). This would be further compounded by the fact that most of the southern Indians have a dark complexion, which may reduce the synthesis of vitamin D in the skin on exposure to sunlight (44). Symptoms of LM closely resemble those of IBS (45, 46). Hence, it remains to be studied in a larger sample size whether IBS is more common in southern India, whether LM in IBS is more common in southern India, and whether the restriction of milk would improve the symptoms of LM. The frequency of IBS in the Indian population varied from 4.2% to 7.5% (47, 48).

The dosage of choice has been 50 g lactose in most studies carried out to determine LM with either the LTT or lactose HBT or both; this was also true in Indian studies (9, 25–27). However, there have been studies in India (49) and elsewhere (46, 50–52) that have used lower lactose doses (20 or 25 g) and found that

TABLE 4
Frequency of genotypes among the subjects with normal or abnormal lactose tolerance test (LTT) and lactose hydrogen breath test (HBT) results[1]

Genotype	Subjects
	n (%)
Abnormal LTT and lactose HBT results for LM (*n* = 100)	
CC	100 (100)[2]
CT	0 (0)
TT	0 (0)
Normal LTT and lactose HBT results for LP (*n* = 31)	
CC	1 (3.2)
CT	25 (80.7)
TT	5 (16.1)
Abnormal LTT results for LM and normal lactose HBT results for LP (*n* = 18)	
CC	14 (77.8)[3]
CT	4 (22.2)
TT	0 (0)
Normal LTT results for LP and abnormal lactose HBT results for LM (*n* = 4)	
CC	3 (75)
CT	1 (25)
TT	0 (0)

[1] LM, lactose malabsorption; LP, lactase persistence.
[2] Significantly different from *CC* genotype for normal LTT and lactose HBT results for LP, $P < 0.00001$ (chi-square test).
[3] Significantly different from *CC* genotype for normal LTT results for LP and abnormal lactose HBT results for LM, $P < 0.00001$ (Fisher's exact test).

TABLE 5
Prevalence of lactose malabsorption (LM) in apparently healthy volunteers from different regions of India[1]

Region, city, and reference	Sample size	Reported frequency of LM	Test used to diagnose LM
	n	%	
Northern India			
Delhi (25)	124	27	LTT (50 g lactose)
Chandigarh (26)	150	50	Lactose HBT (50 g lactose)
Chandigarh (27)	40	42.5	Lactose HBT (50 g lactose)
Lucknow (21)	53	77	LTT and lactose HBT (50 g lactose)
Lucknow (present study)	77	73.7	LTT, lactose HBT (25 g lactose), and genetic test
Southern India			
Bangalore (present study)	76	89.5	LTT, lactose HBT (25 g lactose), and genetic test
Pondicherry (25)	30	67	LTT (50 g lactose)
Trivandrum (25)	30	67	LTT (50 g lactose)

[1] A person was considered to have LM if the lactose tolerance test (LTT) or the lactose hydrogen breath test (HBT) result was abnormal or if the genetic test indicated lactate nonpersistence.

these are acceptable doses for determining LM; all of these studies used lactose HBT. Because a 50-g dose is considered nonphysiologic, we used 25 g in the present study. This is the first study in which the LTT and lactose HBT were conducted with a 25 g dose of lactose and in which the results were validated with the genetic test. Previous studies on the prevalence of LM in apparently healthy volunteers from various cities in India, determined by LTT, lactose HBT, or both in comparison with the results of the present study are summarized in **Table 5**.

The LTT and lactose HBT are 2 tests used widely to determine LM. Both of these tests are time consuming because they involve dietary preparation and fasting overnight; the LTT takes >0.5 h and the lactose HBT takes 3 h of patient time. The genetic test does not require dietary preparation, a challenge with lactose, or fasting and does not induce LM symptoms, such as diarrhea and abdominal pain. Patient time is minimal with genetic testing because only a venous blood sample is needed for PCR-RFLP, sequencing, or automated methods for C/T-13910 genotyping by using real-time PCR, which are now available (53, 54). However, PCR-RFLP and real-time PCR may not be widely available in India, and the presence of a genetic marker may not always translate into physiologic effects on the target organ.

Evidence exists that the C/T-13910 variant has a functional role in the expression of the LPH gene (55–58). The mRNA content of the LPH in the intestinal mucosa is several times higher in individuals with the LP trait (*C/T* or *T/T*) than in individuals with the LNP trait (nonpersistent allele; *C/C*) (59).

We conclude that the frequency of LM is higher among healthy populations in southern India than in northern India. The southern Indian population more often developed symptoms after a lactose load. Considering genotype as the gold standard, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of the LTT and lactose HBT with a 25-g lactose load was found to be quite high.

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The authors' responsibilities were as follows—JB: conducted genetic tests and helped prepare the manuscript; SK: conducted the LTT and lactose HBT at both centers and participated in analyzing and writing the manuscript; PB: oversaw and guided the genetic test and edited the manuscript; JHP: conducted the genetic tests; and UCG: designed the study, supervised the LTT and lactose HBT and data analysis, and critically wrote and revised the manuscript. All authors contributed to the review of the manuscript. JB, PB, and JHP are employees of Biotools Technologies Pvt Ltd, which is a commercial organization providing facilities for genetic tests for lactose malabsorption. SK and UCG had no conflict of interest to disclose.

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