Comparison of a Real-Time Polymerase Chain Reaction Assay for Lactase Genetic Polymorphism With Standard Indirect Tests for Lactose Malabsorption

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Background & Aims: There is a discrepancy in outcome between the lactose tolerance and breath hydrogen tests for lactose malabsorption. The availability of a validated genetic test for lactase polymorphism allows a reevaluation of these tests. Methods: Thirty healthy adults participated in a 50-g lactose challenge test at a university clinic. Blood was drawn for genetic and timed blood glucose testing (2 hours), and breath hydrogen was measured (4.5 hours). Lactase genetic polymorphism was assessed by a real-time polymerase chain reaction assay. Participants completed a diet questionnaire, and symptoms were recorded during the lactose challenge. Sensitivity and specificity were calculated for each indirect test. The 2-way kappa coefficient between these tests was evaluated. Student t test and Wilcoxon rank sum test were used to compare variables. Results: The lactose tolerance test as a standard had an 87.5% sensitivity and 92.7% specificity for genetic status. Only a moderate agreement between lactose tolerance test and breath hydrogen test was observed (2-way kappa coefficient, .53; 95% confidence interval, .22–.83). When genetic status was used as standard, symptoms had a moderate sensitivity and specificity. Lactase tolerance test had very good sensitivity, and specificity. The breath test had excellent sensitivity and specificity. Conclusions: Both indirect tests independently have good to very good sensitivities and specificities for genetic lactase status. The noted disagreement likely reflects variables that affect the tests independently of intestinal lactase status. The value of these tests in the light of the availability of genetic testing is discussed.

The phenotypic dichotomy of lactose digestion and malabsorption is governed by the most common genetic trait worldwide. Whereas persistence of lactase phlorizin hydrolase (LP, lactate-persistent status) is considered to be a dominant genetic trait,1 its loss in adulthood is recessive,2 but the majority of the world’s population become lactase nonpersistence (LNP).3 The consequences of this dichotomy have been linked to multiple effects, some beneficial and some detrimental.4 However, the most obvious perceived effect of LNP status is the development of abdominal symptoms when lactose-containing products are consumed irregularly. The uniqueness of lactose-causing symptoms with dairy food intake is still somewhat unclear because apparent LP subjects can also experience symptoms on lactose challenge tests.5,6 During the last several decades a number of tests for lactose malabsorption were developed in an effort to identify individuals who might benefit from reduced lactose intake. A direct test such as the analysis of intestinal enzyme levels is accurate and to date is considered the gold standard for diagnosis, but it is not practical for large-scale screening. Therefore indirect tests have been developed. The 2 most common tests used today require measurements of blood glucose (lactose tolerance test [LTT]) or exhaled breath hydrogen (hydrogen breath test [BHT]) after a lactose challenge.7 The latter test has surpassed the LTT as a first-line clinical tool. There is some discrepancy between outcomes of the 2 tests when they are compared,7–9 and more recently there has been some questions as to whether the BHT reflects true LNP status.10

During the last several years a Finnish group has developed a genetic test that accurately identifies LP and LNP subjects.11,12 The gene for lactase phlorizin hydrolase (LCT) resides on chromosome 2(2q21),13 but its DNA sequence does not readily distinguish LP/LNP dichotomy.11,14 Instead, a cytosine to thymine mutation, approximately 14-kilobase pairs upstream from the LCT locus, associates completely with promotion of the LP/LNP status.12,15 This C/T-13910 locus polymorphism controls LCT transcription.14 The C/T-13910 mutation can be identified by real-time polymerase chain reaction (PCR) analysis.15,16 The C/C genotype is 100% associated with a reduction to <10 μg/g intestinal lactase. In a predominantly European population, the T/T genotype is also very close to 100% associated with ≥10 μg/g of intestinal lactase. The correlation of the C/T heterozygote to intestinal lactase is 97%.12 Therefore, the genetic test has been validated against intestinal lactase; however, it also does not predict symptoms. The availability of this new test allowed us to reevaluate the reliability of the 2 existing, most commonly used indirect tests for lactose malabsorption with the C/T polymorphism.

Methods and Participants

This study was approved by the Research and Ethics Committee of the Sir Mortimer B. Davis Jewish General Hospital. All participants signed written consent. Healthy, nonpregnant subjects without obvious functional bowel symptoms were

Abbreviations used in this paper: BHT, breath hydrogen test; LCT, lactase phlorizin hydrolase; LNP, lactase nonpersistence; LP, lactase persistence; LTT, lactose tolerance test; PCR, polymerase chain reaction; ppm, parts per million.
recruited on the basis of results of previous lactose BHTs. Other participants were also sought if their ethnic background suggested high or low likelihood of LP or LNP status. These likelihoods were based on previously published population data.\(^3\),\(^17\),\(^18\). For example, Asians or peri-Mediterranean peoples have a high likelihood of LNP status (>20%), whereas northern Europeans have a low likelihood of LNP status (≤20%) and a high likelihood of LP status (>80%). Subjects were also required to have been off any pro-kinetic or anti-kinetic drugs (eg, domperidone or narcotics) or antibiotics during the month preceding recruitment.

We included 30 participants. All were asked to consume a low carbohydrate supper the night before, although water was permitted. They were asked to refrain from smoking and to perform only quiet activities during testing. Each person was asked to fill out a previously validated questionnaire\(^19\) targeting 27 items of lactose-containing foods for the previous 3 days before the tests. The intake of mean daily lactose (g) was independently calculated from the results of other tests. Each person then underwent the 3 tests run simultaneously. Blood was drawn for genetic status and glucose. Breath hydrogen was measured. The latter 2 tests were continued after a 50-g lactose challenge dissolved in 200–250 mL of water (25%–20%).

**Lactase Genetic Test**

An indwelling cannula was inserted into the antecubital vein, and blood (20 mL) was drawn. DNA was prepared by using the DNA Isolation Kit from Gentra Systems (Minneapolis, MN). We used a real-time PCR assay based on fluorescence resonance energy transfer\(^15\),\(^16\) and the LightCycler DNA Master Hybridisation Probes kit (Roche Diagnostics, Mannheim, Germany) for analysis of the C/T genetic polymorphism. The forward primer GCTTGGTAAGCATTGTAGT and the reverse primer GTTGAGCGAAAGATGGG were used for PCR amplification. The sensor probe ATGTAGCCCCTGGCCT is labeled with fluorescein at the 3' end, and the anchor probe CCTCTGCGCTGGCAATACAGATAA is labeled with fluorescein at the 3' end. The mutation is detected by melting curve analysis.

**Lactose Tolerance Test**

Blood is drawn via the indwelling cannula, which is heparinized. After a baseline measurement, blood is drawn at 30, 60, and 120 minutes. Blood glucose is measured by a standard colorimetric method, which oxidizes glucose to glacial form (Roche Diagnostics, Indianapolis, IN). A flat 2-hour glucose curve signifies lactose malabsorption and is defined by a failure to rise above the baseline by more than 1.1 mmol/L.\(^20\)

**Breath Hydrogen Test**

Breath hydrogen in parts per million (ppm) is measured by using a validated hand-held H2 chemical sensor (EC60 gasotlyzer; Bedfont Scientific Ltd, Rochester, Kent, United Kingdom). This instrument has a range of 0–2000 ppm.\(^21\),\(^22\) At each time interval 3 exhaled breaths are measured, and the mean is used to define the interval. The baseline (all <20 ppm) is subtracted from values recorded at each subsequent 30-minute interval up to 4.5 hours. A definite positive value is defined as 20 ppm above baseline,\(^9\),\(^20\) and a probable positive is defined as 10 ppm above the baseline.\(^23\)

**Symptom Score**

Symptoms are also recorded at the same time intervals as breath hydrogen. Each symptom associated with sugar malabsorption—bloating, gas, and cramps—assigned a score of 0–3. In this scheme, 0 means no symptoms, 1 is mild, 2 is moderate, and 3 is severe. Diarrhea is scored as 0 (none) or 1 (present). The range was 0 to a maximum of 90 (9 + 3 + 3 + 9 = 1). However, no patient was expected to achieve such a score.

**Statistical Analysis**

Outcomes of tests were tabulated, and sensitivity and specificity were calculated against genetic status. Two-way kappa statistics were used to determine agreement between the 2 indirect tests. The kappa statistic measures the agreement beyond chance. Values of kappa between .4–.6 are considered to represent moderate agreement and values between 0.6–0.8 to be representative of substantial agreement. Student t test was used to compare total symptom scores between the LNP and LP groups. Wilcoxon rank sum test was used to compare daily lactose intake between symptomatic and asymptomatic subjects and among different ethnic groups. All tests were 2-tailed, and statistical significance was accepted at P < .05.

**Results**

The 18 women and 12 men in the group had a mean age of 26.7 ± 9.4 years (range, 19–61 years). None were of African origin. On the basis of the questionnaire there were 15 with a northwest European, 6 with Mediterranean, and 9 with Asian background. The genetic test suggested that 15 subjects were C/C (LNP), 12 subjects were C/T (LP), and 3 subjects were T/T (LP). The LTT showed that 14 of 15 genetically analyzed LNP subjects and 2 of 15 genetically analyzed LP subjects failed to increase blood glucose above 1.1 mmol/L. If we accept the LTT to define lactose digestion status, comparison with genetic analysis shows a sensitivity of 87.5% (14/16) and specificity of 92.7% (13/14). The 2-way kappa coefficient between LTT and BHT, however, was .53 (95% confidence interval, .22–.83), showing only a moderate level of agreement. Therefore we will consider the genetic test as the standard for further comparisons.

The results of BHT, symptom scores, and LTT are displayed in Table 1. In brief, there were no subjects with a BHT >20 ppm incorrectly classified as LP (Figure 1A), and only 1 subject with a BHT <10 ppm was incorrectly classified as being LNP. Subjects with BHT between 10–20 ppm appeared to be a mix of LNP and LP (Figure 1B). Symptoms were more severe and frequent in the LNP group than the LP group (P = .0003) but were less sensitive and specific for differentiating between LNP and LP on the basis of genetic analysis than either the BHT or LTT. Table 2 lists sensitivities and specificities for indirect tests and symptom scores. Overall, both BHT (when subjects with results of 10–19 ppm are included) and LTT have similar high sensitivity, but BHT has higher specificity. Although excluding subjects with criteria of 10–19 ppm reduces sensitivity, specificity of the BHT remains 100%.

In Table 3 the intake of daily lactose-containing foods is compared with genetic status, subjects with or without symptoms, or estimated ethnic distribution. A trend toward statistical significance of increased lactose intake in nonsymptomatic subjects is noted (P = .08).
Discussion

The results of our study describe the outcome of lactose maldigestion tests in a select multiethnic group. We used a newly described genetic test as a comparison with 2 existing indirect tests. With the LTT as a standard, there were very good sensitivity and specificity for genetically defined LNP status. However, there was only a moderate agreement between LTT and BHT. With the genetic test as standard, both LTT and BHT independently related well to LNP status. The LTT was somewhat more sensitive, and the BHT was more specific. The LNP group had significantly more symptoms than the LP group, but symptoms were only moderately sensitive and specific for genetic status. There is a suggestion that symptomatic subjects, especially Asians, consumed less daily lactose than asymptomatic ones.

The genetic test defines LNP/LP status by virtue of the level of intestinal lactase. The residue of enzyme allows small quantities of lactose to be digested. Indeed, a recent study in Japanese women estimated that a single dose ingestion of less than 10 g of lactose will not induce diarrhea.24 However, larger quantities will not be split into glucose and galactose and will reach the lower intestine. Here bacteria (mainly Clostridium and Bacteroides species in LNP subjects who consume dairy foods intermittently) metabolize it into short-chain fatty acids, carbon dioxide, methane, and hydrogen.27–29 These divergent fates of lactose in LNP subjects allow the indirect tests of maldigestion. In the first instance, administering larger doses of lactose overwhelms residual lactase and leads to intestinal hurry, which further reduces lactose intestinal contact time. As such, there is a failure to increase blood glucose significantly above the baseline. In the second instance, hydrogen that diffuses across the intestinal and ultimately the pulmonary membranes can be measured in the exhaled breath. In addition to qualitatively distinguishing LNP/LP status, the indirect tests have been used to quantify digested (as above24) or maldigested lactose.30 In the case of the former outcome, insulin response has also been reported.24 The BHT for lactose maldigestion was popularized by Metz et al8 and has been compared with other indirect tests, including the LTT in an older publication.7 Newcomer et al7 used quantified intestinal lactase to sucrase ratio as a gold standard

Table 1. Outcomes of BHT, Symptom Score, and LTT Tabulated as Absolute Numbers

<table>
<thead>
<tr>
<th></th>
<th>LNP</th>
<th>LP</th>
</tr>
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<tbody>
<tr>
<td>BHT ≥20 ppm</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>10–19 ppm</td>
<td>2</td>
<td>3a</td>
</tr>
<tr>
<td>0–9 ppm</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11b</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>LTT ≤1.1 mmol/L</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>&gt;1.1 mmol/L</td>
<td>1</td>
<td>13</td>
</tr>
</tbody>
</table>

NOTE. Results of all 30 participants are shown. Lactose digestion phenotype is defined by genotype (C/C = LNP, C/T and T/T = LP).

aFirst time interval when test became positive was under 3 hr for CC subjects but well over 3 hr for T/T subjects. The 1 C/T subject met criteria for positivity for ≥10 ppm within 3 hr and reached ≥20 ppm on a single peak beyond 3 hr.

bTotal symptom score was 12.9 ± 12.3 (mean ± standard deviation) in the LNP group vs 1.5 ± 3.5 in the LP group (P = .0003).

Figure 1. (A) Result of a composite standard breath hydrogen curve (ppm) plotted against minutes is shown for 12 subjects with genotype C/C (homozygous mutants, LNP). All subjects met criteria for levels ≥20 ppm. (B) Composite results of 5 subjects (2 C/C, 2 T/T, and 1 C/T) who met criteria of positivity at ≥10 ppm are shown. The 1 C/T subject also achieved criteria at ≥20 ppm late into the test. Results of the 2 subjects with genotype T/T (phenotype LP) represent false-positive outcomes, whereas the subject with genotype C/T was probably also falsely elevated.

Table 2. Sensitivities and Specificities for Indirect Tests and Symptom Scores

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHT (≥20 ppm vs all)</td>
<td>12/15 (80)</td>
<td>15/15 (100)</td>
</tr>
<tr>
<td>BHT (≥20 ppm vs &lt;10 ppm)a</td>
<td>12/13 (92.3)</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>Symptoms (yes/no)</td>
<td>11/15 (73.3)</td>
<td>12/15 (80)</td>
</tr>
<tr>
<td>LTT (&lt;1.1 mmol/L)</td>
<td>14/15 (93.3)</td>
<td>13/15 (86.7)</td>
</tr>
</tbody>
</table>

NOTE. For each test, sensitivity and specificity for lactose maldigestion assessed by genetic analysis are presented as absolute counts (%) of subjects because of small sample size.

aThese figures recognize that individuals with BHT between 10–19 ppm have an equivocal test. This represented only 5/30 subjects in our sample.
to classify LNP and LP subjects. They found that 76% of LNP participants had the expected flat glucose curve in response to lactose challenge (24% false elevation), whereas 4% of LP subjects had a false flat curve (96% appropriately elevated). The BHT provided complete separation between LP and LNP status by 2 hours. This study established the BHT as the most convenient noninvasive test for lactose malabsorption.

Normal cutoff values for breath hydrogen and the time of appearance have been debated. The standard positive test (hence LNP status) is an increase of 20 ppm above the baseline.

A result of 10–19 ppm above baseline is considered more sensitive by some, but others believe that it is likely not related to lactose malabsorption. In the current report we used both definitions for qualitative purposes. However, use of the ≥10-ppm definition led to misclassification of 3 subjects as LNP. The 10-ppm definition led to misclassification of 3 subjects as LNP. The other subjects failed to reach the ≥20-ppm cutoff value but achieved ≥10 ppm within 3 hours. Therefore a cutoff value of 10 ppm is more sensitive and should be interpreted as suspicious for LNP status. Its accuracy might be enhanced by knowledge of ethnic background. However, for diagnosis, retaining the ≥20-ppm criteria restricted to 2–3 hours remains the most specific test.

Quantitative intestinal enzyme measurement has been the primary comparator for genetic and indirect tests. Whereas genetic analysis is unlikely to vary, both the LTT and BHT can be affected by factors unrelated to lactase enzyme levels. In the case of glucose measurement, gastric emptying, intestinal transit time, and metabolism of glucose (eg, diabetes) can alter results. In the case of breath hydrogen, use of antibiotics, intestinal motility, drugs, or physical effects can alter measurements. Colonic adaptation defined as the observation of improved symptoms of lactose malabsorption and decreased BHT results, as a result of expanded colonic bacterial populations (most likely of the lactic acid–producing variety) with increased metabolic capacity for lactose, might lead to false-negative interpretations. Alternatively, postulated bacterial overgrowth in patients with irritable bowel syndrome might result in false-positive results. These and other different conditions likely explain the discrepancy observed between the 2 indirect tests noted here and reported in the literature.

Nevertheless, independently both tests compare favorably to genetic status. The clinical consequence of these findings is the confirmation of the appropriate current use of these indirect tests. Historical symptoms of lactose intolerance retain a moderate sensitivity and specificity for LNP/LP status. The LTT increases these. However, the sensitivity of the BHT can be increased by choosing the lower cutoff value of 0 ppm, as predicted by Stroechi et al. Furthermore, the BHT here has excellent specificity. In addition, the physiologic variables affecting these indirect tests can be exploited, when genetic status is known. For example, glucose and insulin response to lactose in either LNP or LP subjects might be evaluated by the LTT. Similarly, BHT might be used to assess function and change in lower intestinal microflora in response to dairy food consumption in either of the 2 cases.

Limitations of this study should be noted. First, it is a small study, and the validity and applicability of its outcome will have to be further evaluated in larger groups. Second, we enrolled a select group, and as such, the results might not apply to unselected patients.

In conclusion, both the LTT and BHT independently closely reflect LNP/LP status in a select group of subjects without known functional gastrointestinal disorders. All 3 tests might be useful under a variety of clinical or research situations. Further work is required to place in proper perspective the biologic role of the most common known human genetic trait, lactase deficiency.

### References

9. Pimentel M, Kong Y, Park S. Testing to evaluate irritable bowel

### Table 3. Estimated Average Daily Lactose Intake (g/d)

<table>
<thead>
<tr>
<th>Genetic status</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Median (25th, 75th Quartiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>15</td>
<td>21.8 ± 34.1</td>
<td>12.4 (5.5, 20.0)</td>
</tr>
<tr>
<td>C/T</td>
<td>12</td>
<td>25.7 ± 18.2</td>
<td>19.6 (13.5, 31.3)</td>
</tr>
<tr>
<td>T/T</td>
<td>3</td>
<td>15.6 ± 11.2</td>
<td>11.2 (4.5, 31.2)</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>14.1 ± 12.6</td>
<td>12.6 (6.0, 17.8)</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>27.3 ± 32.4</td>
<td>32.4 (11.9, 28.4)</td>
</tr>
<tr>
<td>Ethnic group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NWE</td>
<td>15</td>
<td>22.8 ± 17.5</td>
<td>21.0 (12.5, 31.2)</td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>33.4 ± 52.8</td>
<td>14.7 (5.5, 20.0)</td>
</tr>
<tr>
<td>Asian</td>
<td>9</td>
<td>14.1 ± 16.4</td>
<td>8.5 (6.0, 13.8)</td>
</tr>
</tbody>
</table>

SD, standard deviation.

*One CC subject consumed 140.2 g lactose/d; if excluded, the mean intake is 12.92 ± 22.94.
*No vs Yes, P = .08 (trend only).
*NWE ancestry from northern and western Europe; M ancestry from Mediterranean region (eg, Italy, Greece).
syndrome correlates with lactulose testing and may not reflect true lactose malabsorption. Am J Gastroenterol 2003;98:2700–2704.


