Lactase persistence/non-persistence variants, C/T\textsubscript{13910} and G/A\textsubscript{22018}, as a diagnostic tool for lactose intolerance in IBS patients

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**Abstract**

**Background:** Irritable bowel syndrome (IBS) is a symptom-based disorder characterized by abdominal pain related to altered bowel habit. We evaluated the predictive power of 2 genetic markers of hypolactasia, C/T\textsubscript{13910} and G/A\textsubscript{22018}, in IBS patients with and without lactose intolerance in order to gain insight into the role of lactose intolerance in IBS.

**Methods:** Seventy five patients (59F/16M, mean age: 49.6± 14.2 years) with an IBS diagnosis based on Rome II criteria and 272 healthy individuals, where 74 (58F/16M, 54.1± 10.9 years) were matched-controls, were evaluated. IBS and healthy individuals were genotyped for the C/T\textsubscript{13910} and G/A\textsubscript{22018} polymorphisms nearby the lactase-phlorizin hydrolase gene. Hydrogen breath test (HBT) with gas chromatography was performed in IBS patients to assess for lactose intolerance.

**Results:** Of the 75 IBS patients, 28 (37%) were defined as lactose intolerants. The grade/severity of symptoms after an oral lactose load were positively correlated to the expiratory H2 excretion ($P<0.001$). Alleles and genotypes frequencies from C/T\textsubscript{13910} and G/A\textsubscript{22018} were not significantly different between IBS patients and control individuals ($P>0.05$;NS). Presence of the C and G allele were positively associated with a higher expiratory hydrogen excretion and more intense gastrointestinal symptoms ($P<0.001$). Considering these polymorphisms as a diagnostic test for lactose intolerance in IBS patients, presence of the CC and GG genotypes were estimated to have, a sensitivity of 100% and 96%, respectively; and a specificity of 83% and 79%, positive predictive value of 100% and 97%, and negative predictive value of 100% and 97%.

**Conclusions:** In IBS patients, genotyping of C/T\textsubscript{13910} and G/A\textsubscript{22018} polymorphisms predicts gastrointestinal symptoms after lactose ingestion and are a diagnostic tool for lactose intolerance.

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**Keywords:** Irritable bowel syndrome; Lactose intolerance; Polymorphism

1. Introduction

Irritable bowel syndrome affects 5% to 20% of the Western population [1], accounting for a high health of medical care seeking and economic burden [2,3]. Adult-type hypolactasia is also a very common condition, autosomal inherited, characterized by lactase deficiency [4]. As lactose remains undigested in intestinal lumen, it increases fluid secretion in small intestine and gas production (e.g., carbon dioxide, hydrogen, methane) by bacterial fermentation in the colon. Consequently, lactose malabsorption could lead to gastrointestinal symptoms that mimic IBS, which include diarrhea, colicky, bloating and flatulence, a disorder defined as lactose intolerance [5].

The role of hypolactasia and lactose intolerance in IBS has been extensively discussed [6–16]. One major implication is that within a group of IBS patients a subgroup of lactose intolerants would be candidate for specific management with a lactose restriction diet. However, studies testing such approach have shown conflicting results [7,8,12,13], in part, due to methodological differences and limitations regarding the characterization of these conditions [17].

Recently, Enattah et al. described 2 DNA variants, C/T\textsubscript{13910} and G/A\textsubscript{22018}, located upstream from the gene encoding the Lactase enzyme, linked to lactose intolerance and IBS.
lactase-phlorizin hydrolase (LPH), that are genetic markers for lactase deficiency [18]. To our knowledge, the correlation between IBS, genetically-determined hypolactasia and functionally-expressed lactose intolerance has never been evaluated. The aim of this study was to verify the predictive power of C/T_13910 and G/A_22018 genetic variations in IBS patients and correlate these genetic findings with the lactose intolerance phenotype.

2. Methods

2.1. Population and study design

Seventy-five patients (59F/16M, mean age: 49.6±14.2 years) with the diagnosis of Irritable Bowel Syndrome (IBS) established on the basis of Rome II criteria and after the exclusion of other gastrointestinal disorders were enrolled in this study. This population was composed of 3 different ethnic groups, based on morphological criteria: 72% white, 22.7% mulatto and 5.3% black. One of the studied patients had a prior history of small bowel resection. No studied patient had a prior diagnosis of inflammatory bowel disease. All these patients were referred for hydrogen breath test to evaluate lactose intolerance. Also, peripheral blood samples were collected to perform DNA genotyping for the C/T_13910 and G/A_22018 variants. There were no problems in the DNA extraction phase and all selected individuals were genotyped.

In order to compare the allele and genotype frequencies of our IBS population we also genotyped 272 healthy individuals (154F/118M, mean age: 40.0±10.9 years, 84% white, 14.1% mulatto and 1.9% black) for the C/T_13910 and G/A_22018 polymorphisms. From this group, 74 individuals (58F/16M, mean age: 54.1±10.9 years, 81.1% white, 16.2% mulatto and 2.7% black), entitled by independent technicians. Also, the PCR products for these variants from each group were randomly assigned by computer to be matched to the IBS group for gender, age and ethnicity. The study was approved by the Scientific/Ethics Committee from the University of São Paulo School of Medicine and informed consent was obtained from all patients and control individuals studied.

2.2. Hydrogen breath test (HBT)

To perform HBT, patients should not have recently taken antibiotics/probiotics and should avoid gas-producing foods the day before the test. More specifically, patient orientations included: 1) no prior antibiotic 30 days prior to the test; 2) on the day before the test an anti-fermentative diet was prescribed and 3) no food consumption was allowed 12 h prior to the test (only water consumption); 4) on the day of the test no smoking was allowed and no physical activity was allowed.

To test for differences in various characteristics, ANOVA and χ² test were used, respectively, for continuous and categorical variables. Allele and genotype frequencies among study participants and Hardy-Weinberg equilibrium (HWE) for the distribution of genotypes were calculated with the Chi-square test using the Statistical Package StatView for Windows ver.5.0 (SAS Institute Inc., SAS.

![Fig. 1. Gastrointestinal symptoms and hydrogen breath test (HBT) in IBS patients (N=75).](image)

Fig. 1. Gastrointestinal symptoms and hydrogen breath test (HBT) in IBS patients (N=75). Results are expressed as the percentage (%) of lactose absorbers (expiratory H2 concentration >20 ppm above basal level) and lactose malabsorbers (expiratory H2 concentration ≥20 ppm above basal level) according to the degree of gastrointestinal symptoms (no, mild, moderate or severe) after lactose ingestion. Gastrointestinal symptoms were more prevalent and intense in lactose malabsorbers than absorbers (χ² test; p<0.001).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>IBS</th>
<th>Controls</th>
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<tbody>
<tr>
<td>C/T_13910</td>
<td>102</td>
<td>102</td>
<td>71.8</td>
<td>70.8*</td>
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<tr>
<td>G/A_22018</td>
<td>40</td>
<td>42</td>
<td>28.2</td>
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<table>
<thead>
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<th>Genotype</th>
<th>Controls</th>
<th>IBS</th>
<th>Controls</th>
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<tbody>
<tr>
<td>CC</td>
<td>37</td>
<td>34</td>
<td>52.1</td>
<td>47.2*</td>
</tr>
<tr>
<td>CT</td>
<td>28</td>
<td>34</td>
<td>39.4</td>
<td>47.2*</td>
</tr>
<tr>
<td>TT</td>
<td>6</td>
<td>4</td>
<td>8.5</td>
<td>6*</td>
</tr>
</tbody>
</table>

Chi-square test was performed comparing frequency: IBS vs. control. *p value >0.05 (NS = not significant).

After an overnight fast, end-alveolar air samples were collected before and 60, 90, 120, 150 and 180 min after an oral load of 25 g lactose in 250 ml water. Expired H2 concentration was immediately measured, in parts per million (ppm), using gaseous chromatography (model CM2, Quinton Instrument Company, Milwaukee, WI). Expiratory H2 excretion during the test was quantified as the area under the curve (AUC) and expressed in ppm x min. Lactose malabsorption (LM) was defined as an increase in H2 concentration of at least 20 ppm above basal level and lactose intolerance (LI) if symptoms (e.g., flatulence, meteorism, diarrhea, distension, abdominal pain) occurred during the test. At the end of HBT, the overall gastrointestinal symptoms were recorded and graded as mild, moderate or intense.

2.3. Genotype determination for C/T_13910 and G/A_22018

From each individual enrolled in this study, 5-ml venous blood samples were drawn into tubes containing EDTA. Genomic DNA was extracted from peripheral blood leukocytes using a standard salting-out procedure. C/T_13910 and G/A_22018 variants were analyzed through polymerase chain reaction (PCR) amplification followed by restriction enzyme digestion assays. Primers used to amplify the fragments encompassing these DNA variants were: sense 5′-ccc tgt taaggactcctaagtgca-3′ and antisense 5′-cttagtaggtgtggatgagggaa-3′ for the _13910 locus and sense 5′-aac ccc ggg ggc gct gga gga gaa-3′ and antisense 5′-ccc acc tea gec tct tga gtgtg-3′ for the _22018 locus. Digestion products were visualized by electrophoresis on a 3% agarose gel stained with ethidium bromide and the information stored in digital form. All genotyping determinations were conducted blinded regarding group (cases and controls) and lactose intolerant status. Quality control for these assays was assessed by randomly selecting representative samples to be regenotyped by independent technicians. Also, the PCR products for these variants from each sample were purified and bi-directional direct sequenced using fluorescence-based dye-exoxy-terminator cycle sequenced (Big Dye, Applied Bioystem) and analyzed in an automated DNA sequencer (ABI 377, Perkin-Elmer).

2.4. Statistical analysis

To test for differences in various characteristics, ANOVA and χ² test were used, respectively, for continuous and categorical variables. Allele and genotype frequencies among study participants and Hardy-Weinberg equilibrium (HWE) for the distribution of genotypes were calculated with the Chi-square test using the Statistical Package StatView for Windows ver.5.0 (SAS Institute Inc., SAS.

Table 1

| Allele and genotype distribution of C/T_13910 and G/A_22018 polymorphisms in IBS patients (n=75) and controls (n=74) |
|---|---|---|---|---|
| Number | Controls | IBS | Controls | IBS |
| C/T_13910 | | | | |
| 13910C | 102 | 102 | 71.8 | 70.8* |
| 13910T | 40 | 42 | 28.2 | 29.2* |
| Genotype | | | | |
| CC | 37 | 34 | 52.1 | 47.2* |
| CT | 28 | 34 | 39.4 | 47.2* |
| TT | 6 | 4 | 8.5 | 6* |

Chi-square test was performed comparing frequency: IBS vs. control. *p value >0.05 (NS = not significant).

After an overnight fast, end-alveolar air samples were collected before and 60, 90, 120, 150 and 180 min after an oral load of 25 g lactose in 250 ml water. Expired H2 concentration was immediately measured, in parts per million (ppm), using gaseous chromatography (model CM2, Quinton Instrument Company, Milwaukee, WI). Expiratory H2 excretion during the test was quantified as the area under the curve (AUC) and expressed in ppm x min. Lactose malabsorption (LM) was defined as an increase in H2 concentration of at least 20 ppm above basal level and lactose intolerance (LI) if symptoms (e.g., flatulence, meteorism, diarrhea, distension, abdominal pain) occurred during the test. At the end of HBT, the overall gastrointestinal symptoms were recorded and graded as mild, moderate or intense.

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Campus Drive, Cary, NC). Sensitivity, specificity, positive and negative predictive values for the CT_13910 and G/A_22018 polymorphisms, assuming a genetic recessive mode of action (CC vs. CT+TT and GG vs. GA+AA) in the population with IBS, were calculated considering lactose hydrogen breath test (HBT) as the gold-standard. A \( P < 0.05 \) was considered significant.

3. Results

3.1. Evaluation of lactose intolerance by hydrogen breath testing in IBS patients

Of the 75 IBS patients, 31 (41%) had lactose malabsorption from which 28 (90%) had gastrointestinal symptoms and, therefore, were defined as lactose intolerants. A negative test was found in 44 (59%) patients and, compared to positive HBT, only 8 (18%) complained of gastrointestinal symptoms (\( P < 0.001 \); Fig. 1). A positive correlation was found between the grade/severity of symptoms and the expiratory H2 excretion during the test (AUC) (\( P < 0.001 \)). As expected, the AUC in positive HBT was significantly higher than in negative HBT (\( P < 0.001 \)). No correlation was found regarding age, gender and ethnic distribution compared to HBT result (data not shown).

3.2. C/T_13910 and G/A_22018 in IBS and healthy controls

Allele and genotype frequencies from cases and control populations are shown in Table 1. Both C/T_13910 and G/A_22018 were in accordance with the Hardy-Weinberg Equilibrium. Frequencies of alleles and genotypes were not significantly different between IBS patients and control individuals.

3.3. Phenotype lactose intolerance and genotype C/T_13910 and G/A_22018 in IBS

In IBS, the results of hydrogen breath test to evaluate lactose malabsorption were compared to the variations in C/T_13910 and G/A_22018 (Fig. 2). Presence of the C allele, from C/T_13910, or the G allele, from G/A_22018, were positively associated with a higher expiratory hydrogen excretion during HBT and the presence of more intense gastrointestinal symptoms (\( P < 0.001 \)). The C/C_13910 was detectable in 100% (26/26) and GG_22018 in 96% (27/28) of lactose intolerant patients (Table 2). Considering the C/T_13910 polymorphism as a diagnostic test for lactose intolerance in these patients, presence of the CC genotype is estimated to have a sensitivity of 100%, specificity of 83%, positive predictive value of 76%, and negative predictive value of 97%.

4. Discussion

The identification of specific polymorphisms as genetic markers linked to IBS is a potentially valuable tool in its diagnosis and therapeutic management. In 2002, Ennattah e al. described 2 DNA variants associated with hypolactasia [18].

![Image](image_url)

Fig. 2. Lactase persistence/non-persistence variants and hydrogen breath test (HBT) A) Expiratory hydrogen excretion during HBT, i.e., AUC was significantly higher in alleles C and G, respectively from C/T_13910 and G/A_22018 variants (\( p < 0.001 \)); B) Expiratory H2 excretion at different time intervals after lactose ingestion in genotypes CC, CT and TT from C/T_13910 variant. Note that H2 excretion in ppm in CC genotype, at 60, 90, 120, 150 and 180 min, was clearly higher than in CT and TT genotypes.

### Table 2

<table>
<thead>
<tr>
<th>Lactose Intolerants</th>
<th>Lactose Tolerants</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N ) (%)</td>
<td>( N ) (%)</td>
<td>( N ) (%)</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>---------</td>
</tr>
<tr>
<td>C/T_13910</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>26 (100.00)</td>
<td>34 (47.22)</td>
</tr>
<tr>
<td>CT</td>
<td>0 (0.00)</td>
<td>34 (47.22)</td>
</tr>
<tr>
<td>TT</td>
<td>0 (0.00)</td>
<td>4 (5.56)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (100.00)</td>
<td>46 (100.00)</td>
</tr>
<tr>
<td>G/A_22018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>27 (96.43)</td>
<td>37 (49.33)</td>
</tr>
<tr>
<td>GA</td>
<td>1 (3.57)</td>
<td>32 (42.67)</td>
</tr>
<tr>
<td>AA</td>
<td>6 (12.77)</td>
<td>6 (8.00)</td>
</tr>
<tr>
<td>Total</td>
<td>28 (100.00)</td>
<td>75 (100.00)</td>
</tr>
</tbody>
</table>

Absolute numbers (percentages) are expressed.
which can lead to lactose intolerance and, consequently, to clinical symptoms that resembles IBS. In this study we evaluate the presence of these mutations associated to hypolactasia in IBS patients with and without lactose intolerance (LI).

It is important to highlight that, in our group of IBS patients, the percentage of LM (41%) was high, considering smaller rates shown in other studies (6–24%) [7–10,12,16]. Possible explanations for this discrepancy could be due to patient ethnic heterogeneities and/or differences in the methodologies applied to define LM [17]. In the literature, the prevalence of LM in the general population is up to 15% among Northern Europeans and 80% to 100% in Africans and Orientals [19]. In this regard, the studies that evaluated LM in IBS were mostly done in Northern European countries.

In Brazil, although there is no precise data, Seva-Pereira et al. evaluated 80 healthy subjects of 3 different ethnic groups and reported a prevalence of LM that ranges from 45% to 100% [20]. We investigated LM in 75 IBS Brazilian patients, where 72% were white, 22.7% mulatto and 5.3% black. Interestingly was that, confirming our previous observation [21], no correlation was found regarding ethnicity (white vs non-white) and the hydrogen breath test result, i.e., the presence or not of lactose malabsorption. Previously, Rasinpera et al. demonstrated a considerable difference in the prevalence of LM when they analyzed 2 distinct white populations from Finland, the Finnish (14.7% of LM) and the one classified as other whites (75% of LM), that were subjects mostly originated from the Mediterranean area [22]. This finding pointed out important issues that should be taken into account when examining LM in ethnically diverse populations. It is well-known that Brazil is a multi-racial country with an admixed population and, this fact, might explain the similar prevalence of LM between white and non-white (mulatto and black) patients.

Another relevant point that should be considered in this work is that we used hydrogen breath test (HBT) to evaluate LM and lactose intolerance (LI). This test is considered less invasive when compared to the biochemical assay of lactase/sucrase ratio from small-intestinal biopsy specimens; moreover, HBT is accurate and the test of choice to evaluate LI in clinical grounds [23]. However, Pimentel et al. [24] and Nucera et al. [25] suggested that positive HBT reflects bacterial overgrowth and not LM, in IBS patients. It is worth to emphasize that the analysis of lactose absorption/tolerance through HBT depends upon several factors, such as the amount of lactose ingested, gastrointestinal transit time, intestinal lactase enzyme content, intestinal microflora, the response of the large intestine to an osmotic load and the sensitivity of the intestine to gas production and excretion [26,27]. In our study, the results of HBT clearly demonstrated a significant increased in hydrogen excretion in a subset of IBS patients, defined as lactose malabsorbers. Likewise, most of IBS lactose malabsorbers had symptoms during the test, i.e., they were lactose intolerants, and confirming previous report [28], there was a correlation between the amount of hydrogen excretion and the severity of gastrointestinal symptoms. These data highlight 2 important issues: first, fermentation process secondary to lactose ingestion affected a considerable percentage of IBS patients and this may reflects hypolactasia and/or bacterial overgrowth; second, the increased hydrogen excretion and worsen of severity of gastrointestinal symptoms may be explained by 2 distinct pathophysiological mechanisms that lead to IBS: abnormal gas handling [29,30] and visceral hypersensitivity [31]. In this regard, we can hypothesize that, in IBS patients, hypolactasia leads to excessive gas production, accumulation of gas in specific areas of the intestine and this process, in turn, could be responsible for gastrointestinal symptoms (e.g., abdominal pain, discomfort) due to the visceral hypersensitivity. Although we have not specifically tested for bacterial overgrowth in our studied population, we observed very few tested individuals with baseline hydrogen values above 10 ppm (an indirect marker of bacterial overgrowth). In this scenario it is unlikely that bacterial overgrowth can bias the observed results in our population.

Recently, 3 studies compared clinical tests of lactose tolerance with the C/T_13910 DNA variant associated with adult primary hypolactasia [32–34]. These studies were mostly done in European descendents patients with suspicion or diagnosis of lactose malabsorption. Bodlaj et al. [32] retrospectively investigating 54 Caucasian patients with positive HBT found that the CC genotype, associated with low intestinal lactase levels, was present in only 50%. Otherwise, Ridefelt et al. [33] and Högenauer et al. [34] found the association of lactose malabsorption with the CC genotype in >90% of patients. In the present study we have also found in IBS patients an extremely high association between both C/T_13910 and G/A_22018 DNA variants and the HBT results. To our knowledge, this is the first report of the association of these functional variants and the lactose malabsorption phenotype in IBS patients.

The decision to investigate this association in the IBS population was based on 2 aspects. First, IBS diagnosis was done according to Rome II criteria and, when necessary, subsidiary exams were performed to exclude other gastrointestinal disorders, which imply a null or extremely low probability to have a case of secondary hypolactasia in this population. In this sense, this explains in part the reason for the observed high sensibility and specificity of DNA testing for lactose malabsorption. Second, and more important, the role of lactose intolerance in IBS is still controversial. Therefore, in order to gain insight in this issue, we investigated in IBS patients the 2 variants highly associated with hypolactasia, described by Enattah et al. Interestingly, C and G alleles, respectively from the C/T_13910 and G/A_22018 polymorphisms, demonstrated a strong correlation with higher expiratory hydrogen excretion and the presence of more intense gastrointestinal symptoms after an oral load of lactose.

In conclusion, we extended the previous observation of the high sensitivity and specificity of DNA testing for lactose intolerance in a population with IBS. Moreover, we demonstrated that 2 specific alleles have functional relevance in the context of exacerbating IBS symptoms. In view of these data, genotyping of these markers should be considered in patients with IBS. This use may lead to more specific and cost-effective management options for this particular subgroup of patients identified through DNA testing.
Acknowledgments

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References


