ORIGINAL PAPERS

Concordance of Genetic and Breath Tests for Lactose Intolerance in a Tertiary Referral Centre

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Abstract

Background & Aims. Lactase non-persistence causes gastrointestinal symptoms after milk ingestion. Hydrogen breath test (BTH) and genotyping of a single nucleotide polymorphism (SNP) C>T 13,910 base pairs upstream of the lactase gene represent potential methods for diagnosis of this autosomal-recessive trait. The aim of the study was to compare the results of both tests in detecting lactose non-persistence in a tertiary referral centre. **Patients.** A group of 58 patients admitted to a German university hospital for symptoms suggesting lactose intolerance**. Methods**. BTH after lactose ingestion (50 g) and SNP -13,910C>T genotyping using single nucleotide primer extension (SNaPshot) technology (CC genotype - lactase non-persistence; TC/TT genotypes - lactase persistence). **Results**. Overall, 17 (29%) patients had a positive and 41 (71%) had a negative BTH result; 15 (26%) patients were CC-positive and 43 (74%) were CC-negative [28 (48%) TC; 15 (26%) TT]. The genotype frequencies did not deviate from the Hardy-Weinberg equilibrium. In the CC-positive group, concordance between both tests was 100%. In contrast, in the CC-negative group concordance was 95%, and positive BTH results could be attributed to other gastrointestinal pathologies in two patients. BTH had 100% negative predictive value, 88% positive predictive value, 100% sensitivity and 95% specificity, as compared to genetic testing. **Conclusions**. In carriers of the CC-genotype, BTH and genotyping correlate perfectly, and the genetic test provides an unambiguous result. In BTH-positive individuals with a negative genetic test there is good reason to suspect secondary causes of lactase deficiency.

Key words

Breath test - lactase - lactose intolerance - single nucleotide polymorphism.

J Gastrointestin Liver Dis
June 2008 Vol.17 No 2, 135-139

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Introduction

Adult-type hypolactasia (also known as primary lactose malabsorption or lactase non-persistence) is the world's most common enzyme deficiency. It is due to the decline of the activity of the small intestine brush border lactase-phlorizin hydrolase (LCT), the disaccharidase involved in the hydrolysis of milk's main carbohydrate lactose [1]. Though all newborns display an adequate expression of LCT, most adults worldwide do not express the enzyme when milk is no longer the main dietary product. As a result of this autosomal-recessive trait, lactose, which is normally digested in proximal to mid-jejunum, reaches the colon where it is fermented by bacteria to fatty acids and gas, causing bloating and diarrhoea [2]. In a minority of individuals, the same symptoms are due to a secondary lactase deficiency that is caused by distinct pathologies of the small intestine (e.g., giardiasis, celiac disease or Crohn's disease) [3]. Several authors have suggested that consuming small amounts of milk does not exert any effects in lactose non-persistent individuals, yet this condition is apparently the major reason for avoiding milk products worldwide [4-7]. On the other hand, the majority of Europeans are able to digest large quantities of lactose throughout their life, a condition called lactase persistence.

In 2003, a Finnish study unraveled the genetic basis for lactose intolerance, demonstrating the link between lactose persistence and a single nucleotide polymorphism (SNP) C>T located 13,910 base pairs (bp) upstream of the LCT gene in intron 13 of the minichromosome maintenance 6 (MCM6) gene [8]. In fact, the -13,910C>T polymorphism appears to govern LCT transcription [9]. Several studies validated that the genotypes TC and TT are associated with lactase persistence, whereas the CC-genotype correlates with hypolactasia [7,9,10]. Additionally, other polymorphisms (e.g. LCT –13907C>G, LCT –13915T>G) have been tested for their involvement in the onset of lactose intolerance [11]. Though these studies provide relevant data, lactose intolerance is still primarily investigated by the hydrogen breath test (BTH). This well-known and widely accepted procedure measures the exhaled amount of hydrogen produced in the process of fermentation once the patient is

challenged with lactose [12,13].

As genetic testing has gained attention in the field of lactose malabsorption, the aim of this study was to compare it with BTH for the diagnosis of lactase non-persistence. In this respect, we genotyped the -13,910C>T variant in a group of patients referred to a tertiary referral centre for suspected lactose intolerance.

Methods

Patients

The study group comprised 58 consecutive patients (25 males, 33 females; median age 41 years, range 18 - 82 years) recruited prospectively between April 2005 and July 2007. Patients were referred to our department with non-specific gastrointestinal symptoms consistent with lactose intolerance (i.e., bloating, abdominal pain, diarrhea). All participants signed an informed consent form, and the study was conducted according to a study design approved by the local ethical committee.

Genetic test

Peripheral venous blood samples for DNA testing were obtained from all patients. DNA was isolated using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany). The genotyping procedure consisted of polymerase chain reaction (PCR) amplification and SNP detection of the -13,910C>T variant using SNaPshot minisequencing (Applera, Norwalk, CT). For PCR amplification, we used forward primer 5'-CTGCGCTGGCGGCAATACAGATA-3' and reverse primer 5'-GCAGGGCTCAAAGAACAATC- 3'. The PCR mixture contained 0.5 µM primers, PCR Buffer (Invitrogen, Karlsruhe, Germany), 1.5 mM MgCl2, 0.2 mM dNTPs each, 1 U Taq polymerase and 20 - 100 ng of DNA in a total volume of 10 µl. Cycling conditions were 94°C for 3 min, followed by 32 cycles of 30 sec at 94°C, 30 sec at 57°C and 75 sec at 72°, and 10 min at 72°C as final extension step. The PCR product was incubated for 60 min at 37°C with 5 U alkaline phosphatase from calf intestine (Cip) and 2 U exonuclease 1 to digest primers and dNTPs, followed by enzyme deactivation at 75°C for 15 min. SNaPshot thermal cycling was performed in a 10 µl mixture containing 5 µl Multiplex Ready Reaction mix, 0.2 µM SNaPshot primer 5'-(AAA)6AACCTTTGAGGCCAGCC-3' and 3 µl of the PCR product (25 cycles of 10 sec at 96°C, 5 sec at 50°C, and 30 sec at 60°C). The products were dephosphorylated by incubation with 1 U/µl Cip, as described above. All products were separated on the ABI 310 automated DNA sequencer and analysed with GeneScan software (Applera).

Hydrogen breath test (BTH)

BTH was performed after at least 12 hours overnight fasting. All patients were obliged to restrain from cigarette smoking before the test. Additionally, individuals who underwent colonoscopy or were taking any antibiotics in the fortnight before the test were excluded from the study. The test was performed after ingestion of 50 g of lactose diluted in 300 ml of water. The amount of exhaled hydrogen was measured in parts per million (ppm) before lactose ingestion (baseline), every 10 minutes during the first hour and every 20 minutes during the second and third hour of the test. In addition, the occurrence of gastrointestinal symptoms after the ingestion of lactose was monitored by questionnaires. The result was regarded as positive if the hydrogen excretion was ≥ 20 ppm higher than baseline in at least two subsequent measurements. All tests were performed on the H2 breath test apparatus Stimotron (Wendelstein, Germany).

Statistical analysis

Consistency with Hardy-Weinberg equilibrium was assessed using exact tests (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Positive predictive value (PPV), negative predictive value (NPV), specificity and sensitivity of BTH against genotyping were calculated.

Results

Concordance between BTH and genetic test

As shown in Table I and Figure 1, 17 (29%) patients had positive and 41 (71%) had negative BTH results. Genotyping revealed that 15 (26%) patients were CC-positive, indicating lactose non-persistence. Accordingly, 43 (74%) patients were CC-negative, which in turn is a genetic hallmark of

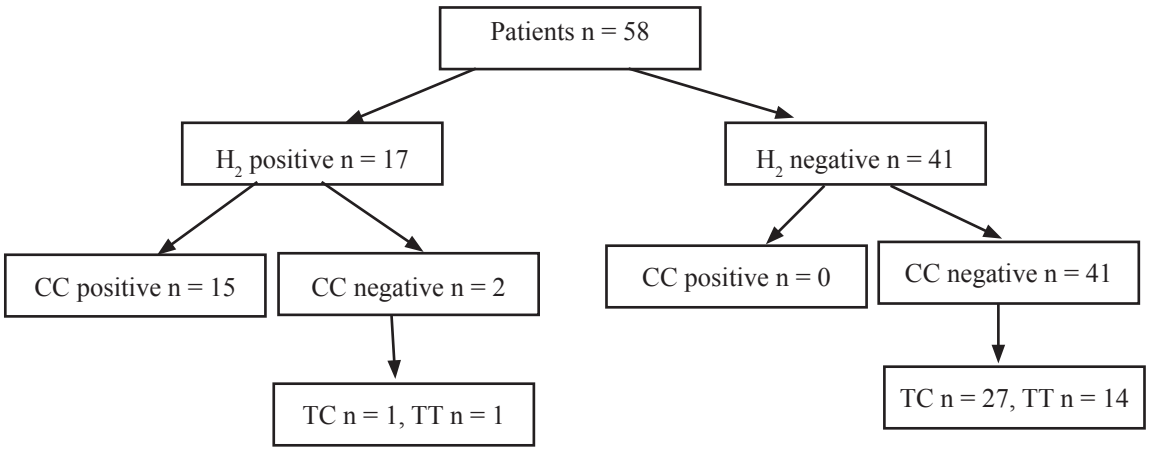

Fig 1. Results of BTH and genotyping assays (layout adapted from Büning et al [16]. For details see Results. H_2 positive and H_2 negative indicates hydrogen peak ≥ 20 ppm and < 20 ppm over baseline after 80 minutes, respectively

lactose persistence. Among these CC-negative individuals, 15 (26%) were TT homozygotes and 28 (48%) carried the heterozygous genotype TC, respectively. Figure 2 indicates that all genotypes did not deviate from the Hardy-Weinberg equilibrium.

The comparison of the BTH and the genetic tests indicates 100% concordance between both procedures in CC-positive patients (Table I).

Table I. Summary of BTH and genotyping results

	BTH positive n=17 (29%)	BTH negative n=41 (71%)
CC-positive n=15 (26%)	n = 15	n = 0
CC-negative n=43 (74%)	n = 2	n = 41

BTH - hydrogen breath test; BTH positive - hydrogen peak ≥ 20 ppm; BTH negative - hydrogen peak < 20 ppm above baseline after 80 min.

Discordance between BTH and genetic test

In the CC-negative group, 2 of 43 (5%) subjects tested positive in BTH, whereas the remaining 41 patients of this group showed negative results. Table II shows that sensitivity and NPV of BTH reached 100%; specificity was 95%, and PPV was 88%. These results indicate that a negative BTH is highly accurate in detecting lactase persistent subjects (carrying the T allele), whereas a positive BTH identifies all lactase non-persistent subjects (those who do not carry the T allele) and a subgroup of lactase persistent subjects in whom lactose intolerance is secondary to other causes.

Table II. BTH in comparison to -13,910 C > T genotype

Sesitivity	100 %
Specificity	95%
NPV	100%
PPV	88%

PPV - positive predictive value; NPV -negative predictive value.

The clinical records of the two patients with negative genetic test but positive BTH were further investigated, as they were suspected of suffering from secondary lactose intolerance. The first patient (genotype TT), a 41-year-old woman, had been diagnosed with Crohn's disease one year before the study and was in the active phase at the time of admission (as documented by ileocolonoscopy and CRP = 29.8 mg/l). Figure 3 depicts the BTH of the second patient (genotype TC, 35-year-old woman). The first hydrogen peak was detected 20 minutes after lactose ingestion (40 ppm above baseline), suggesting bacterial overgrowth. The second peak was detected 40 minutes later, yet it was only 20 - 25 ppm above baseline and lasted only for 2 subsequent measurements. Thus, we assume that bacterial overgrowth caused the discrepant results in this patient. In fact, the patient reported symptoms of irritable bowel syndrome (IBS) such as recurrent abdominal pain and changes in bowel habits.

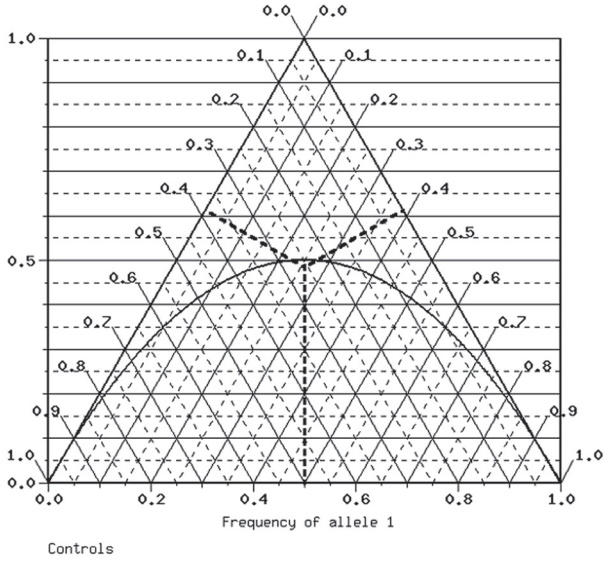

Fig 2. De Finetti diagram for SNP -13,910 C > T. The diagram illustrates genotype and allele frequencies. The frequencies of homozygous genotypes are plotted on the left and right diagonal axes, the frequency of heterozygous patients is plotted on the vertical axis on the left, and the allele frequencies are depicted by the intersection of the vertical dotted line with the bottom perpendicular (allele 1: -13,910C). The genotype frequencies plot on the parabola in the diagram, indicating that they are in Hardy-Weinberg equilibrium (Hardy-Weinberg parabola). The diagram was plotted using the software package developed by T.M. Strom and T.F. Wienker (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

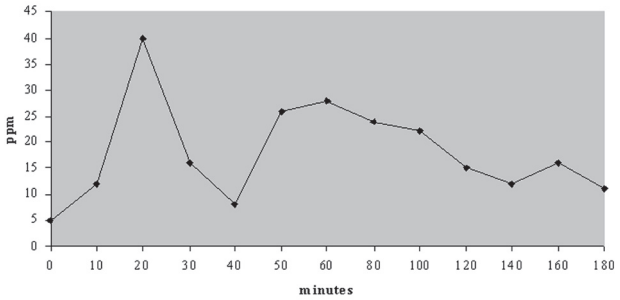

Fig 3. BTH results of patient 2. For details see Results

In addition, this patient reported a long-lasting history of cigarette smoking. Abdominal ultrasound and colonoscopy with inspection of the terminal ileum were normal.

Discussion

The aim of this study was to compare the accuracy of -13,910C>T variant genotyping and lactose BTH for diagnosis of adult-type hypolactasia. The results demonstrate 100% match between BTH results and CC-positive genotypes for detection of lactose non-persistence (Table I, Fig. 1). On the other hand, BTH and genotyping did not show perfect agreement in CC-negative individuals. Among these individuals, two subgroups can be discriminated: (i) a large number of individuals who are CC-negative and have negative BTH and (ii) a small group of CC-negative patients who suffer from secondary lactase deficiency or

other conditions distorting the BTH results and who should undergo further clinical analysis.

In this study, the first patient suffered from active Crohn's disease, which can cause lactose intolerance symptoms and positive BTH results [14]. The second patient displayed several symptoms suggesting IBS. The relationship between IBS and lactose intolerance was investigated by Pimentel et al [15]. According to their study, bacterial overgrowth in the small intestine may be responsible for false positive BTH results in IBS patients [15]. In addition, the patient was a smoker and might not have followed the recommendation not to smoke: cigarettes are known to affect BTH results [16]. Furthermore, heterozygous individuals (TC) express lower amounts of LCT in enterocytes compared to TT carriers [9, 17]. As the second patient carried the genotype TC, even mild intestine abnormalities or bacterial overgrowth might have overwhelmed the existing diminished LCT activity.

The de Finetti diagram (Fig. 2) indicates that there is no deviation from the Hardy-Weinberg equilibrium in this study. Genotype frequencies are affected by several factors, such as inbreeding, selection or genetic drift. Hence, in the case of genotyping patients in a tertiary referral centre, we might expect a deviation towards CC-negative genotypes due to patient selection. Yet the absence of this effect indicates that more stringent selection of patients referred for genotyping is warranted. Interestingly, Büning et al have recently published a paper based on a large set of German patients [18]. In their study, 166 subjects were genotyped: 108 (66%) patients were CC-positive and 58 (34%) patients were CC-negative. In this report, the frequency of lactase non-persistence was higher than the general population prevalence (15 - 20%). This might be attributed to patient selection, yet we were not able to compare their results with ours in terms of Hardy-Weinberg equilibrium as the number of individuals with TC and TT genotypes was not reported by Büning et al [18]. Comparative results have been found by Kerber et al [19]. In this report, BTH and the CC genotype underlying lactose intolerance showed an almost perfect match (97.4% concordance). Interestingly, among heterozygotes, age was the factor that contributed to discordant tests. Similar to our second patient, TC carriers at age 31-65 years displayed significantly more positive BTH results when compared to younger participants. The study by Bodlaj et al [20] provided a thorough list of possible intestinal conditions that may underly secondary hypolactasia and characterized the patients with discrepancies between BTH and genotyping, relating them to a selection based solely on positive BTH results and not on symptoms or medical history.

On the other hand, the high prevalence of lactase non-persistence in the general population should urge physicians to be restrictive when diagnosing primary lactose intolerance based solely on BTH results. Misdiagnosis of adult-type hypolactasia in CC-negative individuals might result in long lasting restraint from milk products. In this case it would not relieve symptoms, yet contribute to osteoporosis and other related complications. Thus, we propose that each patient with complaints concerning lactose consumption should be investigated first by genetic tests and subsequently by additional invasive procedures to clarify if the symptoms are related to other pathologies of the gastrointestinal tract. Since the costs of genetic testing do not exceed BTH, this strategy might also be cost-effective. In case of secondary lactase deficiency, accurate diagnosis and therapy will lead to reduction of lactose-related complaints and can even restore lactase activity. On the other hand, appropriate dietary recommendations are warranted for lactose intolerant individuals, since they are prone to show decreased calcium levels due to lower milk intake and impaired calcium absorption [2,21].

In **conclusion**, this study demonstrates that genotyping and BTH results match perfectly in the group of lactose non-persistent individuals, yet if a patient carries a CC-negative genotype and secondary lactase deficiency is suspected, further investigations are warranted to identify the underlying diseases.

Conflicts of interest

None to declare.

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