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Excellent agreement between genetic and hydrogen breath tests for lactase deficiency and the role of extended symptom assessment

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Clinical manifestations of lactase (LCT) deficiency include intestinal and extra-intestinal symptoms. Lactose hydrogen breath test (H_2-BT) is considered the gold standard to evaluate LCT deficiency (LD). Recently, the single-nucleotide polymorphism $C/T_{-13\,910}$ has been associated with LD. The objectives of the present study were to evaluate the agreement between genetic testing of LCT $C/T_{-13\,910}$ and lactose H_2-BT, and the diagnostic value of extended symptom assessment. Of the 201 patients included in the study, 194 (139 females; mean age 38, range 17–79 years, and 55 males, mean age 38, range 18–68 years) patients with clinical suspicion of LD underwent a 3–4 h H_2-BT and genetic testing for LCT $C/T_{-13\,910}$. Patients rated five intestinal and four extra-intestinal symptoms during the H_2-BT and then at home for the following 48 h. Declaring H_2-BT as the gold standard, the $CC_{-13\,910}$ genotype had a sensitivity of 97 % and a specificity of 95 % with a κ of 0·9 in diagnosing LCT deficiency. Patients with LD had more intense intestinal symptoms 4 h following the lactose challenge included in the H_2-BT. We found no difference in the intensity of extra-intestinal symptoms between patients with and without LD. Symptom assessment yielded differences for intestinal symptoms abdominal pain, bloating, borborygmi and diarrhoea between 120 min and 4 h after oral lactose challenge. Extra-intestinal symptoms (dizziness, headache and myalgia) and extension of symptom assessment up to 48 h did not consistently show different results. In conclusion, genetic testing has an excellent agreement with the standard lactose H_2-BT, and it may replace breath testing for the diagnosis of LD. Extended symptom scores and assessment of extra-intestinal symptoms have limited diagnostic value in the evaluation of LD.

$CT_{-13\,910}$: Genetic testing: Lactose intolerance: Lactase deficiency: H_2-breath test: Hypolactasia: Abdominal pain: Bloating: Diarrhoea

Worldwide, over 5 billion humans are estimated to suffer from lactase (LCT) deficiency, which is the most common cause of lactose intolerance. The geographical distribution of lactose intolerance is variable depending mostly on heritage: in Asian countries, close to 100 % of the general population is considered lactose intolerant, whereas in Northern Europe, prevalence reaches about 10–20 % increasing towards the south[1]. Lactose is a disaccharide physiologically cleaved into glucose and galactose by LCT located on the luminal membrane of the enterocytes located in the proximal small bowel. Reduced expression and/or activity of LCT leads to an accelerated intestinal transit and degradation of lactose by colonic bacteria into SCFA, H_2 and other metabolites implicated in the pathophysiology of classical abdominal symptoms such as diarrhoea, bloating, borborygmi and abdominal pain.

Most laboratories employ a lactose H_2-breath test (H_2-BT) in diagnosing LCT deficiency (LD). This test exploits the fact that H_2, a by-product of bacterial lactose metabolism, is absorbed through the intestinal mucosa into the venous blood, and transported past the liver into the alveoli of the lungs, from where it is exhaled[2,3]. Several studies correlating the amount of exhaled H_2 with LCT deficiency documented a good sensitivity (76–94 %) and specificity (77–96 %) of the BT[2].

In 2002, Enattah *et al.*[4] described single-nucleotide polymorphism 13 910 bp above the structural gene coding for the enzyme LCT on the short arm of chromosome 2q.21-22. This polymorphism consists of the nucleotide switch of T for C, resulting in variants CC, CT or $TT_{-13\,910}$. Enattah demonstrated $CC_{-13\,910}$ to be a good predictor of intestinal LCT activity loss. A recent study[5] showed a lower sensitivity of genetic testing of only 75 % when compared with H_2-breath testing based on ingestion of lactose.Other genetic polymorphisms, with the most prominent one being $G/A_{-22\,018}$ identified in European and Asian subjects, have also been shown to be of importance, but they have not been commercially marketed

Abbreviations: BT, breath test; LCT, lactase.

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to date[4]. Recently, polymorphisms especially in the sub-Saharan African population have demonstrated local gene traits[6]. Genetic tests fail to detect secondary causes of relative LCT deficiency such as inflammatory or malabsorptive bowel disease, in which reduced mucosal absorptive area, insufficient contact time between substrate and enzyme, and also reduced mucosal expression of LCT lead to incomplete degradation of lactose[7]. Besides classical symptoms of lactose intolerance, extra-intestinal symptoms such as headache, fatigue and muscle pain have been suggested to occur in response to ingestion of LCT[8–10].

The aims of the present study were (1) to compare the results of H_2-BT against those of the genetic test; (2) to evaluate the utility of collecting data on intestinal and extra-intestinal symptoms for up to 48 h after oral ingestion of lactose.

Methods

Patients

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki. All study subjects provided written informed consent before being enrolled in the present study. The study was approved by the Ethics Committee of the University Hospital Zurich and of the Canton of Zurich (EK-1225). Consecutive patients referred to our tertiary referral centre for suspected lactose intolerance were included in the study. Subjects were required to be at least 16 years old. Patients with antibiotic use in the past 2 weeks before the procedure, with active chronic or acute inflammatory bowel disease and with drug or alcohol abuse were excluded from the study.

Lactose hydrogen breath test

To minimise basal H_2 values, patients were instructed to consume a carbohydrate-deficient diet (especially avoiding beans, soya products, lentil, peas and whole-grain breads) 1 week before the testing, and to fast at least 12 h before the test. Smoking and physical activity were to be avoided at least 1 h before the test and during the entire test procedure. The oral cavity was decontaminated with 2 × 20 ml of Hextril solution (Pfizer, Zurich, Switzerland). End-expiratory H_2 values were measured offline using a stationary H_2 detection system (Acutronic Medical Systems, Hirzel, Switzerland). For adequate sample collection, patients were instructed to perform two empty breath manoeuvers before each H_2 breath sample collection in order to remove dead air space in the respiratory tree. Patients then exhaled normally, and samples were collected mid-expiration using 20 ml plastic syringes. After determining the baseline H_2 concentration, patients received 50 g lactose powder, or an equivalent of 1 mg/kg body weight was given for patients weighing less than 50 kg. The lactose powder (Kantonsapotheke Universitätsspital Zürich, Switzerland) was dissolved in 150–200 ml of water. Mid-expiratory H_2 breath samples were collected every 15 min for 4 h. At each time point, two breath samples were drawn from each patient, the higher of which was used for analysis. The collected air samples were analysed within 5 min after collection.

The lactose H_2-BT was considered positive (i.e. indicative of LCT deficiency) if there was a rise of at least 20 ppm in H_2 concentration above baseline in at least two consecutive measurements, starting minimum 30 min into the test.

Symptom questionnaire

Patients were asked to rate five typical symptoms of lactose intolerance (nausea, bloating, diarrhoea, borborygmi and abdominal pain) and four extra-intestinal symptoms (headache, dizziness, fatigue, and muscle and joint pain) on a 9-point Likert symptom scale ranging from 1 (non-existent) to 9 (worst ever). For the duration of the study symptoms, rating was done every 15 min concomitant with H_2 measurements. After this, patients were asked to rate both intestinal and extra-intestinal symptoms every 4 h for 48 h after the intake of lactose. Patients were asked to omit values during their sleeping period to avoid any influence of sleep-disturbance effects on symptom recording. Patients left the hospital after 4 h, and they were instructed to refrain from lactose intake (after being given dietary instruction including hidden lactose) for 48 h. Patients were provided pre-stamped envelopes to mail the completed symptom questionnaire to our institute.

Lactulose hydrogen breath test

In patient with discrepant results between genetic test and H_2-BT, a lactulose test was performed either to exclude bacterial overgrowth in those with positive H_2-BT and negative genotype (see below), or to exclude H_2 non-secretory status in patients with negative H_2-BT and positive genotype (see below). Pre-test preparation was identical to that of the lactose BT. Patients were asked to drink 25 g of lactulose powder dissolved in 150–200 ml water, and mid-expiratory samples were taken every 15 min for 4 h. The test was considered positive for bacterial overgrowth when there was a rise in H_2 peak concentration of more than 20 ppm above baseline at least 15 min before measurement of a colonic peak of at least 10 ppm above baseline[11]. Non-excretion indicating a lack of H_2-producing intestinal flora was considered in patients with a rise not exceeding 10 ppm above baseline.

Genetic testing

Venous blood samples were drawn from patients into EDTA tubes (Braun, Melsungen, Germany). Blood samples were stored refrigerated at 4°C for a maximum of 5 d before genetic analysis. Genotyping of the LCT $C/T_{-13\,910}$ polymorphism was done in the diagnostic laboratory of the Institute of Clinical Chemistry of the University Hospital Zurich using melting curve analysis on a Roche LightCycler (Rotkreuz, Switzerland) according to the previously described method by Stolba *et al.*[12]. Accuracy and robustness of the assay were evaluated in the laboratory using sequenced control DNA in repeated experiments.

Statistics

Sensitivity, specificity, and positive and negative predictive values were calculated between results from H_2-BT and

genetic testing. Agreement was calculated using Cohen's κ. Symptom intensity was compared using ANOVA with *post hoc* Bonferroni's correction for multiple testing. Differences were considered statistically significant if $P<0{\cdot}05$.

Results

Patient demographics

In the present study, 201 patients were included. Seven patients who had high basal H_2 values or had denied genetic testing after consenting to the study were excluded from the analysis. Remaining patients (total 194: 139 females, mean age 38, range 17–79 years, and 55 males, mean age 38, range 18–68, $P=0{\cdot}926$) referred for testing of suspected lactose intolerance underwent a lactose H_2-BT and genetic testing for LCT C/T_{-13910}. Patients were largely Northern European (154 Northern Swiss and German, and 5 Eastern Europeans), and furthermore, twenty-six patients were Southern Europeans (including Southern Switzerland), eight were of Asian descent (one Chinese and seven Indian) and seven patients were from Southeast Europe and the Middle East.

Lactose hydrogen breath test

Of all the patients referred for the evaluation of lactose intolerance, sixty-three patients (32·5 %) tested positive for LCT deficiency based on exhaled H_2 values after ingestion of lactose (Table 1). There were no age ($P=0{\cdot}226$)- or sex ($P=0{\cdot}865$)-specific differences between the patients with normal LCT activity and LCT deficiency.

Genetic test v. *lactose hydrogen breath test*

Sixty-eight (35·1 %) patients were CC_{-13910} homozygotes, which is the genotype associated with LCT deficiency. Another sixty-eight (35·1 %) patients were CT_{-13910} heterozygotes, and fifty-eight (29·9 %) patients were TT_{-13910} homozygotes indicating normal LCT activity (Table 1).

There was an excellent agreement between the genetic analysis and the H_2-BT with only nine (4·6 %) discrepant results. Using the lactose H_2-BT as the gold standard, only two patients were misclassified as LCT sufficient (i.e. false negative) in the genetic test, leading to an excellent sensitivity and negative predictive value in diagnosing LCT deficiency (Table 1). Overall, the genetic analysis identified 35·1 % of the patients as LCT deficient, while the H_2-BT identified 32·5 % as LCT deficient.

Table 1. Genotype* and lactose hydrogen breath test (H_2-BT) results

n 194	CC_{13910}	CT_{13910}	TT_{13910}
H_2-BT (+)	61 (31·4 %)	1 (0·5 %)	1 (0·5 %)
H_2-BT (−)	7 (3·6 %)	67 (34·5 %)	57 (29·4 %)

* Sensitivity of genotype C/T_{13910} compared with H_2-BT was 97 %, specificity was 95 %, positive predictive value was 90 % and negative predictive value was 98 %. Cohen's κ for agreement was 0·9.

Discrepant results – lactulose breath test

Of the 194 patients included in the analysis, nine (4·6 %) patients had discordant results between lactose H_2-BT and genetic analysis. These patients were clinically reassessed, and eight patients agreed to undergo the lactulose H_2-BT. Table 2 and Fig. 1 show individual patient exam stratification and results.

Seven of nine (78 %) patients with initially discrepant test results were CC_{-13910} homozygotes indicating LCT deficiency, but they had a negative lactose BT.

Upon focused re-assessment, three patients remembered having used antibiotics shorter than 4 weeks before the test (*Helicobacter pylori* eradication, chronic urinary tract infection), which could have interfered with the test, leading to a false-negative H_2-breath test. However, when tests were performed 2 and 4 months after the initial lactose breath test, two of these three patients who underwent a lactulose breath test exhibited a hydrogen-producing colonic flora, possibly reflecting restoration of formerly suppressed colonic flora. Two patients were identified as non-H_2 producers by lactulose BT. One patient had a H_2 elevation of more than 20 ppm from baseline at only one time point, very little symptoms and only a slight elevation during the lactulose BT. Finally, one patient had a false-positive genetic test as all other test results spoke in favour of lactose deficiency: non-significant H_2 elevation, absence of symptoms and a positive lactulose BT indicating H_2-producing flora.

Among the two patients with genotypes indicating normolactasia but abnormal lactose BT, one patient had small intestinal bacterial overgrowth and the other patient had intercurrent bouts of inflammatory bowel disease resulting in secondary lactose intolerance.

Symptom analysis – overall interpretation

There were no differences between sexes in symptom distribution (all $P>0{\cdot}05$). Patients (both males and females) with genotype CC_{-13910} were symptomatic in 75 % of the cases. Thirty-eight percentage of women were symptomatic with genotype CT_{-13910} *v.* 24 % of men, and 38 % of women were symptomatic with genotype TT_{-13910} *v.* 33 % of men.

Symptom analysis – intestinal symptoms

A completed 48 h symptom questionnaire was available from 95 % of the patients (185/194). Patients with CC_{-13910} homozygous genotypes (sixty-four complete symptom sets available) reported more intense intestinal symptoms (borborygmi, bloating, abdominal pain and diarrhoea) than patients with CT_{-13910} heterozygous genotypes (fifty-five complete symptom sets available) and TT_{-13910} homozygous genotypes (fifty-six complete symptom sets available). Symptoms became intense 45 min after lactose intake and lasted up to 12 h. Peak intensity was reached after 4–8 h of lactose ingestion as indicated in Fig. 2.

There was a fair agreement between symptomatic hydrogen breath test and CC_{-13910} genotype (κ 0·365, $P<0{\cdot}001$). Of the patients with a positive genetic test, 75 % (fifty-one) with genotype CC_{13910} were considered symptomatic compared

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Table 2. Discordant patient details

	Patient characteristics							
	Sex	Age (years)	Genotype	Lactose H_2-breath test results	Symptom results	Lactulose H_2-BT results	Additional clinical information	Diagnosis
1	Male	43	CC_{13910}	(−)	(+)	(−)	None	Non-H_2 producer, LI
2	Female	61	CC_{13910}	(−)	(+)	(−)	None	Non-H_2 producer, LI
3	Female	32	CC_{13910}	(−)	(+)	(+)	Lactulose test performed 4 months later, antibiotic use before the BT	False-negative H_2-test, LI
4	Male	32	CC_{13910}	(−)	(+)	(+)	Lactulose test performed 2 months later, *Helicobacter pylori* eradication before the BT	False-negative H_2-test, LI
5	Female	31	CC_{13910}	(−)	(−)	(+)	Lactulose test performed after 1 month	False-positive genetic test, LT
6	Female	66	CC_{13910}	(−)	(+)	Not performed	Admitted to intermittent antibiotic treatment due to recurrent urinary tract infection	False-negative H_2-test, LI
7	Male	29	CC_{13910}	(−, +) weak	(−, +) weak	(+, −) weak	H_2 only once above 20, lactulose test slightly positive, very little symptoms formally categorised negative	Low H_2 producer, false-negative H_2-test, possible lactase persistence in transition to LI
8	Female	54	CT_{13910}	(+)	(+)	(+)	Intermittent bouts of inflammatory bowel disease	Secondary lactose intolerance, LI
9	Female	26	TT_{13910}	(+)	(−)	(+)	Lactulose test with two peaks, first peak after 30 min	Small intestinal bacterial overgrowth, LT

H_2-BT, hydrogen breath test; LI, lactose intolerance, LT, lactose tolerance.

with 33·8 % (twenty-three) with genotype CT_{13910} and 36·2 % (twenty-one) with genotype TT_{13910} ($P<0·01$).

Symptom analysis – extra-intestinal symptoms

Patients with genotypes indicating LCT deficiency reported similar intensity of dizziness and muscle and joint pain compared with those with genotypes indicating normal LCT activity. Patients with genotype CC reported more intense fatigue 4–12 h after ingestion of lactose compared with those with genotype CT/TT. The intensity of extra-intestinal symptoms are summarised in Fig. 3.

Discussion

In the present study, we have reported on the relationship between genetic testing and lactose H_2-BT in patients evaluated at a Swiss tertiary referral centre. Additionally, in the present study, we have also reported on the intensity of intestinal and extra-intestinal symptoms characteristic for lactose intolerance in LCT-deficient and non-deficient individuals. We found an excellent agreement, sensitivity and specificity between results from testing for genetic polymorphism LCT C/T_{-13910} and lactose H_2-BT. In patients with discrepant results, a careful history and lactulose H_2-BT clarified the diagnosis. With regard to intestinal symptoms, patients with LCT deficiency reported higher symptom intensity for 2–10 h after oral ingestion of lactose for all symptoms except for nausea. LCT-deficient patients and those with normal LCT activity reported similar intensities of extra-intestinal symptoms with the exception of fatigue. Increased fatigue 4–16 h after oral ingestion of lactose was most likely the result of the increased intestinal symptoms rather than being directly related to lactose intolerance.

The present results are consistent with a previous report by Krawczyk *et al.*[13] who evaluated fifty-eight patients for lactose intolerance in a German tertiary referral centre. They found a 100 % agreement between lactose H_2-BT and genotype CC_{-13910}. Considering the genetic test as the gold standard, they reported an excellent sensitivity and negative predictive value of the H_2-BT.

Nine patients (4·6 %) in our group had discrepant results in the lactose H_2-BT. The most common reason (33 %) for the discrepant results was the prior use of antibiotics leading to suppressed intestinal flora and a 'non-H_2 producer' status.

Few of our patients had a false-positive result. Colonisation of the proximal intestinum with H_2-producing bacteria termed small intestinal bacterial overgrowth can lead to false-positive BT results[14]. We diagnosed one patient with small intestinal bacterial overgrowth; the other patient's positive lactose H_2-BT despite negative genetic test was most likely attributable to intermittent bouts of inflammatory bowel disease, which was not recognised at study inclusion[7].

Högenauer *et al.* reported a lower sensitivity (75 % opposed to 97 % in our subjects) in the detection of LCT deficiency when comparing genetic test and lactose H_2-BT as the gold standard[5]. Differences in test design (in our study patients had to reach a δ H_2 peak above 20 ppm in at least two consecutive measurements) and studied population have to be taken into account when interpreting these data. Similar to our findings, Högenauer reported a high specificity in

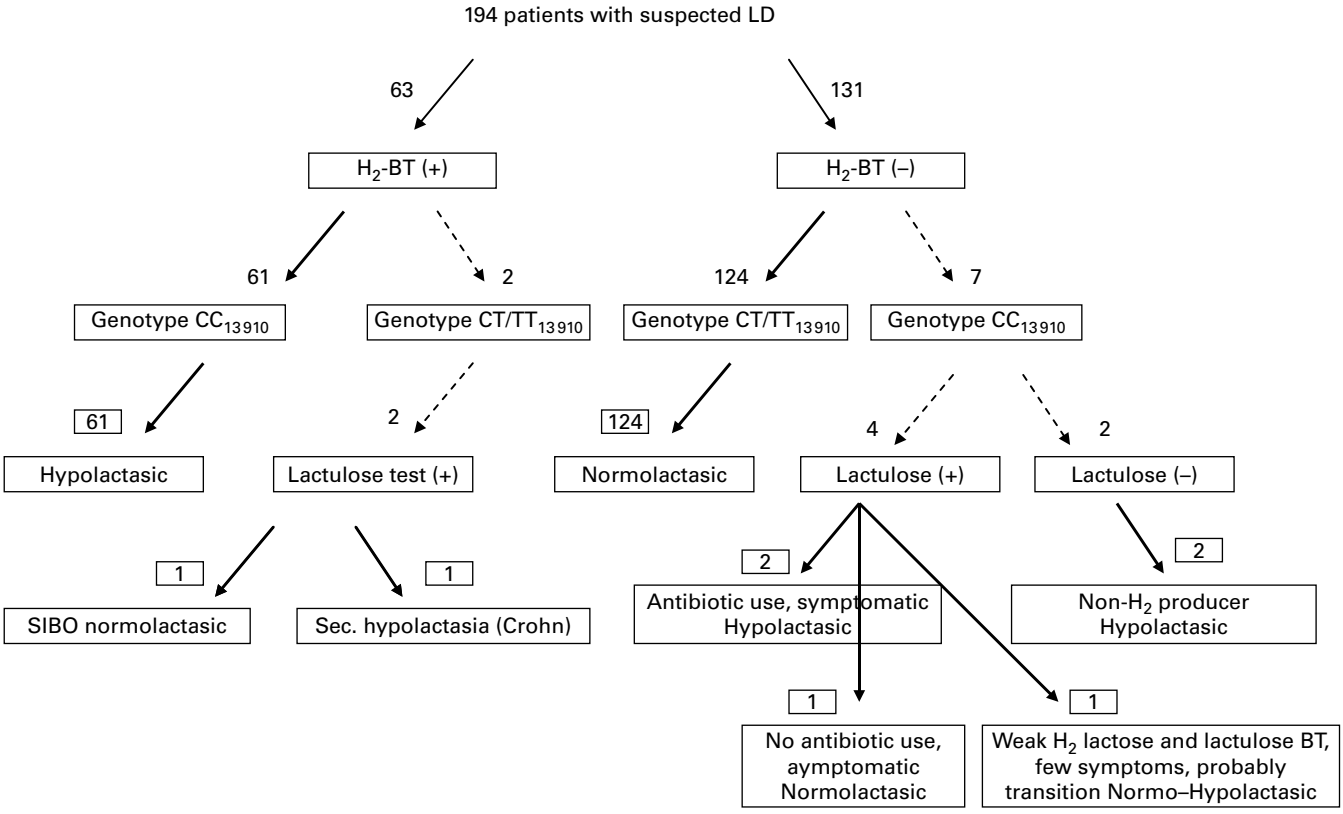

Fig. 1. Flowchart showing patient results and stratification. ⟶, Diagnosis; - - - →, discrepant results. LD, lactase deficiency; H_2-BT, hydrogen breath test.

diagnosing LCT deficiency in patients with genotype CC_{-13910}. One patient with negative lactose H_2-BT and normal lactulose H_2-BT had a positive genetic test. This may be explained by LCT persistence into young adulthood as shown in a recent two-decade follow-up study in a Finnish population[15]. Furthermore, epigenetic modulation of the DNA with consecutive changes in functional properties or gene–gene interactions have to be discussed, and since we did not test for other genetic polymorphisms possibly associated with LCT persistence such as G_{22018}, C_{-14010}, G_{-13907} and G_{-13915}, we cannot rule them out[4,16]. A second patient with negative H_2-BT, few symptoms and slightly elevated breath H_2 concentrations had a positive genetic test. This patient was considered a low-H_2 producer, but transition into LCT deficiency as mentioned earlier cannot be excluded. A technical limitation in the diagnosis of the LCT C/T_{-13910} genotype has been discussed in systems employing LightCycler diagnosis and melting curve analysis[17]. However, since our population was largely European and five of seven patients with genotype CC_{13910} and negative BT were clearly symptomatic indicating lactose intolerance, we believe that this should not affect our diagnoses and derived statements. Adding to the finding of previous investigators who compared genetic testing and phenotype, our data show a high negative predictive value of 98 % for genetic testing, supporting a low likelihood of LCT deficiency in patients with genotypes CT and TT_{13910}.

Symptom analysis is an important part of establishing a clinical diagnosis of lactose intolerance. Symptoms typically attributed to lactose intolerance include abdominal pain, nausea, diarrhoea, bloating and borborygmi. In addition to these, it has been suggested that systemic extra-intestinal symptoms such as headache and light headedness (up to 86 %), loss of concentration and short-term memory loss (82 %), fatigue (63 %) and muscle and joint pain (71 %) as well as allergic reactions (40 %, including eczema, pruritus and airway reaction) could be the results of LCT deficiency[8–10]. In addition to the typical intestinal symptoms, we chose four extra-intestinal symptoms (headache, fatigue, dizziness, and muscle and joint pain) and a time frame of 48 h to explore symptoms which may occur late after oral ingestion of lactose.

For typical intestinal symptoms, the results were consistently different for all the symptoms except for nausea. Patients with genotype indicative of LCT deficiency had more intense symptoms starting from 45 min (bloating) to 90 min (diarrhoea) and lasting 8 h (abdominal pain) to 28 h (borborygmi) after oral ingestion of lactose. Roughly one-third of our patients with lactose persistence had typical symptoms of lactose intolerance although showing LCT persistence. Interestingly, one-quarter of the patients without adequate LCT production remained largely asymptomatic. It has been shown that there is a considerable overlap between irritable bowel syndrome and lactose intolerance plus the placebo effect as patients may have been biased to test positive for different reasons. On the other hand, it has been shown that most patients with LCT deficiency do not develop symptoms after intake of usual servings of lactose (12·5 g as in one cup of milk)[18]. Even the considerably larger dose of 50 g, which is considered adequate in a Northern European population, only showed a sensitivity of 75 % to detect

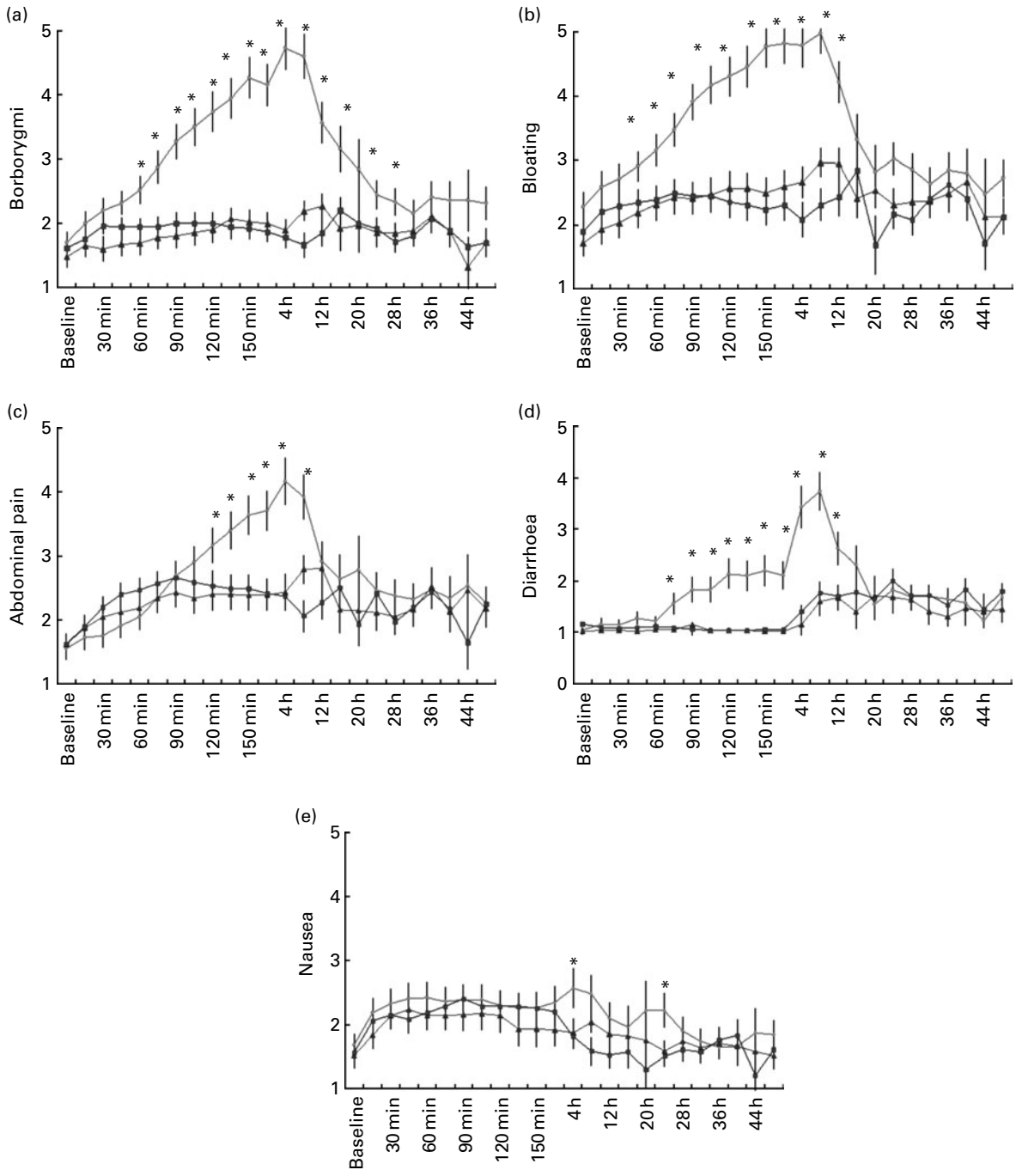

Fig. 2. Intestinal symptom scores over 48 h according to genotyping for lactase C/T$_{-13910}$. (a) Borborygmi, (b) bloating, (c) abdominal pain, (d) diarrhoea and (e) nausea. —●—, Genotype CC; –▲–, genotype CT; –■–, genotype TT. * Mean values were significantly different ($P<0{\cdot}05$).

hypolactasia by symptoms. This underlines the individual perception of symptoms and may also be caused by a certain tolerance of larger lactose doses, as portion sizes increase around the globe[19], and evidence exists that patients with lactose intolerance can adapt over time to larger doses of lactose by adaptation of their microflora[20].

There was no difference in symptom expression between genotypes CT$_{13910}$ and TT$_{13910}$ at any time point. Conversely to this, Matthews *et al.*[10] reported differences in sensitivity of H_2 lactose testing between genotypes CT and TT$_{13910}$ in a large patient population. The reason for this discrepancy is unclear, and it may be due to patient population as they examined patients from an irritable bowel syndrome cohort possibly suffering in part from underlying motility disorder or small intestinal bacterial overgrowth leading to secondary lactose intolerance of false-positive results. Furthermore, the authors performed 6 h H_2 recordings, whereas we tested patients for a maximum of 4 h. Though our time window of symptom assessment of 48 h was clinically important, in our patient population clinically suspected to have lactose intolerance in the first 6 h or later up to 48 h, differences in neither intestinal symptoms nor extra-intestinal symptoms were noted based on

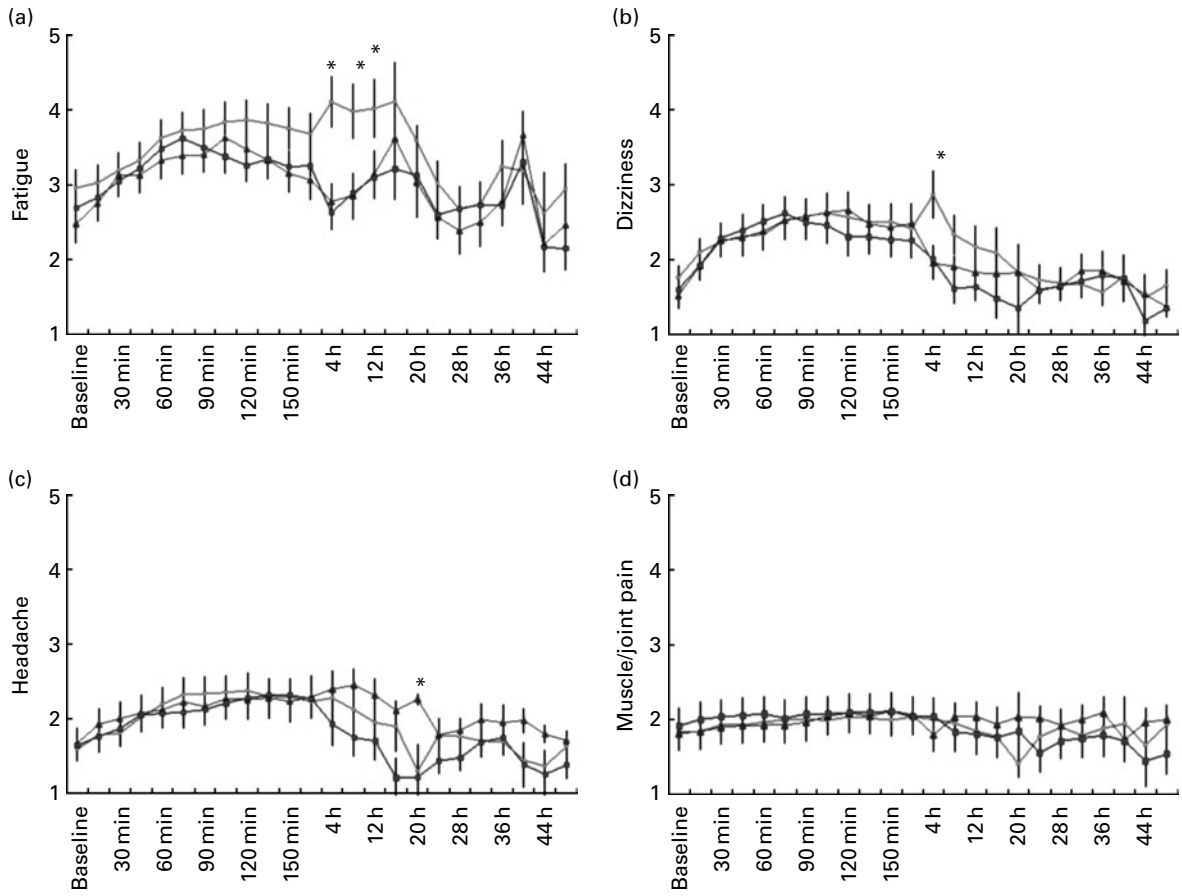

Fig. 3. Extra-intestinal symptom scores over 48 h according to genotyping for lactase C/T_{-13910}. (a) Fatigue, (b) dizziness, (c) headache and (d) muscle/joint pain. —●—, Genotype CC; –▲–, genotype CT; –■–, genotype TT. * Mean values were significantly different ($P<0{\cdot}05$).

difference in genotypes CT and TT_{13910}. Kuokkanen *et al.*[21] noted different levels of intestinal brush border LCT with 4–9 U/g in CC_{-13910} carriers and with 13–49 and 18–87 U/g in CT and TT carriers, respectively. We believe that the observed large overlap in LCT expression between CT and TT_{-13910} carriers explains the similar symptom pattern in patients, and that it does not seem to translate into clinically significant differences in our patient population. This is in accordance to the data reported by Högenauer *et al.*[5], although they showed a higher percentage of CT-carrying patients than of TT_{-13910}-carrying patients with lactose H_2-BT indicating LCT deficiency.

Extra-intestinal symptoms did not yield different results based on genetic testing for LCT deficiency either during the initial 4 h window, where symptoms were quantified every 15 min, or in the 44 h thereafter. Only fatigue was noted more often in patients with LCT deficiency 4–16 h after ingestion. This may be due to a vagal nerve system response due to increased intestinal activity with respect to the osmotic challenge by undigested lactose or a reaction to typical intestinal symptoms during the first hours of the test that may be painful and stressful in patients with lactose intolerance.

The long duration of classical symptoms was somewhat surprising, as from a mechanistic standpoint, undigested lactose passes quicker through the intestine due to its osmotic properties. This may be explained by two mechanisms. First, apart from lactose residues still causing mechanical symptoms, the concept of a local reaction via impairment of the endoplasmic reticulum in the intestinal wall after provocation with large amounts of lactose emerged, which may be comparable to changes induced by viral infections[22]. Secondly, direct effects on the mucosa comparable to those caused by bacterial enterotoxins have been discussed, but have not been validated yet[10,23].

In conclusion, our data show that the testing of LCT C/T_{-13910} genotype could become the first-line test for investigating patients with symptoms suggestive of lactose intolerance. The length and self-limitation of symptoms provoked by a lactose H_2-BT are clinically important information to be shared with the patients, and if needed, pain and/or antispasmodic medication should be provided. Extended symptom questionnaires do not yield meaningful information on patients with LCT deficiency.

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involved in the data analysis and finalisation of the manuscript. B. S. was involved in the study design and data management. O. G., A. v. E. and M. F. were involved in the study design and finalisation of the manuscript. R. T. was involved in the data analysis and writing of the manuscript. The authors have no conflicts of interest. The present study was supported by the Swiss Foundation for Nutritional Research (Schweizerische Gesellschaft für Ernährung; SGE), grant number 358.

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