European Journal of Clinical Nutrition (2009) 63, 909–912
 0954-3007/09 $32.00
www.nature.com/ejcn

SHORT COMMUNICATION

Prevalence of adult-type hypolactasia as diagnosed with genetic and lactose hydrogen breath tests in Hungarians

D Nagy[1], E Bogácsi-Szabó[1], Á Várkonyi[2], B Csányi[1], Á Czibula[1], O Bede[2], B Tari[2] and I Raskó[1]

[1]*Institute of Genetics, Biological Research Centre of Hungarian Academy of Sciences, Szeged, Hungary and* [2]*Department of Paediatrics, Albert Szent-Györgyi Medical University, Szeged, Hungary*

The prevalence of adult-type hypolactasia varies ethnically and geographically among populations. A C/T_{-13910} single nucleotide polymorphism (SNP), upstream of the lactase gene, is known to be associated with lactase non-persistence. The aim of this study was to determine the prevalence of lactase-persistent and non-persistent genotypes in the Hungarian population, the age at onset and the applicability of the lactose H_2 breath test in comparison with genetic screening. The prevalence of the C/C_{-13910} genotype among adults was 37%. Hypolactasia starts to appear at around 5 years of age. Over the age of 12 years, almost all of those with a C/C_{-13910} genotype have lactase non-persistence. The C/C_{-13910} genotype was closely associated with a positive lactose H_2 breath test in symptomatic children, whereas the lactase-persistent genotypes correlated better with a negative H_2 test in a control group. In conclusion, supplementary non-invasive breath and genotyping tests furnish a perfect clinical diagnosis.
European Journal of Clinical Nutrition (2009) **63,** 909–912; doi:10.1038/ejcn.2008.74; published online 21 January 2009

Keywords: lactose intolerance; single nucleotide polymorphism C/T_{-13910}; H_2 breath test; specificity; sensitivity; positive and negative predictive value

Introduction

Adult-type hypolactasia (ATH, lactase non-persistence) is an autosomal recessive trait with ethnic and geographical variations in its prevalence and age at onset (Swallow, 2003; Rasinperä *et al*., 2004). A single nucleotide polymorphism (SNP), upstream of the human lactase gene (Enattah *et al*., 2002), has various enhancing effects on the lactase gene expression (Olds and Sibley, 2003; Lewinsky *et al*., 2005). In individuals with T/T_{-13910} and C/T_{-13910} or with the C/C_{-13910} variant, lactase persistence or non-persistence may develop, respectively. In Hungary, the standard method of ATH diagnosis is the lactose H_2 breath test (HBT). We determined the prevalence of lactase-persistent and non-persistent genotypes in the Hungarian population, and the age at onset of ATH, and compared the genotypes with the HBT in symptomatic and asymptomatic children.

Correspondence: Dr D Nagy, Institute of Genetics, Biological Research Centre of Hungarian Academy of Sciences, POB 521, H-6701, Szeged, Hungary.
E-mail: nagydor@gmail.com
Contributors: DN and EBS contributed equally to this study.
Received 10 March 2008; revised 25 September 2008; accepted 2 December 2008; published online 21 January 2009

Materials and methods

Three groups of 296 Caucasian-Hungarian individuals were analysed. Group 1 consisted of 82 persons (46 females, 36 males; mean age: 11 years; range: 2–19 years) with ATH-related symptoms. In all, 15% of these subjects exhibited coeliac disease, 9% giardiasis, 3% inflammatory bowel disease and 7% bacterial or viral intestinal infection, whereas group 2 comprised 104 randomly-selected control individuals (58 females, 46 males; mean age: 11 years; range: 2–20 years) with no abdominal symptoms and no previous gastrointestinal diseases . Both groups underwent HBT and genetic screening. The allele frequencies were established from group 3, 110 randomly-selected volunteers (age: 19–26 years).

HBT was performed after the ingestion of 1 g of lactose/body weight kilogram (max. 50 g) in a 10% aqueous solution.

If a secondary lactase deficiency, such as coeliac disease, was suspected in the background of positive HBT results ($\geqslant$20 p.p.m. at 120 min after lactose load), despite the presence of lactase-persistent genotypes, small intestinal biopsies were obtained.

Coeliac disease was detected histologically from the intestinal samples of 12 patients who displayed sero-positivity for IgA anti-endomysial antibodies and/or IgA anti-gliadin antibodies and tissue transglutaminase. The histological staging was carried out according to the Marsh system: type 1, 2, 3a, 3b or 3c (Oberhuber, 2000).

DNA was extracted from hair roots or buccal smears, using the Chelex-based method (Walsh *et al*., 1991). A restriction enzyme recognition site including, SNP, was introduced into the PCR product by using the derived cleaved amplified polymorphic sequence (dCAPS) method (dCAPS Finder 2.0 program, forward primer: 5′-GGCAATACAGATAAGATAATG GAG-3′; reverse primer: 5′-CCTATCCTCGTGGAATGCAGG-3′; mismatching nucleotide underlined). PCR amplification reactions accorded to the AmpliTaq Gold DNA Polymerase protocol (Applied Biosystems, California, USA). The 119 base pair (bp) PCR products were cleaved separately by NlaIV or HinfI endonucleases (Fermentas, Ontario, Canada), which resulted in the case of the C allele in 96-, 23-bp-long, and in that of the T allele in 97-, 22-bp-long fragments, and were run on an 8% native polyacrylamide gel with 1 negative (no template) and 3 positive controls (CC, CT and TT$_{-13910}$) and visualized by UV transillumination.

The PCR products of the subjects with discrepant genotype and phenotype results were sequenced to confirm or disprove the results of the restriction fragment length polymorphism (RFLP) and to discover other possible mutations in the background of the discrepancy. Sequencing was conducted under BigDyeTM Terminator Version 3.1 cycling conditions. The products were purified by ethanol precipitation and analysed on an Automatic Sequencer 3730xl (Applied Biosystems).

Statistical analysis

The GraphPad Prism version 4.00 for Windows software (GraphPad Software, San Diego, California, USA) was used. Parametric data were calculated by variance analysis and the unpaired Student's *t*-test with the Welch correction when the variances between the pairs of groups differed significantly ($P<0.05$ in the F-test). Data are expressed as means ± standard error of the mean (means ± s.e.m.). HBT results were compared with the genotype by the Fisher exact test. $P<0.05$ was considered to be statistically significant.

Results

The prevalences of the C/C$_{-13910}$, C/T$_{-13910}$ and T/T$_{-13910}$ genotypes were 73, 26 and 1% in group 1, as compared with 38, 50 and 12% in group 2, and 37, 48 and 15% in group 3. The allele frequencies associated with lactase persistence (T$_{-13910}$) and non-persistence (C$_{-13910}$) in group 3 were 37.8 and 62.2%, respectively.

The individuals of group 1 and 2 with different genotypes for the HBT were subdivided into three age groups to evaluate the age at onset (Table 1). In all, 12 patients had type 3 coeliac disease. Five of the 12 patients had the C/C$_{-13910}$ genotype and 7 others had the C/T$_{-13910}$

Table 1 Comparison of the C/T$_{-13910}$ genotypes with the H_2 breath test results in the age—subgroups of patients (group 1) and controls (group 2)

	C/C$_{-13910}$ genotype				*C/T$_{-13910}$ and T/T$_{-13910}$ genotypes*			
	Group 1 (n = *60*)		*Group 2* (n = *40*)		*Group 1* (n = *22*)		*Group 2* (n = *64*)	
	HBT (n *(%)*)				*HBT* (n *(%)*)			
	Positive	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*	*Positive*	*Negative*
Total (*n*)	54 (90)[a]	6 (10)	23 (58)	17 (42)	3 (14)[b]	19 (86)[c]	2 (3)[d]	62 (97)
<5 years	0	3 (100)[e]	1 (14)[f]	6 (86)	1 (14)[d]	6 (86)	0	12 (100)
5–12 years	29 (100)	0	11 (65)	6 (35)	1 (20)	4 (80)	1 (5)	21 (95)
>12 years	25 (89)	3 (11)[g]	11 (69)	5 (31)[g]	1 (10)	9 (90)	1 (3)	29 (97)
12–14 years	8 (80)	2 (20)	6 (86)	1 (14)	1 (20)	4 (80)	0	11 (100)
14–16 years	8 (89)	1 (11)	0	2 (100)	0	3 (100)	0	10 (100)
>16 years	9 (100)	0	5 (71)	2 (29)	0	2 (100)	1 (11)	8 (89)

Abbreviation: HBT, H_2 breath test.
The results for the age subgroup >12 years are discussed. The severity of coeliac disease was classified according to the Marsh system: type 1, 2, 3a, 3b or 3c.
[a]Coeliac disease was diagnosed in four subjects, aged 6 (type 3c), 7 (type 3b), 16 (type 3b) and 17 years (type 3a).
[b]Coeliac disease was diagnosed in one subject aged 12 years (type 3c) and giardiasis in two others aged 7 and 14 years.
[c]Coeliac disease was diagnosed in six subjects aged 3 (type 3b), 3 (type 3b), 10 (type 3b), 12 (type 3c), 13 (type 3b) and 17 years (type 3a).
[d]Secondary lactose malabsorption was developed because of a virus infection.
[e]Coeliac disease was diagnosed in one subject aged 4 years (type 3b).
[f]Adult-type hypolactasia-related symptoms were enhanced by intestinal infection.
[g]The exhaled H_2 levels were close to the cut-off level (17–19 parts per million) in two cases in group 1 and in three cases in group 2.

genotype. Among the patients with the C/C$_{-13910}$ genotype, 1 (20%) had type 3a, 3 (60%) had type 3b and 1 (20%) had type 3c coeliac disease. One C/T$_{-13910}$ genotyped patient had type 3a (14%), 4 had type 3b (57%) and 2 had type 3c (29%). Subjects with coeliac disease and negative HBT results were on a gluten-free diet.

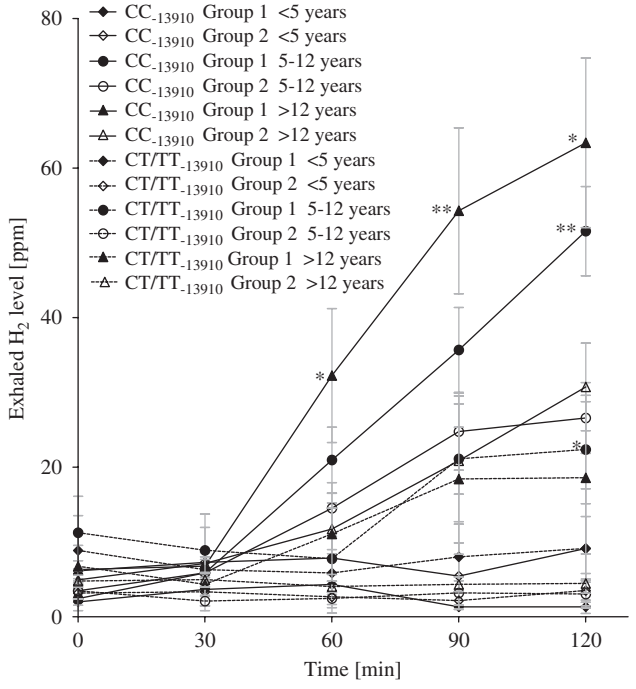

Figure 1 Means ± s.e.m. lactose H_2 breath test data at 0, 30, 60, 90 and 120 min after lactose intake for the genotypes in the patients (group 1, $n=82$) and the controls (group 2, $n=104$). Asterisks denote significant differences (*$P<0.05$; **$P<0.01$) between the group 1 and 2 data.

A significant difference in the mean exhaled H_2 level was measured between groups 1 and 2 (Figure 1). The 120-min sampling point of the HBT results proved to be the most reliable in comparison with the genotypes in groups 1 and 2, both in the 5–12-year (group 1: $P<0.01$, group 2: $P<0.001$) and in the >12-year subgroup (group 1: $P<0.001$, group 2: $P<0.001$). The positive and negative predictive values, and the sensitivity and specificity of the HBT at 120 min in groups 1 and 2, together with the results of previous studies, are presented in Table 2.

Five of the 33 sequenced samples showed different results from those of RFLP. The correct C/T$_{-13910}$ genotypes based on sequencing are presented in Table 1.

Discussion

The 37% incidence of ATH in the Hungarian population corresponds to the overall level in Europe (Sahi, 1994); it is rarely manifested at <5 years. The coincidence of the C/C$_{-13910}$ genotype with a positive HBT increases with age. The symptoms develop at around the age of 12 years; all those in group 1 aged >16 years had ATH-related symptoms, whereas in the control group the coincidence never reached 100%.

The C/C$_{-13910}$ genotype displayed a close association with a positive HBT in group 1, whereas the lactase-persistent genotypes correlated better with a negative HBT in group 2. The patients had significantly higher H_2 levels than the controls from 60 min (Figure 1), presumably as a result of a lactase deficiency enhancement, primarily because of additional diseases that do not exist in group 2.

Despite introducing sequencing as an independent genotyping method, we were not able to clarify the discrepancies between the genotypes and the HBT results, except in 5 cases. The genotyping error rate of the RFLP in our study is

Table 2 Evaluation of the HBT and genetic screening in our patients (group 1) and controls (group 2) and in previous studies

		Number of		*Sensitivity*	*Specificity*	*Positive PV*	*Negative PV*
		Children	*Adults*	*% (95% CI)*		*% (95% CI)*	
Our study[a]		186	—	77 (68–85)	94 (87–98)	94 (86–98)	78 (69–85)
Total		82	—	90 (79–96)	86 (65–97)	95 (85–99)	76 (55–91)
Group 1		104	—	58 (41–73)	97 (89–100)	92 (74–99)	78 (69–87)
Group 2							
Newcomer *et al.* (1975)[b]	United States	—	50	100	100	—	—
Arola *et al.* (1988)[b]	Finland	—	63	69	96	—	—
Högenauer *et al.* (2005)[c]	Austria	—	123	75	99	—	—
Büning *et al.* (2005)[c]	Germany	—	166	91.4	96.0	98.1	82.8
Schirru *et al.* (2007)[c]	Sardinia	—	84	100	96	98	100
Bernardes-Silva *et al.* (2007)[c]	Brazil	—	75	100	83	76	100
Krawczyk *et al.* (2008)[a]	Germany	—	58	100	95	88	100
Mottes *et al.* (2008)[c]	Italy	43		91	55	—	—

Abbreviations: CI, confidence interval; HBT, H_2 breath test; PV, predictive value.,
[a]HBT results were compared with genotyping.
[b]HBT results were compared with the lactase activity of intestinal biopsy samples.
[c]Genotyping results were compared with the HBT.

15%, which is concordant with previous results (Hosking *et al*., 2004; Hüebner *et al*., 2007).

The discrepancy between the C/C$_{-13910}$ genotype and the HBT results at >12 years may be explained by an abnormal colon bacterial metabolism or glucose metabolism, a slow intestinal transit or hypolactasia later in life.

The positive HBT results conflicting with the genotypes may be a consequence of accelerated intestinal transit, secondary lactose malabsorption because of inflammation, coeliac disease, giardiasis, bacterial or viral infections, a carrier status of a congenital lactase deficiency (Järvelä *et al*., 1998) or other genetic factors (Enattah *et al*., 2008). Nevertheless, we were not able to detect any other SNP responsible for the ATH-related symptoms in the close vicinity of the C/T$_{-13910}$ variant.

With regard to the sensitivity, specificity, and positive and negative predictive values of the HBT, our results correspond more or less to those in previous papers. The slight differences between our own and the previous data might be explained by the differences in the HBT methodology, the number, the nationality or the age of the subjects, and the analysis (Table 2).

The HBT has several disadvantages: it is time-consuming, a lactose intake may cause symptoms, physiological and pathological factors may influence the result and there can be a lack of distinction between primary and secondary lactose malabsorption. The DNA genotyping is exact, but provides no information on the manifestation of the symptoms after a lactose intake; it is suitable for the prediction of ATH even in childhood and for dietary intervention. We suggest a supplementary use of the two tests to achieve a correct diagnosis.

Acknowledgements

We thank Mrs Mária Radó and Mrs Gabriella Lehõcz for their skilled technical assistance, and Sándor Kocsubé and László Nagy of the Department of Microbiology, Faculty of Sciences, University of Szeged for their useful advice. This work was supported by grant OM-00050/2004 of the Hungarian National Research and Development Programs. The sampling was approved by the Ethical Committee of the Medical Faculty, and the subjects or their parents gave their informed consent.

References

Arola H, Koivula T, Jokela H, Jauhiainen M, Keyrilainen O, Hola T *et al*. (1988). Comparison of indirect diagnostic methods for hypolactasia. *Scand J Gastroenterol Suppl* **23**, 351–357.

Bernardes-Silva CF, Pereira AC, Fátima Alves da Mota G, Krieger JE, Laudanna AA (2007). Lactase persistence/non-persistence variants, C/T_13910 and G/A_22018, as a diagnostic tool for lactose intolerance in IBS patients. *Clin Chim Acta* **386**, 7–11.

Büning C, Genschel J, Jurga J, Fiedler T, Voderholzer W, Fiedler EM *et al*. (2005). Introducing genetic testing for adult-type hypolactasia. *Digestion* **71**, 245–250.

Enattah NS, Jensen TG, Nielsen M, Lewinski R, Kuokkanen M, Rasinperä H *et al*. (2008). Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *Am J Hum Genet* **82**, 57–72.

Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Järvelä I (2002). Identification of a variant associated with adult-type hypolactasia. *Nat Genet* **30**, 233–237.

Hosking L, Lumsden S, Lewis K, Yeo A, McCarthy L, Bansal A *et al*. (2004). Detection of genotyping errors by Hardy-Weinberg equilibrium testing. *Eur J Hum Genet* **12**, 395–399.

Högenauer C, Hammer HF, Mellitzer K, Renner W, Krejs GJ, Toplak H (2005). Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. *Eur J Gastroenterol Hepatol* **17**, 371–376.

Hüebner C, Petermann I, Browning BL, Shelling AN, Ferguson LR (2007). Triallelic single nucleotide polymorphisms and genotyping error in genetic epidemiology studies: MDR1 (ABCB1) G2677/T/A as an example. *Cancer Epidemiol Biomarkers Prev* **16**, 1185–1192.

Järvelä I, Enattah NS, Kokkonen J, Varilo T, Savilahti E, Peltonen L (1998). Assignment of the locus for congenital lactase deficiency to 2q21, in the vicinity of but separate from the lactase-phlorizin hydrolase gene. *Am J Genet* **63**, 1078–1085.

Krawczyk M, Wolska M, Schwartz S, Gruenhage F, Terjung B, Portincasa P *et al*. (2008). Concordance of genetic and breath tests for lactose intolerance in a tertiary referral centre. *J Gastrointestin Liver Dis* **17**, 135–139.

Lewinsky RH, Jensen TGK, Møller J, Stensballe A, Olsen J, Troelsen JT (2005). T$_{-13910}$ DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity *in vitro*. *Hum Mol Genet* **14**, 3945–3953.

Mottes M, Belpinati F, Milani M, Saccomandi D, Petrelli E, Calacoci M *et al*. (2008). Genetic testing for adult-type hypolactasia in Italian families. *Clin Chem Lab Med* **46**, 980–984. (abstract).

Newcomer AD, McGill DB, Thomas PJ, Hofman AF (1975). Prospective comparison of indirect methods for detecting lactase deficiency. *N Engl J Med* **293**, 1232–1236.

Oberhuber G (2000). Histopathology of celiac disease. *Biomed Pharmacother* **54**, 368–372.

Olds LC, Sibley E (2003). Lactase persistence DNA variant enhances lactase promoter activity *in vitro*: functional role as a cis regulatory element. *Hum Mol Genet* **12**, 2333–2340.

Rasinperä H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H *et al*. (2004). A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut* **53**, 1571–1576.

Sahi T (1994). Genetics and epidemiology of adult-type hypolactasia. *Scand J Gastroenterol Suppl* **202**, 7–20.

Schirru E, Corona V, Usai-Satta P, Scarpa M, Oppia F, Lorgia F *et al*. (2007). Genetic testing improves the diagnosis of adult type hypolactasia in the Mediterranean population of Sardinia. *Eur J Clin Nutr* **61**, 1220–1225.

Swallow DM (2003). Genetics of lactase persistence and lactose intolerance. *Annu Rev Genet* **37**, 197–219.

Walsh PS, Metzger DA, Higuchi R (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* **10**, 506–513.