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RAPID COMMUNICATION

Molecularly defined adult-type hypolactasia in school-aged children with a previous history of cow's milk allergy

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Abstract

AIM: To assess the role of lactase non-persistence/persistence in school-aged children and their milk-related symptoms.

METHODS: The genotypes for the C/T$_{-13910}$ variant associated with lactase non-persistence/ persistence were determined using PCR-minisequencing in a group of 172 children with a mean age of 8.6 years (SE = 0.02, 93 boys) participating in a follow-up study for cow's milk allergy. The parents were asked to assess their children's milk consumption and abdominal symptoms.

RESULTS: The presence of allergy to cow's milk was not associated with the C/C$_{-13910}$ genotype related with a decline of lactase enzyme activity during childhood (lactase non-persistence). The frequency of the C/C$_{-13910}$ genotype (16%) was similar to published figures for the prevalence of adult-type hypolactasia in Finland. The majority of the children (90%) in this series consumed milk but 26% of their families suspected that their children had milk-related symptoms. Forty-eight percent of the children with the C/C$_{-13910}$ genotype did not drink milk at all or consumed a low lactose containing diet prior to the genotyping ($P < 0.004$ when compared to the other genotypes).

CONCLUSION: Analysis of the C/T$_{-13910}$ polymorphism is an easy and reliable method for excluding adult-type hypolactasia in children with milk-related symptoms. Genotyping for this variant can be used to advise diets for children with a previous history of cow's milk allergy.

Key words: Adult-type hypolactasia; Primary lactose malabsorption; Genetic testing; Cow's milk allergy

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INTRODUCTION

Lactase deficiency (LD, lactase non-persistence) is the most common cause of milk intolerance in children and adolescents world-wide[1]. It plays a significant role in recurrent abdominal pain in populations with common consumption dairy products[2]. Immunologically mediated adverse reaction to ingested proteins of cow's milk is referred to as cow's milk allergy (CMA), the symptoms of which usually appear under one year of age[3]. CMA affects 1.9%-3.2% of infants[4] though recent studies suggest that allergy to cow's milk protein may have an impact on abdominal symptoms at schoolage also[5]. CMA is easily differentiated from LD if it manifests at an early age with skin and/or respiratory symptoms[6]. In cases of CMA that present with gastrointestinal symptoms, the clinical picture may overlap with symptoms caused by low lactase activity especially at schoolage, when the downregulation of lactase enzyme activity occurs[7].

The differential diagnosis of milk-related symptoms in children is difficult to establish because of the variability of clinical symptoms and inaccurate diagnostic laboratory tests[8]. The diagnosis of lactose malabsorption is based on the measurement of disaccharidase activities in intestinal biopsy specimens, a method which is not suitable for every day clinical practice. The indirect commonly used lactose-tolerance test (LTT) is not reliable in children and results in up to 30% of false positive results, thus reducing its value in clinical use[9]. In addition, children with normal lactose digestion complain of symptoms during LTT[10].

Recently, a C to T single nucleotide polymorphism residing 13 910 base pairs upstream of the lactase-phlorizin

hydrolase (LCT) gene has been shown to associate with lactase persistence/non-persistence, the C/C-13910 genotype defining lactase non-persistence as well as C/T-13910 and T/T-13910 genotype lactase persistence[11]. The association of the C/T-13910 variant with disaccharidase activities and lactase/sucrase ratio (L/S) has been verified in a total of >600 intestinal biopsy specimens[7, 11, 12]. In Finnish children (8-20 years of age) the mean level of lactase activity among subjects with the C/C-13910 genotype is 6.5 U/g protein. The C/T-13910 genotype is 29.9 U/g protein and 50.0 U/g protein respectively, showing a trimodal distribution of the lactase activity[7]. Functional evidence for the C/T-13910 variant in regulation of lactase activity has been obtained in several studies[12-15] and a greater increase in LCT promoter activity was reported for the T-13910 variant[13, 14]. The down-regulation of intestinal lactase varies according to ethnicity but the differences at the timing of down-regulation are not marked and most commonly start to appear around 5-6 years of age[7]. At the age of 12 years, all children with the C/C-13910 genotype have low lactase activity in their intestines[7].

The aim of the present study was to evaluate the role of lactase non-persistence in milk consumption and milk-related clinical symptoms by analysing the C/T-13910 genotypes of lactase persistence/non-persistence in a group of 172 school-aged children from eight to nine years of age with or without a previous history of CMA.

MATERIALS AND METHODS

Subjects

This study was part of a prospective follow-up study of children with a history of CMA diagnosed at a mean age of seven months [16]. The present study group comprised 172 school-aged children (mean age 8.6 years; SE = 0.02, 93 boys, 79 girls) who were clinically examined during August 2003-March 2004 at the Helsinki University Central Hospital, Helsinki, Finland. Ninety-three children (54%) had a previous diagnosis of CMA and 79 children comprised the control group. All these children were subjects in the study by Saarinen and collaborators[17], in which 6209 unselected infants born between August 1994 and November 1995 in the Helsinki region were followed up from birth for the development of CMA. The presence of CMA was confirmed by a challenge test[17]. At the time of the present visit, the families were asked about the children's milk consumption and possible milk-related symptoms and those agreeing to participate in genetic testing of adult-type hypolactasia were included in the study. IgE-mediated hypersensitivity to cow's milk was measured by skin prick test. A diameter >3 mm exceeding the negative control was considered as a positive response[18]. Those children who were still avoiding milk due to previous CMA were re-challenged by cow's milk[16]. A supplementary questionnaire on the amount of milk consumed and possible abdominal symptoms during the preceding week was mailed later. Celiac disease was screened as previously described[19].

Ethics

The study was approved by the Ethical Committee of the Hospital for Children and Adolescents, University of Helsinki. The families/children gave their informed consent.

Genotyping

DNA was isolated from blood by phenol-chloroform extraction as previously described[20] and DNA fragments spanning the C/T-13910 variant were amplified using one biotinylated primer and one unbiotinylated primer (primer sequences available on request). Briefly, PCR amplifications were carried out in a 50-μL volume with genomic DNA (100 ng), primers (20 ng), dNTPs (200 μmol/L), and 0.5 U of Taq polymerase in a standard buffer (Dynazyme, Finnzymes, Espoo, Finland). The PCR cycle conditions were as follows: an initial round of denaturation at 94 ℃, then 35 cycles at 94 ℃ for 30 s, at 53 ℃ for 30 s, at 72 ℃ for 1.25 min and a final extension at 72 ℃ for 10 min. Ten μl of the PCR product was captured in a streptavidin-coated microtiter well (Thermo Electron, Vantaa, Finland) and two parallel minisequencing reactions were carried out for each PCR-product. The minisequencing reaction contained 10 pmoles of the minisequencing primer (primer sequence available on request), 0.1 μL of tritium-labelled dNTP (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK), and 0.05 U of DNA polymerase (Dynazyme, Finnzymes, Espoo, Finland). The microtiter wells were incubated for 15 min at 56 ℃ and finally the wells were washed. The detection primer was eluted and the eluted radioactivity was measured in a liquid scintillation counter (Rackbeta 1209, Wallac, Finland) as previously described [21].

Statistical analysis

The Mann-Whitney *U* test and Spearman's rank correlation test were used for nonparametric comparisons. Fisher's exact 2-sided test was also used.

RESULTS

Twenty-six percent of the families (45/172) suspected that their children had milk-related symptoms at 8-9 years of age at the time of this study. A significantly greater proportion of these came from those diagnosed to have CMA (76%, 34/45) at a mean age of seven months[17] than those serving as controls ($P < 0.003$).

Skin-prick test with cow's milk was positive (> 3 mm) in 12% of the children undergoing these tests (20/168) and all these children had a previous history of CMA (Table 1). At the time of this study, the challenge test with cow's milk was positive in 11 of the 16-challenged children[16].

The frequency of C/C-13910 genotype defining adult-type hypolactasia was 16% in the total study group of 172 children. There was no correlation with the genotype and positive reaction in the skin prick test or food challenge. Of the 27 children with the C/C-13910 genotype, two were challenged with cow's milk at the time of this study and one of them had a positive challenge for cow's milk. The milk consumption of different genotypes is presented in Table 2. Screening for celiac disease was negative in 170/172 of the children. The two children with a positive screening test consumed milk and reported no abdominal symptoms. The genotypes in these two children were C/

Table 1 Milk consumption and abdominal symptoms in school-aged children with a history of cow's milk allergy[1] and their controls

	Previous allergy[1]	Controls
Questionnaire completed	92% (n=86)	97% (n=77)
Milk product consumption	80% (n=69)	100%[a] (n=77)
Milk consumption < 1 dL/d	36% (23/69)	16%[b] (12/77)
Low lactose content in diet	14% (10/69)	8% (6/77)
Abdominal symptoms: Flatulence	13% (11/86)	8% (6/77)
Loose stools	5% (4/86)	4% (3/77)
Skin prick test >3 mm to cow's milk	22%[2] (20/91)	0 (0/77)

[1]milk challenge positive at a mean age of 7 mo; [a]P < 0.0001 *vs* milk consumption; [b]P < 0.02 *vs* milk product consumption.

Table 2 Milk consumption and abdominal symptoms in school-aged children genotyped for adult-type hypolactasia

	T/T1-13910	C/T[1]-13910	C/C[2]-13910
Total number of children			
n=172	n=68	n=77	n=27
Milk products consumed	95%	95%	93%
(n=163)	(54/62)	(67/74)	(25/27)
Drinks milk < 1 dL/day	22%	18%	44%[a]
(n=146)	(12/54)	(12/67)	(11/25)
Low lactose content in the diet	7%	4%	36%[b]
(n=146)	(4/54)	(3/67)	(9/25)
Skin prick test >3 mm to cow's milk	13%[5]	11%[5]	12%[3]
(n=168)	(9/67)	(8/75)	(3/26)

[1]lactase persistence; [2]defines adult-type hypolactasia; [a]P < 0.02 *vs* genotypes associated with lactase persistence (T/T-13910 plus C/T-13910); [b]P < 0.002 *vs* T/T-13910 genotype or C/T-13910 genotype; [3]each of these prick positive children had a diagnosis of cow's milk allergy at a mean age of seven mo[17].

Table 3 Abdominal symptoms during a one-week period in school-aged children genotyped for adult-type hypolactasia

	Total	T/T[1]-13910	C/T[1]-13910	C/C[2]-13910
Questionnaire completed	n=163	n=62	n=74	n=27
Abdominal symptoms	(n=38)	15% (n=9)	27% (n=20)	33%[a] (n=9)
Flatulence	(n=17)	5% (n=3)	12% (n=9)	19%[b] (n=5)
Periumbilical pain	(n=10)	6.5% (n=4)	5.4% (n=4)	7.4% (n=2)
Loose stools	(n=7)	3.2% (n=2)	5.4% (n=4)	3.7% (n=1)
Upper epigastric pain	(n=7)	5% (n=3)	4.0% (n=3)	3.7% (n=1)
Constipation	(n=5)	0	5.4% (n=4)	3.7% (n=1)
Dyspepsia	(n=1)	0	1.4% (n=1)	0

[1]lactase persistence; [2] defines adult-type hypolactasia; [a]P=0.05, [b]P<0.04 *vs* T/T-13910 genotype.

Table 4 Probable changes in milk consumption of families after receiving the results of genetic testing for adult-type hypolactasia in school-aged children

	T/T[1]-13910	C/T[1]-13910	C/C[2]-13910
Questionnaire completed	94% (62/68)	97% (74/77)	100% (27)
No change in milk consumption	89% (n=54)	81% (n=61)	59%[3] (n=16)
Less lactose containing milk	0	0	33% (n=9)
More lactose containing milk	8% (n=5)	11% (n=8)	0
Not decided	5% (n=3)	7% (n=5)	7% (n=2)

[1]lactase persistence; [2]defines adult-type hypolactasia; [3]33% of the families consumed low lactose containing diet prior to genetic testing.

T-13910 and T/T-13910 (data not shown).

Fifty-two children out of the total study group of 172 children drank less than one dL of milk per day or did not consume milk products. The C/C-13910 genotype defining adult-type hypolactasia was present in 25% (13/52) and CMA in 21% (11/52, confirmed in a challenge test) of these children. Each child with CMA had a previous diagnosis of CMA. Milk consumption did not have any significant effect on body mass index (BMI, kg/m^2) and BMI was similar in children with different genotypes for adult-type hypolactasia (data not shown).

The questionnaire on daily milk consumption and abdominal symptoms was filled in by 95% (163/172) of the families (Table 3). Adult-type hypolactasia was suspected in 9% (15/163) of the children by their parents but was confirmed by genotyping in not more than 20% of these children (3/15 with the C/C-13910 genotype). One of these three children with the C/C-13910 genotype had a diagnosis of lactose malabsorption based on intestinal biopsy at the age of 7 years and the other two were noticed to have lactose-related symptoms at the age of 1.5 and 7 years. Two of the 27 children with the C/C-13910 genotype did not consume milk because of CMA. Eighty percent of the children (20/25) with the C/C-13910 genotype who had no CMA at this age had a low lactose containing diet at home or drank quantities of milk less than one dL/day already prior to the genetic testing (Table 2). A similar diet with milk restriction was practiced more rarely among children with genotypes associated with lactase persistence (31/119 of the children with C/T-13910 or T/T-13910 genotypes who answered the question and did not have allergy to milk, P< 0.004). Children with the C/C-13910 genotype did not report significantly more abdominal pains than children with the C/T-13910 or T/T-13910 genotypes but there was a difference in the presence of flatulence (P< 0.04, Table 3). Among the children with the C/C-13910 genotype, 4/15 children had milk restriction beyond two years of age because of milk allergy. Two of these four children experienced lactose-related symptoms.

The acceptance of genetic testing was good, as only two families did not participate in the study. Based on the interviews of the families among the children with the C/C-13910 genotype, 30% of the families reported to have another family member with symptoms of adult-type hypolactasia. Thirteen percent of the families of the total series and one third of the children with the C/C-13910 genotype reported that the result of the genetic test for adult-type hypolactasia had a probable effect on their milk consumption (Table 4). Of the families with genotypes associating with lactase persistence (C/T-13910 or the T/T-13910 genotype), 10% reported that the result of the genetic test was helpful in avoiding unnecessary restrictions on milk consumption.

DISCUSSION

Our results show that the overlap of IgE-mediated cow's milk allergy, CMA, and lactase non-persistence is unlikely

to occur at schoolage. The prevalence of adult-type hypolactasia in our study was 16% corresponding to the reported frequency in our population[7,11,22,23] and was not associated with CMA but correlated with the consumption of low lactose containing diets and the presence of flatulence. All children with a positive skin prick test for cow's milk at this age (12%) had a previous diagnosis of CMA, settled at a mean age of seven months as previously reported in detail[16]. It is noteworthy that not a single child in the non-CMA group turned IgE-positive for milk at schoolage, further confirming that IgE-mediated reactions with cow's milk develops at an early age. Tolerance to cow's milk develops in the majority of children with CMA (70%) by the age of three and eight to nine years. At the time of the present study, 85% of the children recovered from CMA[16].

It was reported that one third of the Finnish children at the age of eight years with the genotype C/C$_{-13910}$ have a high intestinal lactase activity[7] and are unlikely to develop symptoms caused by adult-type hypolactasia. It should however be borne in mind that in children the decline of intestinal lactase occurs slowly and depends on ethnicity [7]. Based on our previous study about the timing of down-regulation of lactase activity[7], we can estimate that about 10% (0.63 × 16%) of the children being studied have a reduced lactase activity (<10 U/g/protein) and all the others have a high lactase activity (>10 U/g/protein). In the present study, 60% of the children with the C/C$_{-13910}$ genotype consumed a low lactose containing diet or had milk-related symptoms at this age and 40% of those with the C/C$_{-13910}$ genotype were considered milk-tolerant. The avoidance of milk and consumption of low lactose containing diet was much more common among children with the C/C$_{-13910}$ genotype than among those with the genotypes associated with lactase persistence. This confirms our preliminary finding[7] and suggests that the children have experienced milk-related symptoms triggering a reduced lactose intake.

It is common, however, that children with abdominal dysfunction after consuming milk are unaware of its cause[24, 25]. This was also obvious in our series as the suspicion of milk-related symptoms was not increased in the families of the children with the C/C$_{-13910}$ genotype defining adult-type hypolactasia. However, restricted lactose intake was more common in these families. Although this dietary modification, the children with the C/C$_{-13910}$ genotype reported more flatulence when compared to those with the genotype T/T$_{13910}$ associated with lactase persistence ($P < 0.04$). There was no difference in the frequency of abdominal pain between the children with either CMA or lactose malabsorption and the control children.

It is common that only some individuals who self-report them as lactose intolerants are in fact lactose maldigesters when tested objectively[26-30]. The data on children, however, are limited. In our series, every fifth child suspected not to tolerate lactose was confirmed to have the predisposing genotype for adult-type hypolactasia. The lack of awareness of lactose maldigestion may increase abdominal symptoms in children as reported by Webster *et al*[31] who noticed that after a proper diagnosis of lactose malabsorption, the avoidance of milk products is more rigorous and results in a decrease in complaints. It is unclear whether individuals with adult-type hypolactasia and lactose maldigestion but without obvious symptoms of lactose intolerance should avoid milk or not. A recent double-blind study suggested that lactose maldigesters might benefit from lactose avoidance even though the diagnostic criteria for lactose intolerance are not fulfilled[32]. On the other hand, studies in adults reported that as many as 32% of lactose mal-absorbers experience no symptoms from lactose containing milk products[26], but at present there are no means to predict this at an individual level.

In the present study, 10% of the children with the C/T$_{-13910}$ genotype reported symptoms such as flatulence and loose stools suggestive of milk intolerance. One of these children had CMA (milk challenge positive) but in the other cases the causes for these symptoms were unknown. It is possible that some of these children are carriers of a mutation of congenital lactase deficiency (CLD), a rare congenital disorder which may result in low lactase activity but no disease manifestations in heterozygotes[33, 34]. Celiac disease may cause secondary hypolactasia but screening tests for the disease are negative in these children.

Parents easily suspect milk as a causative agent for abdominal symptoms in countries where dairy products are widely used. In the present study, 26% of the families reported a suspicion of milk-related symptoms in their school-aged children. The majority of these children did not drink milk nor had low lactose containing diets. When milk-related symptoms were suspected, the parents recognized CMA easily due to the previous history of CMA but their suspicion of adult-type hypolactasia was seldom confirmed. Accurate diagnosis of possible milk-related symptoms poses a challenge for clinicians as the elimination of milk and a proper challenge test for milk proteins or tolerance test for lactose are both time-consuming and may give unsatisfactory results[8]. The genetic testing of adult-type hypolactasia performed from a drop of blood was accepted well by the families as 99% of the families agreed to test their children. About 10% of the families reported that a negative result for adult-type hypolactasia was helpful as they may now avoid unnecessary restrictions on milk consumption.

In conclusion, a suspicion of milk-related symptoms is common in everyday clinical practice. The genetic test of C/T$_{-13910}$ polymorphism is reliable in excluding adult-type hypolactasia in children with milk-related symptoms. Genotyping for this variant may help in planning the diet for children with a suspicion of milk-related symptoms.

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