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

Heli Rasinperä, Kristiina Saarinen, Anna Pelkonen, Irma Järvelä, Erkki Savilahti, Kaija-Leena Kolho

Heli Rasinperä, Irma Järvelä, Department of Medical Genetics, University of Helsinki, Finland
Kristiina Saarinen, Erkki Savilahti, Kaija-Leena Kolho, Hospital for Children and Adolescents, University of Helsinki, Finland
Anna Pelkonen, Department of Allergology, Skin and Allergy Hospital, Helsinki University Central Hospital, Helsinki, Finland
Irma Järvelä, Laboratory of Molecular Genetics, Helsinki University Hospital, Helsinki, Finland
Kaija-Leena Kolho, Hospital for Children and Adolescents, University of Helsinki, Finland; The Helsinki University Science Foundation, Helsinki, Finland; The Helsinki University Hospital Research Funding, Helsinki, Finland; The Helsinki University Science Foundation, Helsinki, Finland and The Foundation of Nutrition Research, Helsinki, Finland

Correspondence to: Dr. Kaija-Leena Kolho, Hospital for Children and Adolescents, Box 281, 00290 Helsinki, Finland. kaija-leena.kolho@helsinki.fi
Telephone: +358-9-47174787 Fax: +358-9-47175299
Received: 2005-09-15 Accepted: 2005-10-26

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

Rasinperä H, Saarinen K, Pelkonen A, Järvelä I, Savilahti E, Kolho KL. Molecularly defined adult-type hypolactasia in school-aged children with a previous history of cow’s milk allergy. World J Gastroenterol 2006; 12(14): 2264-2268

http://www.wjgnet.com/1007-9327/12/2264.asp

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 school age 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 school age, 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 13910 base pairs upstream of the lactase-phlorizin
The C/T-1910 variant with disaccharidase activities and lactase/sucrase ratio (L/S) has been verified in a total of >600 intestinal biopsy specimens. In Finnish children (8-20 years of age) the mean level of lactase activity among subjects with the C/C-1910 genotype is 6.5 U/g protein. The C/T-1910 genotype is 29.9 U/g protein and 50.0 U/g protein respectively, showing a trimodal distribution of the lactase activity. Functional evidence for the C/T-1910 variant in regulation of lactase activity has been obtained in several studies and a greater increase in LCT promoter activity was reported for the T-1910 variant. 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. At the age of 12 years, all children with the C/C-1910 genotype have low lactase activity in their intestines.

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-1910 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. 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, 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. 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. Those children who were still avoiding milk due to previous CMA were re-challenged by cow’s milk. 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.

**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 and DNA fragments spanning the C/T-1910 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 °C, then 35 cycles at 94 °C for 30 s, at 53 °C for 30 s, at 72 °C for 1.25 min and a final extension at 72 °C 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-producer. The minisequencing reaction contained 10 pmole 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 °C and finally the wells were washed. The detection primer was eluted and the eluted radioactivity was measured in a liquid scintillation counter (Wallac Beta 1209, Wallac, Finland) as previously described.

**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 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 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.

The frequency of C/C-1910 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-1910 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/C-1910 and T/T-1910.
To 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/m²) 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

---

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

| Previous allergy | Controls |
|------------------|----------|
| Questionnaire completed | 92% (n = 86) | 97% (n = 77) |
| Milk product consumption | 80% (n = 69) | 100% (n = 77) |
| Milk consumption < 1 dL/d | 36% (23/69) | 16% (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% (20/91) | 0 (0/77) |

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

---

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

| T/T-13910 | C/T-13910 | C/C-13910 |
|-----------|-----------|-----------|
| Total number of children | n = 172 | n = 68 | n = 27 |
| Milk products consumed | 95% | 95% | 93% |
| (n = 163) | (54/62) | (67/74) | (25/27) |
| Drinks milk < 1 dL/day | 22% | 18% | 44% |
| (n = 146) | (12/14) | (12/67) | (11/25) |
| Low lactose content in the diet | 7% | 4% | 36% |
| (n = 116) | (4/54) | (3/67) | (8/25) |
| Skin prick test > 3 mm to cow’s milk | 13% | 11% | 12% |
| (n = 108) | (9/76) | (8/75) | (8/76) |

1 lactase persistence; † defines adult-type hypolactasia; ‡P < 0.02 vs genotypes associated with lactase persistence (T/T-13910 plus C/T-13910); † †P < 0.02 vs T/T-13910 genotype or C/C-13910 genotype; each of these prick positive children had a diagnosis of cow’s milk allergy at a mean age of seven mo.

---

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

| Total | T/T-13910 | C/T-13910 | C/C-13910 |
|-------|-----------|-----------|-----------|
| Questionnaire completed | n = 163 | n = 62 | n = 71 | n = 27 |
| Abdominal symptoms | n = 38 | n = 15 | n = 27 | n = 27 |
| Flatulence | n = 17 | 5% (n = 3) | 12% (n = 9) | 19% (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 = 10 | 5% (n = 3) | 4.0% (n = 3) | 3.7% (n = 1) |
| Constipation | n = 5 | 5% (n = 4) | 3.7% (n = 1) | 0 |
| Dyspepsia | n = 1 | 1.4% (n = 1) | 0 |

1 lactase persistence; † defines adult-type hypolactasia; ‡P < 0.05, † †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**

| Total | T/T-13910 | C/T-13910 | C/C-13910 |
|-------|-----------|-----------|-----------|
| Questionnaire completed | 94% (62/68) | 97% (74/77) | 100% (27) |
| No change in milk consumption | 89% (n = 54) | 81% (n = 61) | 59% (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; † defines adult-type hypolactasia; ‡33% of the families consumed low lactose containing diet prior to genetic testing.
to occur at schoolage. The prevalence of adult-type hypolactasia in our study was 16% corresponding to the reported frequency in our population \(^{[11,22,23]}\) 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 \(^{[10]}\). 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 \(^{[10]}\).

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% \((0.63 \times 16\%)\) of the children being studied have a reduced lactase activity \(<10\ \text{U/g protein}\) and all the others have a high lactase activity \((>10\ \text{U/g protein})\). 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 \(^{[1]}\) 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 malabsorbers 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 malabsorption may increase abdominal symptoms in children as reported by Webster \(_{13}\) \(^{[13]}\) 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 malabsorption but without obvious symptoms of lactose intolerance should avoid milk or not. A recent double-blind study suggested that lactose malabsorbers might benefit from lactose avoidance even though the diagnostic criteria for lactose intolerance are not fulfilled \(^{[22]}\). 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 \(^{[23,34]}\). 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.

ACKNOWLEDGMENTS

The authors are grateful to the children and their families for their participation in this study. The authors thank Eija Hämäläinen, Hanna Komu and Sirkku Kristiansen for excellent help in performing the laboratory work.

REFERENCES

1. Semenza G, Auricchio S, Mantei N. Small-intestinal disaccharidases. In: Scriver CR, Beaudet AL, Sly D, Valle D, eds. The metabolic and molecular basis of inherited disease, New York: McGraw-Hill, 2001; 1623-1650
The clinical significance of disaccharide malabsorption.

1. Gudmand-Hoyer E. The clinical significance of disaccharide malabsorption. *Am J Clin Nutr* 1994; 59: 7355-7415

2. Sampson HA. Food allergy. *JAMA* 1997; 278: 1888-1894

3. Sicherer SH. Food allergy. *Lancet* 2002; 360: 701-710

4. Kokkonen J, Haapalaiti M, Laurila K, Karttunen TJ, Maki M. Cow's milk protein-sensitive enteropathy at school age. *J Pediatr* 2001; 139: 797-803

5. Bahn SL. Cow's milk allergy versus cow milk intolerance. *Ann Allergy Asthma Immunol* 2002; 89: 56-60

6. Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela I, Kolho KL. A genetic test which can be used to diagnose adult-type hypoplasctasia in children. *Cyt* 2004; 53: 1573-1576

7. Arola H. Diagnosis of hypoplasctasia and lactose malabsorption. *Scand J Gastroenterol Suppl.* 1994; 202: 26-35

8. Krasilnikoff PA, Gudman-Hoyer E, Moltke HH. Diagnostic value of disaccharide tolerance tests in children. *Acta Paediatr Scand* 1975; 64: 693-698

9. Lebenthal E, Rossi TM, Nord KS, Branski D. Recurrent abdominal pain and lactose absorption in children. *Pediatrics* 1981; 67: 828-832

10. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypoplasctasia. *Nat Genet* 2002; 30: 233-237

11. Kuokkanen M, Enattah NS, Oksanen A, Savilahti E, Orpana A, Jarvela I. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypoplasctasia. *Gen* 2003; 52: 647-652

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

13. Troelsen JT, Olsen J, Moller J, Sjostrom H. An upstream polymorphism associated with lactase persistence has increased enhancer activity. *Gastroenterology* 2003; 125: 1866-1894

14. Arika T, Amemiya K, Nomoto K. Combination therapy of radiation and Sizofiran (SPG) on the tumor growth and metastasis on squamous-cell carcinoma NR-S1 in syngeneic C3H/He mice. *Biotherapy* 1992; 4: 165-170

15. Saarinen KM, Pelkonen AS, Makela MJ, Savilahti E. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. *J Allergy Clin Immunol* 2005; 116: 869-875

16. Saarinen KM, Juntunen-Backman K, Jarvenpaa AL, Kuitunen A, Lope L, Renlund M, Siivola M, Savilahti E. Supplementary feeding in maternity hospitals and the risk of cow's milk allergy: A prospective study of 6209 infants. *J Allergy Clin Immunol* 1999; 104: 457-461

17. Saarinen KM, Savilahti E. Infant feeding patterns affect the subsequent immunological features in cow's milk allergy. *Clin Exp Allergy* 2000; 30: 400-406

18. Sulkkanen S, Halltunen T, Laurila K, Kolho KL, Korponay-Szabo IR, Sarnesto A, Savilahti E, Collin P, Maki M. Tissue transglutaminase autoantibody enzyme-linked immunosorbent assay in detecting celiac disease. *Gastroenterology* 1998; 115: 1322-1328

19. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

20. Syvanen AC, Landegren U. Detection of point mutations by solid-phase methods. *Hum Mutat* 1994; 3: 172-179

21. Enattah NS, Forsblom C, Rasinpera H, Tuomi T, Groop PH, Jarvela I. The genetic variant of lactase persistence C(-13910)T as a risk factor for type I and II diabetes in the Finnish population. *Eur J Clin Nutr* 2004; 58: 1319-1322

22. Rasinpera H, Forsblom C, Enattah NS, Halonen P, Salo K, Victorzon M, Mecklin JP, Jarvinen H, Enholm S, Sellick G, Alazzouzi H, Houlston R, Robinson J, Groop PH, Tomlinson I, Schwartz S Jr, Aaltonen LA, Jarvela I. The C/C-13910 genotype of adult-type hypoplasctasia is associated with an increased risk of colorectal cancer in the Finnish population. *Cyt* 2005; 54: 643-647

23. Barr RG, Levine MD, Watkins JB. Recurrent abdominal pain of childhood due to lactose intolerance. *N Engl J Med* 1979; 300: 1449-1452

24. Scrimshaw NS, Murray EB. The acceptability of milk and milk products in populations with a high prevalence of lactose intolerance. *Am J Clin Nutr* 1988; 48: 1079-1159

25. Carroccio A, Montalto G, Cavena G, Notarbatolo A. Lactose intolerance and self-reported milk intolerance: relationship with lactose malabsorption and nutrient intake. *Lactase Deficiency Study Group. J Am Coll Nutr* 1998; 17: 631-636

26. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics--compensation for lactase insufficiency. *Am J Clin Nutr* 2001; 73: 421S-429S

27. Suarez FL, Savaiiano DA, Levitt MD. A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose insufficiency. *BG2FO4 to treat lactose intolerance. Am J Clin Nutr* 1999; 69: 140-146

28. Vesa TH, Seppo LM, Marteau PR, Sahi T, Korpela R: Role of irritable bowel syndrome in subjective lactose intolerance. *Am J Clin Nutr* 1998; 67: 710-715

29. Webster RB, DiPalma JA, Gremse DA. Lactose malabsorption and recurrent abdominal pain in children. *Dig Dis Sci* 1995; 40: 1506-1510

30. Gremse DA, Creer AS, Vacik J, DiPalma JA. Abdominal pain associated with lactose ingestion in children with lactose intolerance. *Clin Pediatr (Phila)* 2003; 42: 341-345

31. Savilahti E, Launila K, Kuokkanen J. Congenital lactase deficiency. A clinical study on 16 patients. *Arch Dis Child* 1983; 58: 246-252

32. Jarvela I, Enattah NS, Kokkonen J, Varilo T, Savilahti E, Peltonen L. Assignment of the locus for congenital lactase deficiency to 2q21, in the vicinity of but separate from the lactase-phlorizin hydrolase gene. *Am J Hum Genet* 1998; 63: 1079-1085

S- Editor Wang J  L- Editor Wang XL  E- Editor Cao L