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RAPID COMMUNICATION

Lactase non-persistence and milk consumption in Estonia

Margus Lember, Suvi Torniainen, Mart Kull, Riina Kallikorm, Peeter Saadla, Tarvo Rajasalu, Hanna Komu, Irma Järvelä

Margus Lember, Mart Kull, Riina Kallikorm, Peeter Saadla, Tarvo Rajasalu, Department of Internal Medicine, University of Tartu, Tartu, Estonia
Suvi Torniainen, Hanna Komu, Irma Järvelä, Department of Medical Genetics, University of Helsinki, Helsinki, Finland
Irma Järvelä, Laboratory of Molecular genetics, Helsinki University Central Hospital, Helsinki, Finland
Supported by the Estonian Science Foundation grant No. 6452, Sigrid Jusélius Foundation, Helsinki, Finland and Helsinki University Hospital Research Funding, University of Helsinki, Finland
Correspondence to: Margus Lember, Department of Internal Medicine, University of Tartu, Estonia. margus.lember@kliinikum.ee
Telephone: +372-7-318600
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Abstract

AIM: To define the frequency of the C/T-13910 variant associated with lactase persistence/non-persistence trait and to analyze the milk consumption of lactase non-persistent subjects in Estonia.

METHODS: We genotyped 355 Estonians by polymerase chain reaction and direct sequencing. Milk consumption was analyzed by a questionnaire, specially developed to analyze milk consumption and abdominal complaints.

RESULTS: The frequency of the genotype of the C/C-13910 (lactase non-persistence) was found to be 24.8% in native Estonians. No other single nucleotide polymorphisms covering the region of 400 bp adjacent to the C/T-13910 variant were found. Lactase non-persistence subjects were found to consume less milk than lactase persistence subjects.

CONCLUSION: The frequency of lactase non-persistence defined by the C/C-13910 genotype confirms the results of the previous studies based on indirect methods of determining hypolactasia. Milk consumption of lactase non-persistence subjects is consistent with previously reported figures of adult-type hypolactasia in Estonia. However, lactase non-persistence does not prevent the intake of milk in many adults.

Key words: Lactase persistence; Milk; Estonia

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INTRODUCTION

Lactase enzyme hydrolyses lactose to glucose and galactose facilitating their absorption through the gut wall. Adult-type hypolactasia (lactase non-persistence, primary lactose malabsorption) is the most common enzyme deficiency in the world[1]. Clinically, hypolactasia is the main reason of milk intolerance in adults. Individuals with hypolactasia may develop symptoms of abdominal pain, borborygmi, flatulence and diarrhea if they drink milk[1,2]. The symptoms depend on the age of the subject. It has been reported that development of symptoms depends on the quantity of lactose in the diet and individual sensitivity. Individuals with hypolactasia can tolerate moderate quantities of milk without symptoms[3-5]. Many persons but not all with this condition avoid consuming large quantities of milk.

Normally, lactase activity declines after the weaning. However, a mutation has occurred in human history that maintains lactase activity high throughout life[1]. Specifically, a single nucleotide polymorphism (SNP) C/T (rs4988234) residing 13 910 bp upstream from the initiation codon of the lactase gene (LCT) has been shown to be associated with lactase persistence trait in Asian, European and Northern African populations[6-9]. Genotype CC of the C/T-13910 variant defines hypolactasia (lactase activity under 10 U/g per protein) and CT and TT genotypes' lactase persistence (lactase activity over 10 U/g per protein)[10-12]. More recent functional studies have shown that the C/T-13910 variant is associated with the regulation of the LCT gene at transcriptional level[10,11]. It has been shown that the T-13910 allele enhances LCT promoter activity 4 times more than the C-13910 allele[13,14]. A nuclear binding factor OCT-1 has recently been found to bind to the C/T-13910 region, more efficiently to the T- allele than to the C- allele. However, it seems that some other transcription factors are also needed for complete enhancer activity[15]. The excellent correlation of the C/T-13910 variant to the lactase activity[11] has made it a robust marker for routine clinical diagnostics. By using this SNP, hypolactasia can be diagnosed more easily and accurately than by other tests where environmental factors may interfere with and have

an impact on the results[16].

The prevalence of adult-type hypolactasia differs between ethnic groups. In Finland, the prevalence of adult-type hypolactasia is found to be 18% and in Sweden 10%[6,17]. Previously, the reported prevalence of adult-type hypolactasia in Estonians varies from 23% to 32%[1,18-20] and is 57% among Russians living in Estonia[20].

The aim of this study was to figure out the prevalence of adult-type hypolactasia in Estonians by genotyping of the C/T-13910 polymorphism and to find out if there are differences in milk consumption and intolerance in individuals with different genotypes of the C/T-13910 variant. We also studied if other SNPs exist adjacent to the C/T-13910 variant.

MATERIALS AND METHODS

Study population

The study was carried out in Väike-Maarja, Estonia. A random sample (n = 434) of the population aged 25-70 years was drawn, the sample corresponded to the age and gender structure of the general population in Estonia. An informed consent was obtained from the subjects who participated in the study. The study was approved by the Ethics Committee of the Tartu University. Three hundred and sixty-six persons participated in the study (response rate 84%) and gave a blood sample, fulfilled a questionnaire about their health and personal data, including nationality of their grandparents, symptoms of milk intolerance and milk consumption habits. There were 167 males and 199 females, and their average age was 48.9 years. A blood sample was drawn for DNA analysis from each participant. DNA was isolated using the standard procedure. In statistical analysis chi-square test was used.

Genotyping

The DNA fragment spanning the C/T-13910 variant was amplified by polymerase chain reaction (PCR) and analyzed by direct sequencing. The total volume of PCR was 50 μL containing genomic DNA (100 ng), reverse (5' -GTCACTTTGATATGATGAGAGCA-3') and forward (5'-CCTCGTTAATACCCACTGACCTA-3') primers (20 ng each), dNTPs (200 μmol/L) and 0.5 U of Taq polymerase in a standard buffer (Dynazyme, Finnzymes, Espoo, Finland). The PCR was initiated with denaturation at 95℃ for 10 min (during which enzyme was added), then 35 cycles were carried out in following conditions: denaturation at 94℃ for 30 s, annealing at 53℃ for 30 s, extension at 72℃ for 75 s and a final extension at 72℃ for 10 min. The size of PCR products was verified by 1.5% agarose gel electrophoresis with ethidium bromide. The purification of PCR products was done by 2.5 U of shrimp alkaline phosphatase (USB) and 5 U of exonuclease I (New England Biolabs) at 37℃ for 60 min, after which enzymes were inactivated at 80℃ for 15 min. The cyclic sequencing consisted of BigDye 3.1 terminator (Applied Biosystems) according to the manufacturer's instructions with a total volume of 10 μL. Sequencing reaction was as follows: at 96℃ for 1 min, then 25 cycles at 96℃ for 10 s, at 55℃ for 5 s and at 60℃ for 4 min. To remove unincorporated nucleotides, sequencing reaction products were purified by Millipore Multiscreen plates (Millipore, USA) with Sephadex G-50 superfine sepharose (Amersham Biosciences, Sweden). The sequenced products were at first electrophoresed on an ABI 3730 DNA analyzer (Applied Biosystems) and then Sequencing Analysis 5.2 software (Applied Biosystems) was used for base calling. The obtained sequence was analyzed by Sequencher 4.1.4 software (Gene Codes, USA).

Table 1 Frequency of the C/T-13910 variant

Genotype of C/T-13910 variant	Frequency (%)
CC	24.8
CT	47.5
TT	27.7

Table 2 Milk consumption and self-reported milk intolerance in Estonian adult population according to different genotypes of the C/T-13910 variant *n* (%)

Genotype	C/C-13910	C/T-13910	T/T-13910	Total	*P*
Drinking milk (dL/d)					[a]$P < 0.05$
0	24 (31)	34 (23)	21 (24)	79 (25)	
1-2	38 (49)	63 (42)	35 (40)	136 (43)	
3-7	15 (19)	40 (27)	23 (27)	78 (25)	
8 and more	1 (1)	12 (8)	8 (9)	21 (7)	
Symptoms from milk	17 (22)	7 (5)	5 (6)	29 (9)	[b]$P < 0.001$

[a]$P < 0.05$ *vs* C/T-13910 and T/T-13910 in drinking milk; [b]$P < 0.001$ *vs* those without milk intolerance.

RESULTS

The genotype was obtained from 355 subjects. According to their ethnic origin there were 314 Estonians (at least three grandparents being reported to be Estonians) and 41 other nationalities or mixed marriages (mostly Russians, Finns, but also Ukrainians, Swedes, Germans, Polish). In the final frequency analysis, the samples of the abovementioned 314 subjects were included. The frequencies of different C/T-13910 genotypes are presented in Table 1.

We found only two other single nucleotide mutations upstream of the C/T-13910 variant. One was C/T-variant (rs4988233), 101bp upstream of the C/T-13910 variant that was found in four subjects. The other was G/T variant 26bp upstream of the C/T-13910 variant that was found only in one subject. We did not find any polymorphisms downstream of the C/T-13910 variant.

Milk consumption and self-reported milk intolerance in the Estonian adult population with different genotypes are presented in Table 2. The subjects with the C/C-13910 genotype of adult-type hypolactasia drank less milk and reported more often milk intolerance as compared with those with the C/T-13910 or the T/T-13910 genotype.

DISCUSSION

We found that the frequency of adult-type hypolactasia was 24.8% in Estonia. This confirmed the results of the previous studies based on indirect methods of determining hypolactasia, according to which the frequency of hypolactasia varies from 23% to 32%[1]. Our results may be more accurate because of the direct genotyping used for diagnosis in comparison with previously used lactose tolerance tests[16].

The prevalence of adult-type hypolactasia in Europe increases from west to east and from north to south, and is the lowest in Northern Europe[1]. The frequency of 25% is in agreement with this pattern. We have previously shown that the prevalence of the C/C-13910 genotype is 10% in Sweden and 18% in Finland[6,17].

The binding sites of transcription factors usually extend 100-200 bp. In the present study, we found only few other single nucleotide mutations adjacent to the C/T-13910 variant, indicating that the C/T-13910 variant is the only SNP in Estonian population affecting the binding of transcription factor at this binding site, thus confirming the previous findings that this SNP is the only SNP that affects transcription of the LCT gene in all studied populations.

As expected, we found that the consumption of milk was lower in lactase non-persistent subjects than in lactase persistent subjects, suggesting that lactase non-persistence does not prevent the intake of milk. Indeed, only 2/3 of subjects with lactase non-persistence get symptoms after consuming milk and most of the malabsorbers can consume couple of glasses of milk per day, especially during meals[3,21]. This may explain the fairly high rate of milk consumers among genotype CC-13910. Our results concerning milk consumption of CC-13910 genotype subjects are consistent with previously reported results[22,23]. Still, some individuals with hypolactasia may have severe abdominal complaints after milk consumption and it is of utmost importance to diagnose hypolactasia and give recommendations to restrict their fresh milk intake. The restriction of milk consumption is not indicated for those with hypolactasia but without any symptoms after drinking milk.

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