Lactase non-persistent genotype influences on dairy product consumption and gastrointestinal symptoms in Northern Russians

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Abstract

**Objective:** Milk is an important source of nutrients. However the consumption of milk may cause abdominal complaints in lactose intolerant individuals. It is known that frequency of -13910C/C genotype is high among Northern Russians and exceeds the prevalence in northern Europe. In our study we tested two hypotheses 1) subjects with lactase non-persistent genotype (-13910C/C) have more symptoms associated with milk 2) subjects with lactase non-persistence avoid using milk.

**Methods:** In total, 518 students aged 17 to 26 years were randomly selected from different departments of the Northern State Medical University (NSMU) for the genotyping lactase activity defining -13910C/T variant. All subjects filled in a questionnaire about their personal data, self-reported gastrointestinal (GI) symptoms and milk consumption habit.

**Results:** Northern Russians consume very small amount of milk weekly. Among carriers of lactase non-persistent (LNP) genotype there are 10 percent units milk-consumers less than among lactase persistent (LP) persons (p = 0.03). Complaints to GI disorders caused by milk were different between genotypes (p = 0.02). From all analyzed types of food only milk was associated with increased GI symptoms among subjects with LNP genotype (OR =1.95, CI 1.03-3.69)

**Conclusions:** Our study confirmed that even small amounts of milk result in higher frequency of GI disorders in LNP subjects. Hence both hypotheses were verified. In case of increasing milk consumption symptoms may increase need of medical consultations. Consequently it is important either for people themselves or for health care staff to be aware about lactase persistence/non-persistence.

**Key words:** lactose intolerance, adult-type hypolactasia, lactase non-persistence, milk consumption, gastrointestinal complaints
**Introduction**

Milk is an important source of everyday nutrition. Bovine milk is a rich resource of nutrients containing lipids, proteins, amino acids, vitamins, and minerals. Such substances like immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes, and other bioactive peptides are also present in milk [1,2]. Milk has been considered as a major source of calcium as well as the simplest and “cost-effective” way to ingest calcium [3,4]. As far as dairy products also provide other important nutrients, their consumption improves the nutritional quality of the diet appreciably [4,5].

Nevertheless, the consumption of milk has decreased in the last decade in Northern Europe [1,5]. The data about milk consumption in Russia in recent years has been very limited and available only from market analyses that show the decrease in milk consumption in some regions of the country [6]. The milk consumption is low among young Northern Russians [7]. The abovementioned milk properties make it important for everyday nutrition, especially for children. Recently, the national program “School milk” which aims to supply schoolchildren with essential milk daily has been implemented in Russia [8].

Adult-type hypolactasia, characterized by the down regulation of lactase enzyme activity in the intestine during human growth, is inherited as an autosomal recessive trait [9,10]. The first genetic variant associated with adult-type hypolactasia is a one base polymorphism C>T at -13910 (rs4988234) upstream of the lactase encoding gene on chromosome 2 and was identified in 2002 [11]. The -13910 C/T is the most common variant of lactase persistence among Caucasians [12]. Several other population-specific variants nearby have been identified, some of them suggestive [13-16]. The -13910C/T has been shown to be located in OCT-1 binding site and is acting as an enhancer. Nowadays, the genotyping is the most appropriate method for diagnostics of adult-type hypolactasia with 100% specificity [17,18].

Milk contains disaccharide lactose which may cause gastrointestinal problems in adults because of its weak digestion by part of the population. In Northern Europe, lactase persistence is
common, therefore many people may drink milk without any consequences [19]. However in populations where the frequency of lactase persistent genotype is rather low the consumption of milk may cause complaints from digestive tract [20]. The development of symptoms depends on the amount of consumed milk as well as individual sensitivity. It has been shown that subjects with hypolactasia can tolerate moderate quantities of milk, up to 12g of lactose/200 ml of milk. If daily dose of lactose is consumed by small portions and also with a meal, the possibility of symptoms is low [20,21,22].

The most common gastrointestinal (GI) symptoms which characterize the intolerance to lactose are flatulence, diarrhea, gurgling, abdominal distension and abdominal cramping [22,23,24]. The appearance of symptoms is explained clearly by mechanism of lactose utilization. Lactase-phlorizin hydrolase splits the entering lactose into two composed monosaccharides glucose and galactose. The lack of lactase activity leads to the intake of indigested lactose into the bowel. The osmotic effect causes diarrhea [25] while increased level of fermentation in intestine leads to increasing production of gases and therefore flatulence [26,27].

The prevalence of adult-type hypolactasia (lactase non-persistence, -13910C/C) among Northern Russian has been found about 35% [7]. Another study has also shown the prevalence of the -13910C/C genotype among Russians, living in the central part of the country ranges from 36 to 50% [28,29]. This frequency is twice as high as in Finland and in many times higher comparing with other Scandinavian countries [30-33]. It also exceeds the prevalence of -13910C/C genotype among Estonians [34].

Recent studies show that subjects with intolerance to lactose tend to reduce the amount of consuming milk that is predictable as far as they have symptoms after milk consumption [31,35,36,37,38]. In our previous study [7] subjects did not show any differences between genotypes in frequency of GI complaints during life. But we found that the consumption of milk is extremely low among Northern Russian population comparing with other Northern
populations. Does milk consumption influence on the appearance of GI symptoms in population with high frequency of lactase non-persistent genotype but with very low consumption of milk in whole?

In current study we intend to test two hypothesis 1) subjects with lactase non-persistent genotype (-13910C/C) possibly have more symptoms associated with milk 2) subjects with -13910C/C avoid using milk.
Materials and Methods

The students aged 17 to 26 years were randomly selected from different departments of the Northern State Medical University (NSMU) during the period from 2006 to 2008 for the investigation. Blood samples (n=241) or buccal samples (n=277) were taken for genotyping lactase activity defining -13910 C/T variant from 518 subjects in total.

The first group was recruited during the period of 2006-2007 and blood samples were taken for the genotyping. The second group was recruited during the period of 2007-2008 and buccal samples were taken for the same purposes. The mean age for whole group was 19.8±1.72. The women were presented in 78.2% of all participants. The both groups of students were similar concerning to distribution of age and gender.

The study was approved by the Ethic Committee of NSMU (No 08/06 from 29.11.2006). All subjects gave their informed consent for the participation in the study.

Analyses of genotype

Blood samples were genotyped at the Department of Medical Genetics, University of Helsinki. Buccal samples were genotyped at the Forensic Laboratory of University of Tampere.

Genotyping of blood samples was performed as described previously [11]. DNA was amplified by polymerase chain reaction (PCR). We used Taq polymerase (Dynazyme, Finnzymes, Espoo, Finland) with the conditions described elsewhere. The used forward primer was 5’-CCTCGTTAATACCCACTGACCTA -3’ and the reverse primer was 5’-
GTCACCTTGATATGATGAGAGCA-3’ that cover about 400 bp region on both sides of the C/T-13910 variant. The PCR product was verified by 1.5 % agarose gel electrophoresis (with ethidium bromide). The PCR products were purified using Shrimp Alkaline Phosphatase (USB) and Exonuclease I (New England Biolabs) at 37°C for 60min and at 80°C for 15min.

In sequencing BigDye 3.1 terminator (Applied Biosystems) was used according to the manufacturer’s instructions. Sequencing conditions were as follows: at 96°C for 1min, then 25
cycles at 96˚C for 10s, at 55˚C for 5s and at 60˚C for 4min. Sequencing reaction followed
purification by Millipore Multiscreen plates (Millipore, USA) with Sephadex G-50 Superfine
sepharose (Amersham Biosciences, Sweden), electrophoresis by ABI 3730 DNA Analyzer
(Applied Biosystems) and base calling by Sequencing Analysis 5.2 software (Applied
Biosystems). The obtained sequence was analysed using Sequencher 4.6. software (Gene Codes,
USA).

The polymorphism of lactase persistence/non persistence SNP rs4988235 from buccal
samples was determined with TaqMan Human Custom Genotyping Assay from Applied
Biosystems. Assay was performed according to the instructions provided with the assay with
ABI Prism 7900 HT sequence detection system (AppliedBiosystems, California, USA).

**Questionnaire**

All subjects gave written informed consent and filled in a questionnaire about their personal data,
self-reported condition of health, milk consumption habits. We used the questionnaire which was
granted by Sahi T [10].

Gastrointestinal symptoms were estimated by question “Have you ever experienced the
following symptoms and in what frequency?”. The question wasn't intended to clarify the
connection between milk consumption and symptoms. The variables of answers were “Every
day”, “At least one time per week”, “Every second week”, “Sometimes”. If participant has never
had the symptom he left the place empty. We classified the answer “Sometimes” with no
symptoms into one group and marked it “Without any GI symptoms”. The second group
included the rest of variables and was taken for analyses of differences between genotypes.

The food’s influence to appearance of GI disorders was estimated by question “Do you
have any GI disorders if you consume the following type of food?” where six types of food were
included. Subjects were asked to put “Yes” or “No” opposite every type of food.
In the last part of questionnaire subjects were asked to define their everyday milk product consumption from 0 to more than 5 glasses per day. The participants had to choose from “Not at all”, “1-2 glasses”, “3-5 glasses” and “More than 5 glasses” of milk/sour milk per day.

The comparison was made between lactase non-persistent group (-13910C/C) and lactase persistent group (-13910C/T and -13910 T/T) for all abovementioned questions.

**Statistical analysis**

The difference between groups of genotype in milk consumption and others were tested by χ² test. Odds ratios (OR) with 95% confidence interval (CI) for -13910C/C genotype in the logistic regression analysis were calculated for gastrointestinal symptoms in total, symptoms after several types of food and for volume of milk/sour milk consumption.
Results

The prevalence of the -13910C/C genotype (lactase non-persistence, LNP) among young population of North West Russia was found to be 34.7% (Table 1).

About sixty percent of all subjects reported GI symptoms (Table 2). The most frequent symptom was stomachache. However no differences were revealed in frequency of gastrointestinal symptoms in total between LP and LNP genotype groups.

Majority of subjects consumed very small amount of milk daily. They reported the consumption not more than 1-2 glasses of milk per week. Among carriers of LNP genotype there are 10 percent units less milk-consumers in comparison with LP group (46.1 and 56.6%). The differences were statistically significant (p = 0.03). The sour milk consumption did not give significant differences in symptoms between LP and LNP subjects.

The milk consumption had connection to GI disorders in 13.3% of subjects with LNP genotype and in 7.1% of persons with LP genotype (Table 3). Complaints to GI disorders caused by milk consumption were different between genotypes (p = 0.02).

From all analyzed types of food only milk resulted in statistically significant differences in symptoms between LP and LNP subjects (OR =1.95, CI 1.03-3.69) (Table 4). In regression analysis there was not any connection between consuming food and appearance of symptoms besides milk consumption.
Discussion

Young Northern Russians consume very small amounts of milk daily. However among LNP genotype carriers there are still less number of milk-consumers than among LP persons. Our study demonstrates that even if milk is consumed in very small amounts it is the only one type of food having an influence upon the appearance of GI disorders differently by genotype. We can conclude that subjects with -13910C/C have more symptoms from milk and they avoid to use milk. It states that both our hypotheses are verified.

Sour milk consumption has not given such differences. This is in accordance to previously proved finding that sour milk is better tolerated than milk by persons with lactose intolerance [21,39]. Sourmilk contains less lactose because of bacterial fermentation.

All students recruited to the study were considered as healthy. However, only forty percents of them have not reported any GI symptoms and half of them reported stomachache that was the most frequent symptom among all subjects without links to genotype. Possibly this finding requires further investigation of student’s health to clarify the causes of frequent GI disorders among healthy young population.

Regarding to other populations it was shown that only flatulence occurs significantly more frequent among LNP subjects [39, 40]. Jussila J et al [41] showed the loose stool was more frequent symptom in subjects with selective lactose malabsorption.

In our study we were not able to specify the symptoms appeared more frequently in lactose non-persistent subjects. However the results demonstrate that Northern Russians with -13910C/C avoid to use even small amount of milk. Such self-prescribing diminishing of consumed milk is in agreement with the study in Finnish children with the -13910C/C genotype who naturally diminished their usage of milk [31] and also with study in children of other ethnicities [42]. It was also shown that other age groups also tend to reduce their milk consumption because of appearance of symptoms after milk [24, 38].
Since milk is an important source of nutrients there is a number of studies aimed to prove the advantages and disadvantages to milk restriction or elimination from diet. The lack of milk in nutrition of young population is strongly associated with decreased bone mineral density and leads to osteoporosis in older age [42]. There is lack of accurate statistic data about prevalence of osteoporosis among Russian population. However it is obvious that the osteoporosis as well as higher risk of bone fractures connected with it is an actual problem for Russian population [43]. Building peak bone mass during childhood and adolescent can be the best prevention of osteoporosis in older age.

It seems to be quite positive tendency to increase milk consumption among Russian population by providing of federal program [8]. The benefits from the raise of milk consumption are obvious. In the same time our study confirmed that even small amounts of milk result in higher frequency of GI disorders among lactase non-persistent subjects. It is predictable that in case of increasing milk consumption symptoms may appear more frequently and may increase need of medical consultations and care. Therefore it is important either for people themselves or for health care staff to be informed about lactase persistence/non-persistence.
Competing interest

The authors declare that they have no competing interest. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Authors’ contributions

YK designed the research, collected the material, processed the data, and drafted the manuscript;
ST made the genotyping of blood samples;
ST made the genotyping of buccal samples and drafted the manuscript;
IJ designed the research, supervised the research, and helped to draft the manuscript;
PK supervised of analyses of genotypes (buccal samples), and help to draft the manuscript;
MI proposed the study idea, supervised, supported and designed the study, and drafted the manuscript
KM supervised, supported and designed the study, supervised of data processing, and drafted the manuscript

All authors read and approved the final manuscript.
References


8. The national programm “School milk”. [www.schoolmilk.ru]


### Table 1
Prevalence of -13910C/C genotype among young population of North West of Russia

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C-13910</td>
<td>180</td>
<td>34.7</td>
</tr>
<tr>
<td>C/T -13910</td>
<td>238</td>
<td>45.9</td>
</tr>
<tr>
<td>T/T-13910</td>
<td>100</td>
<td>19.3</td>
</tr>
<tr>
<td>Total</td>
<td>518</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2
The frequency of gastrointestinal symptoms (GI) among young people with lactase-persistent/non-persistent genotype (Question “Have you ever experienced the following symptoms?”)

<table>
<thead>
<tr>
<th>GI symptoms</th>
<th>Lactase non-persistence C/C-13910 (n = 180)</th>
<th>Lactase persistence C/T and T/T-13910 (n = 339)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomachache</td>
<td>93  51.7</td>
<td>176  51.9</td>
<td>1.00</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>23  12.8</td>
<td>42  12.4</td>
<td>0.89</td>
</tr>
<tr>
<td>Flatulence</td>
<td>17  9.4</td>
<td>38  11.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Heartburn</td>
<td>14  7.8</td>
<td>23  6.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Nausea</td>
<td>13  7.2</td>
<td>25  7.4</td>
<td>1.00</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3   1.7</td>
<td>12  3.5</td>
<td>0.28</td>
</tr>
<tr>
<td>Without any GI</td>
<td>74  41.1</td>
<td>143 42.2</td>
<td>0.71</td>
</tr>
</tbody>
</table>

### Table 3
The frequency of GI disorders connected to different food stuff (Question “Do you have any GI disorders if you consume the following type of food?”)

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Lactase non-persistence -13910C/C (n = 180)</th>
<th>Lactase persistence -13910C/T and -13910T/T (n = 339)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty</td>
<td>56  31.1</td>
<td>120  35.5</td>
<td>0.33</td>
</tr>
<tr>
<td>Fried</td>
<td>43  23.9</td>
<td>69  20.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Milk</td>
<td>24  13.3</td>
<td>24  7.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Sour milk and kefir</td>
<td>16  8.9</td>
<td>17  5.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Vegetables</td>
<td>10  5.6</td>
<td>14  4.1</td>
<td>0.51</td>
</tr>
<tr>
<td>Fruits</td>
<td>7   3.9</td>
<td>19  5.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Any other food</td>
<td>28  15.6</td>
<td>52  15.4</td>
<td>1.00</td>
</tr>
<tr>
<td>No any food</td>
<td>88  47.6</td>
<td>174 51.6</td>
<td>0.74</td>
</tr>
</tbody>
</table>
Table 4.
Odds ratios (OR, 95% CI) of the lactase non-persistent genotype (-13910C/C) in the logistic regression analysis for gastrointestinal symptoms, symptoms after some food and milk consumption. Statistically significant differences are presented in bold face.

<table>
<thead>
<tr>
<th>GI symptoms</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>2.12</td>
<td>(0.58 – 7.76)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>1.17</td>
<td>(0.62 – 2.21)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1.04</td>
<td>(0.50 – 2.14)</td>
</tr>
<tr>
<td>Stomach pain</td>
<td>1.00</td>
<td>(0.68 – 1.46)</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>0.98</td>
<td>(0.53 – 1.76)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.82</td>
<td>(0.39 – 1.71)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms after some food</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>1.95</td>
<td>(1.03 – 3.69)</td>
</tr>
<tr>
<td>Sour milk and kefir</td>
<td>1.61</td>
<td>(0.75 – 3.43)</td>
</tr>
<tr>
<td>Fried</td>
<td>1.61</td>
<td>(0.97 – 2.67)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>1.25</td>
<td>(0.52 – 3.00)</td>
</tr>
<tr>
<td>Any other food</td>
<td>1.02</td>
<td>(0.59 – 1.75)</td>
</tr>
<tr>
<td>Fatty</td>
<td>0.68</td>
<td>(0.43 – 1.07)</td>
</tr>
<tr>
<td>Fruits</td>
<td>0.52</td>
<td>(0.20 – 1.31)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Milk product consumption</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>1.51</td>
<td>(1.04 – 2.17)</td>
</tr>
<tr>
<td>Sour milk</td>
<td>1.10</td>
<td>(0.76 – 1.60)</td>
</tr>
</tbody>
</table>