



YULIA KHABAROVA

Adult-type Hypolactasia in North-West Russia



ACADEMIC DISSERTATION

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ACADEMIC DISSERTATION

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To Ivan,
Ekaterina and Timosha.
And to my dear mother.

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List of original publications

This thesis is based on the following original publications, referred to in the text by Roman numerals.

- I. Khabarova YA, Torniaainen ST, Nurmi HA, Järvelä IE, Isokoski MK, Mattila KJ. Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia. *World J Gastroenterol.* 2009 Apr 21; 15 (15):1849-53.
- II. Khabarova Y, Tornianen S, Tuomisto S, Järvelä I, Karhunen P, Isokoski M, Mattila K. Lactase non-persistent genotype influences milk consumption and gastrointestinal symptoms in Northern Russians. *BMC Gastroenterol.* 2011 Nov 13; 11:124.
- III. Khabarova Y, Torniaainen S, Savilahti E, Isokoski M, Mattila K, Järvelä I. The -13914G>A variant upstream of the lactase gene (LCT) is associated with lactase persistence/non-persistence. *Scand J Clin Lab Invest.* 2010 Sep; 70(5):354-7.
- IV. Khabarova Y, Grigoryeva V, Tuomisto S, Karhunen PJ, Mattila K, Isokoski M. High prevalence of lactase non-persistence among indigenous nomadic Nenets, north-west Russia. *Int J Circumpolar Health.* 2012 Apr 25; 71:1-6.

Abbreviations

BHT	breath hydrogen test
CLD	congenital lactase deficiency
GI	gastrointestinal
IBS	irritable bowel syndrome
LCT	the official symbol of the gene “lactase”
LM	lactose malabsorption
LPH	lactase-phlorizin hydrolase
LNP	lactase non-persistence/lactase non-persistent
LP	lactase persistence/lactase persistent
LTT	lactose tolerance test
LTTE	lactose tolerance test with ethanol
NAO	Nenets Autonomous Okrug
NSMU	Northern State Medical University
OSP-1	the transcription factor binding strongly to the T ₋₁₃₉₁₀ variant
PCR	polymerase chain reaction
SNP	single nucleotide polymorphism

Summary

Adult-type hypolactasia (primary lactose malabsorption, lactase non-persistence) is the most common enzyme deficiency in humans, presenting in all populations and varying considerably by frequency in different ethnicities. Adult-type hypolactasia may lead to unspecific abdominal complaints such as diarrhea, flatulence, audible bowel, nausea, bloating and abdominal cramping. The condition is genetically determined and inherited as a recessive trait. Homozygous subjects with the C/C-₁₃₉₁₀ genotype evince very low lactase activity in the jejunum, whereas heterozygous C/T-₁₃₉₁₀ subjects and carriers of the T allele in its homozygous variant (T/T-₁₃₉₁₀) maintain a higher lactase activity.

The possibility of persisting lactase in adult age appeared in humans as a mutation. The genetic test allowing determination of the LNP genotype and thus diagnosing adult-type hypolactasia was discovered in 2002.

The prevalence of adult-type hypolactasia among Northern Russians and indigenous Nenets was studied here using genotyping for LCT gene variants as a marker for the condition. We observed that Northern Russians had a 36% prevalence of adult-type hypolactasia, whereas 90% of neighboring native Nenets are lactase non-persistent.

We also studied the influence of C/C-₁₃₉₁₀ on the appearance of GI symptoms and milk consumption among a Russian population. The conclusion was that homozygotes for C/C have more GI symptoms caused by milk. Milk was determined as only one of the tested foods giving rise to symptoms in LNP subjects. Moreover, there were fewer milk-consumers among C/C-₁₃₉₁₀ subjects compared to C/T and T/T genotype carriers.

We discovered the G>A-₁₃₉₁₄ genotype variant upstream of the LCT gene in one of our study subjects who carried C/C in -₁₃₉₁₀ position. We examined members of this person's family and found this mutation in three of them. Previously such a variant had been reported in two persons only, both of them living in Central Europe. However, in the current study the significance of the variant was for the first time investigated and described. We carried out measurement of the lactase activity in the small intestine of our subject and found

that a variant in heterozygous state was associated with increased lactase activity, suggesting that this phenomenon was most likely associated with the G>A-13914 variant.

In the Nenets study the method of concordance of grandparents' origin was applied to establish the number of native Nenets in the study group. We estimated separately the frequency of C/C-13910 among those who had four, three, two and one grandparent of Nenets origin. Such an approach was applied for the first time in a hypolactasia study. It emerged that those who had only Nenets in previous generations had a highest prevalence of hypolactasia, while the frequency among others diminishes gradually in accordance to the number of Nenets ancestors. We used the term ethnicity in the biological sense in our study.

We established that the prevalence of adult-type hypolactasia varies considerably among populations even if they are close geographical neighbors. The frequency of a genotype depends on ethnicity and history of milk usage. The gene flow from other populations exerts an influence on the frequency of lactase non-persistence.

Tiivistelmä

Aikuistyyppin hypolaktasia (primaari laktoosimalabsorptio, laktoosi-intoleranssi) on ihmisen yleisin entsyymipuutos. Sitä esiintyy kaikissa maailman väestöissä. Yleisyys vaihtelee huomattavasti eri etnisten ryhmien välillä. Aikuistyyppin hypolaktasia saattaa johtaa erilaisten vatsavaivojen kuten ripulin, ilmavaivojen, äänekkään suoliston, pahoinvoinnin, turvotuksen ja vatsan kouristuksien esiintymiseen. Hypolaktasia on resessiivisesti periytyvä entsyymipuutostila. C/C-¹³⁹¹⁰-genotyyppiä olevien homotsygoottien yksilöiden ohutsuolen laktaasiaktiiviteetti on erittäin vähäinen. Sen sijaan C/T-¹³⁹¹⁰-heterotsygooteilla sekä T-alleelia kantavilla homotsygooteilla (T/T-¹³⁹¹⁰) suolen laktaasiaktiiviteetti on korkea.

Laktaasientsyymin aktiiviteetin säilyminen aikuisiälle mahdollistui mutaation seurauksena. C/C-¹³⁹¹⁰-genotyypin ja aikuistyyppin hypolaktasian määrittävä geenitesti kehitettiin vuonna 2002.

Aikuistyyppin hypolaktasian esiintyvyyttä pohjoisvenäläisten ja nenetsien alkuperäiskansan keskuudessa tutkittiin tyyppittämällä laktaasigeenin variantteja. Havaitimme, että 36 prosentilla pohjoisvenäläisistä ja 90 prosentilla nenetseistä on hypolaktasian genotyyppi.

Tutkimme myös C/C-¹³⁹¹⁰-geenin yhteyttä ruoansulatuskanavan oireiden ilmenemiseen sekä maidonkulutuksen yleisyyteen venäläisväestössä. Havaitimme, että C/C-homotsygooteilla on enemmän maidosta aiheutuvia ruoansulatuskanavan oireita kuin muilla geenivarianteilla. Maidon lisäksi myös muut testatut ruoka-aineet aiheuttivat oireita C/C-¹³⁹¹⁰-genotyyppiä edustaville tutkittaville. Lisäksi heidän joukossaan oli vähemmän maitotuotteita käyttäviä henkilöitä verrattuna C/T- ja T/T- genotyypin edustajiin.

Löysimme G>A-¹³⁹¹⁴- geenivariantin potilaalta, jolla C/C-genotyyppi oli ¹³⁹¹⁰-positiossa. Muunnos sijaitsee 13914 emäsparin päässä laktaasigeeniä edeltävästä sekvenssikohdasta. Löysimme saman alleelin myös tämän henkilön kolmelta perheenjäseneltä. Aiemmin tämä variantti on löydetty ainoastaan kahdelta keskieuropalaiselta tutkittavalta. Variantin merkitys kuvattiin kuitenkin ensimmäisen kerran vasta käsillä olevassa tutkimuksessa. Mittasimme tutkittavan ohutsuolen laktaasiaktiiviteetin ja huomasimme heterotsygoottivariantin olevan

yhteydessä kohonneeseen laktaasiaktiiviteettiin. Tämä viittaa siihen, että kohonnut laktaasiaktiiviteetti todennäköisesti liittyy nimenomaan G>A-¹³⁹¹⁴-varianttiin.

Nenetsitutkimuksessa määritimme syntyperäisten nenetsien määrän isovanhempien syntyperän perusteella sekä määrittämällä C/C-¹³⁹¹⁰-genotyypin esiintyvyyden niiden nenetsien keskuudessa, joilla oli kaksi, kolme tai neljä nenetsiesi-isää. Tällaista lähestymistapaa käytettiin nyt ensimmäistä kertaa hypolaktasiatutkimuksessa. Havaitimme, että niillä nenetsillä, joilla oli vain nenetsiesi-isiä aiemmissa sukupolvissa oli myös eniten hypolaktasiaa. Muiden nenetsien keskuudessa hypolaktasian esiintyvyys väheni nenetsiesi-isten lukumäärän laskiessa. Käytämme tutkimuksessamme käsitettä *etninen* sen biologisessa merkityksessä.

Aikuistyyppin hypolaktasian esiintyvyys vaihtelee merkittävästi eri väestöissä vaikka ne sijaitsisivatkin maantieteellisesti lähellä toisiaan. Tietyn genotyypin esiintyvyys riippuu etnisestä syntyperästä sekä maitotuotteiden käyttöhistoriasta. Geenivirta muista populaatioista vaikuttaa myös laktoosi-intoleranssin esiintyvyyteen.

Абстракт

Гиполактазия (первичная лактазная мальабсорбция, неустойчивость лактазы) - наиболее частый вариант ферментного дефицита человека, встречающийся во всех популяциях и значительно отличающийся по частоте в разных этнических группах. Взрослый тип гиполактазии может проявляться неспецифическими абдоминальными жалобами, такими как диарея, вздутие и урчание в животе, тошнота, выделение газов и боли в животе. Взрослый тип гиполактазии предопределен генетически и наследуется как рецессивный признак. У гомозигот с С/С-₁₃₉₁₀ генотипом активность лактазы в тощей кишке очень низкая, в то время как у гетерозиготных носителей генотипа С/Т-₁₃₉₁₀ и у гомозигот по Т аллелю (Т/Т-₁₃₉₁₀) сохраняется высокая лактазная активность.

Способность поддерживать стабильный уровень лактазы во взрослом возрасте возникла у человека как мутация. Генетический тест, дающий возможность определить генотип неустойчивости лактазы и диагностировать взрослый тип гиполактазии, был разработан в 2002 году.

Распространенность взрослого типа гиполактазии среди русских, проживающих на Севере и в популяции коренных ненцев, была исследована с помощью метода генотипирования. Частота взрослого типа гиполактазии среди русских северян составила 36%, в то время как у живущих по соседству ненцев - 90%.

Возможное влияние С/С-₁₃₉₁₀ генотипа на возникновение гастроинтестинальных симптомов и потребление молока среди русской популяции было изучено в ходе исследования. Гомозиготы С/С имеют больше гастроинтестинальных симптомов, вызванных приемом молока. Молоко оказалось единственным из протестированных видов продуктов, ведущим к возникновению симптомов у людей с лактазной неустойчивостью. Кроме того, было показано, что среди носителей С/С-₁₃₉₁₀ количество потребителей молока меньше, чем среди С/Т-₁₃₉₁₀ и Т/Т-₁₃₉₁₀ носителей.

G>A-₁₃₉₁₄ вариант генотипа был обнаружен у одной из участвующих в исследовании пациентки с С/С генотипом в -₁₃₉₁₀ позиции. Мы обследовали

семью пациентки и обнаружили G/A мутацию у трех членов этой семьи. Ранее такой вариант генотипа был описан только у двух человек, живущих в Центральной Европе. Возможное значение этого варианта было впервые исследовано и описано в представленной работе. При проведении измерения лактазной активности в тонком кишечнике пациентки было обнаружено, что вариант G/A ассоциирован с повышенной лактазной активностью, что позволяет предположить влияние генотипа G/A⁻¹³⁹¹⁴ на повышение лактазной активности у пациента с C/C⁻¹³⁹¹⁰ генотипом.

При исследовании популяции ненцев был использован метод конкордантности происхождения прародителей для выявления группы нативных ненцев. Отдельно была оценена частота генотипа C/C⁻¹³⁹¹⁰ среди тех, кто имел четыре, три, два или одного прародителя (дедушку или бабушку) – ненца. Такой подход был использован впервые. Наивысшая частота лактазной неустойчивости была выявлена среди ненцев, имеющих только ненцев в предыдущих поколениях, в то время как частоты генотипа среди остальных групп ненцев уменьшались в соответствии с уменьшением количества ненецких предков.

Мы подтвердили тот факт, что распространенность взрослого типа гиполактазии может значительно варьировать в популяциях, даже если они являются близкими территориальными соседями. Частота генотипа зависит от происхождения и истории потребления молока. Приток генов из других популяций оказывает влияние на частоту лактазной неустойчивости.

Introduction

Adult-type hypolactasia (lactase non-persistence, LNP) is the most common enzyme deficiency encountered all over the world. The prevalence of this condition varies considerably among ethnicities, and there are still populations where its prevalence has not been studied.

Adult-type hypolactasia is inherited in an autosomal recessive manner and causes a primary decline in enzyme activity. It was formerly believed that lactase non-persistence manifests in childhood. However, the age at which lactose intolerance manifests can vary between ethnicities. The majority of northern Europeans have the ability to maintain lactase activity and digest lactose throughout their life (lactase persistence).

The lactase persistence mutation is one of the best-known positive mutations, following upon changes in food consumption. The cultural historical hypothesis, suggested by Simoons (Simoons 1970, Simoons 1969), elaborated by McCracken (McCracken 1970, McCracken 1971) and supplemented by Flatz and Rotthauwe (Flatz, Rotthauwe 1973) associated the occurrence of hypolactasia with the history of dairying. Populations traditionally keeping cows and therefore having the milk products as the most desirable food enjoyed a survival advantage. Such peoples probably had more living children than those with hypolactasia and this ensured the spread of this mutation. The duration of the tradition impacts on differences in the prevalence of lactose intolerance among different ethnicities.

The identification of specific nucleotide polymorphisms associated with adult-type hypolactasia (Enattah, Sahi et al. 2002) has made it possible to test whether a patient has lactase-persistent or non-persistent genotype. The genetic variant associated with adult-type hypolactasia, the one-base polymorphism C/T₋₁₃₉₁₀, was identified in 2002. The variant is inherited recessively, the C/C₋₁₃₉₁₀ genotype (C allele in homozygous form) being invariably associated with lactase non-persistence, while the C/T₋₁₃₉₁₀ and T/T₋₁₃₉₁₀ variants are responsible for the persistence of lactase. Subsequently other genotype variants associated with lactase activity were discovered, mostly in the Southern part of the world. Although certain other alleles associated with lactase persistence have also been discovered in Europe, the C/T-

¹³⁹¹⁰ polymorphism remains the most common variant of the lactase persistent/non-persistent genotype for the European population.

Adult-type hypolactasia has been considered to be responsible for the occurrence of certain unspecific abdominal complaints. The most common gastrointestinal symptoms characteristic of intolerance to lactose are flatulence, gurgling, abdominal distension, abdominal cramping and diarrhea. Subjects with hypolactasia can tolerate moderate quantities of milk, up to 12g of lactose/250 ml of milk. If the daily dose of lactose is consumed in small portions and also with a meal, the likelihood of symptoms is very low.

In this work, the prevalence of adult-type hypolactasia (C/C-¹³⁹¹⁰ genotype) among a population in North-West Russia was studied using the genotyping method. We carried out the investigation among Russian and Nenets ethnic groups in order to establish the prevalence of adult-type hypolactasia among these neighboring populations.

The populations of North Russia are among those where the prevalence of lactase persistence/non-persistence has not previously been studied. However, in clinical practice I see many patients who report a variety of gastrointestinal complaints which they associate with milk consumption and consequently exclude milk products from their diet. Whether these subjects with “milk troubles” are lactase non-persistent or some of them simply diagnose themselves as having hypolactasia remains an open question. Since awareness of adult-type hypolactasia is low or even absent among practitioners in the region, no one paid attention to the possibility of hypolactasia as a factor underlying these gastrointestinal complaints. It is thus clearly meaningful to study adult-type hypolactasia in the North of Russia.

1 Review of the literature

1.1 Metabolism of lactose

1.1.1 Lactose in humans

The milk of most mammals contains the carbohydrate called lactose (Arola, Tamm 1994). In human milk lactose is present in amounts up to 7.2 g per 100 ml (Jeness 1979), while the lactose content in cow's milk is estimated to be 4.4–4.7g /100 ml (Campbell, Matthews et al. 2010, Agostoni, Turck 2011). Lactose provides an excellent source of energy, growth and development during infancy (Agostoni, Turck 2011, Lomer, Parkes et al. 2008). Chemically lactose or β -D-galactopyranosyl-(1 \rightarrow 4)-D-glucose is a disaccharide composed of the monosaccharides D-glucose and D-galactose, joined by a β -1,4-glycosidic linkage (Troelsen 2005). The enzyme lactase-phlorizin hydrolase (LPH, lactase, β -galactosidase) is responsible for splitting the entering lactose into two composed monosaccharides glucose and galactose (Fig. 1).

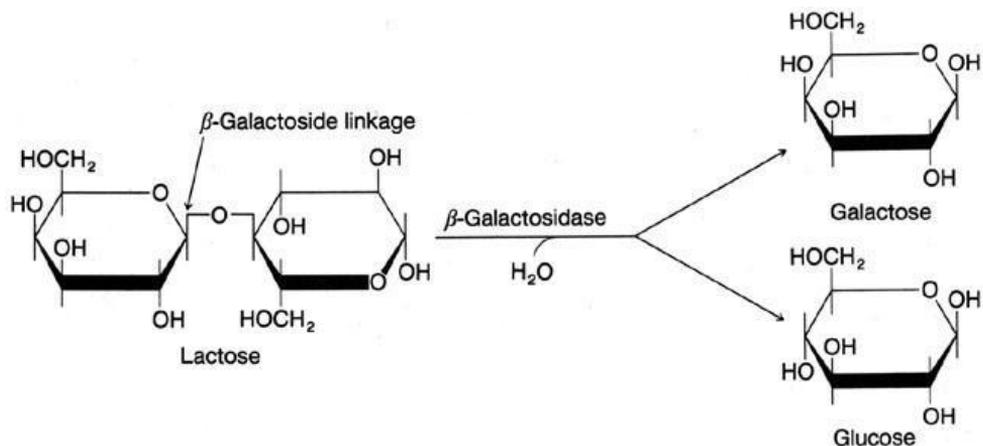


Figure 1. Metabolism of lactose in humans (from Lactose Intolerance, 2012 with some modifications)

These two oligosaccharidases are then absorbed by intestinal enterocytes; glucose is eventually utilized as an energy source whilst galactose is metabolized by the Leloir Pathway into nucleotide sugar UDP-glucose (McSweeney, Fox 2009).

1.1.2 Structure and function of lactase enzyme

The LPH is presented on the apical surface of enterocytes in the brush border of the small-intestinal epithelium (Fig.2). It is anchored by its C-terminal end to the enterocyte surface and the major part of the enzyme molecule protruding into the gastrointestinal lumen (Lomer, Parkes et al. 2008). Such a location renders the enzyme vulnerable in a case of cell damage in comparison to other intestinal disaccharidases which are located deeper (Heitlinger, Rossi et al. 1991, Vesa, Marteau et al. 2000).

The enzyme belongs to a group of intestinal microvillar disaccharidases which also include sucrase-isomaltase and maltase-glucoamilase (Naim, Sterchi et al. 1987). Lactase-phlorizin hydrolase has two strongly associated catalytic sites: β -galactosidase (EC 3.2.1.23) and phlorizin hydrolase (EC 3.2.1.62). The lactase (β -galactosidase) hydrolyses lactose and cellobiose, whereas phlorizin hydrolase splits aryl- and alkyl- β -glycosides such as phlorizin and phlorizin hydrolase (Sebastio, Villa et al. 1989). Of the above mentioned only lactose is significant in terms of nutrition.

In humans the highest activity of lactase has been demonstrated in the jejunum, approximately 50 up to 200 cm distal to the ligament of Treitz (Shils, Shike c2006, Auricchio, Maiuri 1994), the activity being minimal in the ileum.

Lactase is an enzyme typical of mammals only. Its level in the small intestine determines the ability to digest lactose from food without abdominal symptoms.

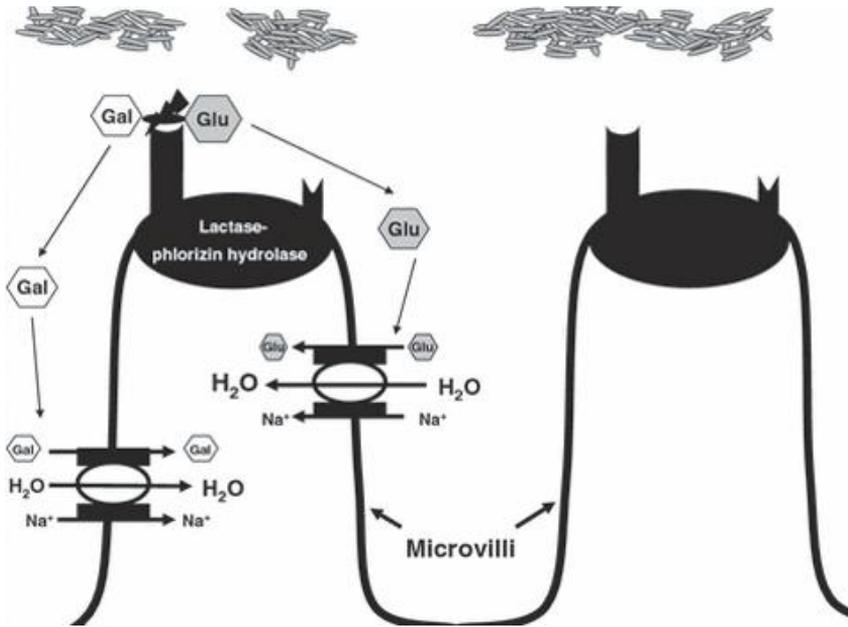


Figure 2. Lactase-phlorizin hydrolase anchored by the C-terminal end in the apical surface of the enterocyte, its major part protruding into the gastrointestinal lumen (Glu – glucose; Gal – galactose) (from Lomer, Parkes et al. 2008)

1.1.3 Metabolism of lactose in hypolactasia

The efficacy of lactose metabolism is determined by a variety of factors apart from lactase activity. Individual differences in lactose intolerance can be related to the amount of lactose presented in the intestine, the speed of gastric emptying and intestinal passage, the capability of the intestinal microflora to ferment lactose and the response of the large bowel to the osmotic load (Arola, Tamm 1994).

As the term hypolactasia implies conspicuously low lactase activity, lactose derived from food cannot be properly split into glucose and galactose and thus remains in the intestine in unchanged form. Unabsorbed lactose passing through the colon has a marked osmotic effect, resulting in water and electrolyte accumulation, speeding transit and softening the stool. Such a mechanism manifests clinically as diarrhea or loose stool (Launiala 1968, Ladas, Papanikos et al. 1982). Unabsorbed lactose is subject to ileac and colonic fermentation by bacterial microflora. The presence of unabsorbed lactose in the intestine leads to the

production of intermediates in the form of lactate and end-product metabolites such as short chain fatty acids (SCFA), hydrogen, methane and carbon dioxide (He, Venema et al. 2008, Levitt, Wilt et al. 2013). These products are responsible for increasing gut transit and pressure in the colon and might thus cause such clinical symptoms as abdominal pain, bloating and flatulence as well as borborygmi (audible bowel).

1.2 Definition and types of hypolactasia

1.2.1 Definition of hypolactasia and related conditions

It is of prime importance to define terms concerning lactose intolerance correctly. The terminology can be presented as follows. *Hypolactasia* or *Lactase deficiency* implies very low lactase activity in the jejunal mucosa (Asp, Dahlqvist et al. 1973, Sahi 1994). Researchers (Sahi 1994, Harrington, Mayberry 2008) emphasize the necessity to distinguish between *lactose malabsorption* and *lactose intolerance*. *Lactose malabsorption* describes a poor capacity to hydrolyze lactose, this being confirmed by lactose tolerance tests. *Lactose intolerance* implies the appearance of symptoms after lactose indigestion.

It would appear that all subjects with lactose malabsorption have symptoms and hence lactose intolerance. However, some subjects with poor hydrolyzing lactose capacity are asymptomatic. Milk intolerance involves the appearance of symptoms after milk consumption, this, however, not always as a result of lactose malabsorption but possibly caused by colonic flora or IBS.

1.2.2 Types of hypolactasia

1.2.2.1 *Congenital lactase deficiency*

Congenital lactase deficiency (CLD) is the most severe, albeit rare form of hypolactasia in newborns. Its prevalence worldwide is low, the highest rate being recorded in the population of Finland (Jussila 1969, Behrendt, Keiser et al. 2009). CLD is an autosomal recessive disorder associated with a complete absence of lactase expression in the intestinal wall (Savilahti, Launiala et al. 1983). Congenital

lactase deficiency manifests with watery diarrhea after the first lactose from breast milk reaches the neonate's intestine. If the condition is undiagnosed and thus untreated, diarrhea leads to severe dehydration and weight loss. A lactose-free diet relieves symptoms and the newborn resumes normal development (Torniainen, Freddara et al. 2009).

1.2.2.2 *Adult-type hypolactasia*

In contrast to the rare congenital lactase deficiency, adult-type hypolactasia is the most common enzyme deficiency worldwide (Sahi 1994). Adult-type hypolactasia (lactase non-persistence, LNP) is inherited in an autosomal recessive manner and causes a primary decline in enzyme activity (Sahi, Isokoski et al. 1973, Sahi 1974, Isokoski, Sahi et al. 1981). A strong positive selection for lactase persistence has been suggested (Simoons 1970, Bersaglieri, Sabeti et al. 2004, Ingram, Mulcare et al. 2009). Subjects with lactase persistence are mutant gene carriers. However, from either an evolutionary or a clinical point of view lactase non-persistence is a normal condition (Jarvela 2005). For the carriers of the lactase non-persistent genotype the decline of lactase activity at a certain age is a normal physiological phenomenon. The majority of northern Europeans are lactase-persistent and preserve the ability to maintain lactase activity and digest lactose throughout life (Holden, Mace 1997, Hollox 2005, Enattah, Trudeau et al. 2007). Among other populations the prevalence of adult-type hypolactasia varies significantly (Simoons, Johnson et al. 1977).

In contrast to the lactase-persistent phenotype the carriers of lactase non-persistence lose the ability to digest lactose in the process of growing, this ultimately resulting in adult-type hypolactasia. In as far as humans belong to the mammals, lactase activity was formerly assumed to be reduced soon after weaning (Simoons 1978, Rossi, Maiuri et al. 1997, Jarvela 2005, Gerbault, Liebert et al. 2011). However, it remains obscure whether lactase activity in fact declines after weaning or later, or as has been assumed during weaning. In many humans lactase remains active in adulthood. According to several studies lactase non-persistent subjects evince a decline in lactase activity at ages from 2 to 20 years (Simoons 1980, Tammur 1991, Wang, Harvey et al. 1998, Swallow 2003, Heyman 2006, Sahi 1994), but it can also manifest on rare occasions after the age of 20 years (Seppo, Tuure et al. 2008). It has been shown that age at the onset of gene down-regulation varies in different populations (Lebenthal, Antonowicz et al. 1975). It is presumed to be later among Whites compared to Blacks and Asians (Sahi 1994, Wang,

Harvey et al. 1998, Heyman 2006). There are no data as to the age of gene down-regulation among the Russian population or any other ethnicities living in the territories of Russia.

1.2.2.3 *Secondary causes of hypolactasia*

Secondary hypolactasia is always linked to injury to the intestinal mucosa and depends on the severity of the injury (Lebenthal, Lee 1980, Heitlinger, Rossi et al. 1991, Nieminen, Kahri et al. 2001, Prasad, Thapa et al. 2008, Siddiqui, Osayande 2011). Since lactase is located more distally on the villus, this enzyme is damaged more severely. While the activity of lactase in secondary hypolactasia is not always reduced to such a low level as in primary variants of the deficiency (Villako, Maaros 1994), it takes longer to recover than the morphological structure of the villi (Troelsen 2005).

The conditions and diseases resulting in secondary hypolactasia have been charted. In the literature all causes have been divided into three groups (Maaros 1991, Swagerty, D.Walling et al. 2002, Ojetti, Nucera et al. 2005):

1. Small-bowel diseases: HIV enteropathy, regional enteritis, celiac disease, Whipple's disease, severe gastroenteritis
2. Multi-system diseases: carcinoid syndrome, cystic fibrosis, diabetic gastropathy, kwashiorkor, Zollinger-Ellison syndrome
3. Iatrogenic causes: chemotherapy, antibiotics such as neomycin, colchicines induction, radiation enteritis, resection of small intestine

Other factors leading to secondary hypolactasia are alcohol (Perlow, Baraona et al. 1977), the presence of parasites such as *Giardia lamblia* (Singh, Bhasin et al. 2000) and *Ascaris lumbricoides* (Carrera, Nesheim et al. 1984). The decrease in the activity of lactase is particularly marked in the case of a combination of malnutrition and protein deficiency (Villako, Maaros 1994).

1.3 Genetics of adult-type hypolactasia

It has now been proved that the activity of lactase in the small intestine is genetically determined. Numerous studies have verified this finding. The first relevant family study was made in Finland in the early 70s. Sahi and associates,

(Sahi, Isokoski et al. 1973, Sahi 1974, Sahi, Launiala 1977) based on an investigation of 326 family members, showed that the decline in small-intestinal lactase activity is controlled by an autosomal recessive trait. As children of ages younger than the manifestation age of hypolactasia in the Finnish population had not developed hypolactasia by the time of investigation they were examined later and had developed hypolactasia as expected (Sahi, Launiala 1977). This finding strongly contributed to the genetic theory of adult-type hypolactasia.

The lactase-persistent trait predominates over the non-persistent trait (Jarvela 2005). In some populations lactase-phlorizin hydrolase activity persists through life, whereas in others the decline in lactase activity develops at a certain age.

The lactase gene (LCT) has been assigned to chromosome 2g21-22 (Harvey, Fox et al. 1993, Kruse, Bolund et al. 1988) and its structure has been characterized (Boll, Wagner et al. 1991). About 200 members of Finnish families previously examined for lactase non-persistence by LTTE were genotyped in order to determine the DNA variant associated with lactase non-persistence (Enattah, Sahi et al. 2002). Other ethnic groups such as Italian, German, South Korean as well as groups from Utah and France were also sampled for the analysis. Based on linkage disequilibrium and haplotype analysis the region associated with lactase persistence has been restricted to a 47kb region outside the LCT gene. Two single nucleotide polymorphisms were identified, cytosine (C) to thymidine (T), residing 13,910 base pairs, and G to A change, residing 22,018 base pairs upstream of the LCT gene (Jarvela 2005). In the study in question all family members with non-persistence were homozygous and had the C/C₋₁₃₉₁₀ genotype, while those with C/T₋₁₃₉₁₀ and T/T₋₁₃₉₁₀ genotypes were lactose-persistent. The strong association between the lactase/sucrase ratio detected in biopsy samples from the small intestine and genotype was shown for all cases (Enattah, Sahi et al. 2002).

As the prevalence of the C/C₋₁₃₉₁₀ genotype among not only Finnish but also participants from other ethnicities was consistent with previous epidemiological data, genotype analysis has been used as a relevant diagnostic test for adult-type hypolactasia. The discovery of the LCT gene has also contributed to the cultural historical hypothesis of hypolactasia. As distantly related populations have been recognized as carriers of the same DNA variants it is conceivable that the persistent trait emerged long before these populations became isolated from each other.

Thus a single nucleotide variant, C/T₋₁₃₉₁₀, located 14 kb upstream of the lactase gene (LCT), represents the most likely variant associated with lactase non-persistence for Northern European populations. The association has been

confirmed in other population samples (Rasinperä, Savilahti et al. 2004). C/T₋₁₃₉₁₀ has been shown to be located at the OCT-1 binding site and acts as an enhancer (Lewinsky, Jensen et al. 2005). The G/A₋₂₂₀₁₈ variant was found to be closely associated with lactase persistence in the Finnish population, with several exceptions for non-Finnish samples (Enattah, Sahi et al. 2002).

Finally, it has been demonstrated that strong positive selection has occurred in an extensive region that includes the LCT gene (Bersaglieri, Sabeti et al. 2004). However, it has been shown that the lactase persistent T₋₁₃₉₁₀ allele is absent in African populations and another allele might be responsible for lactase persistence in Africa.

Mulcare and associates (Mulcare, Weale et al. 2004) were the first to demonstrate that the C-13.9kbT polymorphism is not a predictor of lactase persistence in sub-Saharan Africans. Since then several other specific variants near the C/T₋₁₃₉₁₀ have been identified (Imtiaz, Savilahti et al. 2007, Ingram, Elamin et al. 2007, Tishkoff, Reed et al. 2007, Jensen, Liebert et al. 2011). Mulcare's group investigated Tanzanians, Kenyans and Sudanese and identified three SNPs, G/C₋₁₄₀₁₀, T/G₋₁₃₉₁₅ and C/G₋₁₃₉₀₇ which are associated with lactase persistence. They also established strong evidence of a positive mutation which allows humans to consume milk in adulthood. Imtiaz and colleagues (Imtiaz, Savilahti et al. 2007) showed that T/G₋₁₃₉₁₅ is the founder mutation of lactase persistence in an urban Saudi population and suggested that the lactase non-persistent trait was more likely brought from Africa to the Arabian Peninsula. A study by Ingram and group (Ingram, Elamin et al. 2007) demonstrated that the ₋₁₃₉₁₀ T allele has a very low frequency in many African milk-drinking pastoralist groups, where the lactase persistence phenotype has been reported in high frequency. These data thus suggest that in different populations different allele variants are probably associated with lactase persistence. The convergent evolution of LP in diverse populations most probably reflects different histories of adaptation to milk culture (Enattah, Jensen et al. 2008). Africans and Europeans show similar patterns of lactase persistence, the patterns being however due to different genetic variants in each group (Wooding 2007).

The novel mutation has also been revealed in two European samples (Tag, Schiffers et al. 2007, Tag, Oberkanins et al. 2008). Firstly, in a 37-year-old male patient who had symptoms of adult-type hypolactasia. The ethnic origin of this patient was not clear from the report. He was heterozygous for the C/T₋₁₃₉₁₀ variant (Tag, Schiffers et al. 2007). Another forty-year old Austrian male had also been determined to be a carrier of this novel G/A₋₁₃₉₁₄ variant (Tag, Oberkanins et

al. 2008). In both cases no other tests to determine lactase persistence/non-persistence were carried out, and the significance of this genotype thus remains unclear. Further studies would contribute to a fuller understanding of the place of G/A₋₁₃₉₁₄ in the lactase persistence/non-persistence issue in the European population.

To summarize, the T₋₁₃₉₁₀ allele is 86-100% associated with lactase persistence in the European population (Enattah, Sahi et al. 2002, Poulter, Hollox et al. 2003, Hogenauer, Hammer et al. 2005, Ridefelt, Hakansson 2005), while among African populations other genotypes are responsible for the hereditary persistence of lactase. Some rare genotypes are assumed to be associated with lactase persistence among European samples. However, the role of these alleles remains unknown and further investigations are needed to highlight their implications.

1.4 Epidemiology and prevalence of hypolactasia (historical aspects)

It has long been known that milk can cause gastrointestinal symptoms. Hippocrates first reported gastrointestinal upset in individuals who consumed milk (Wilson 2005). Galen mentioned, almost 2000 years ago, that milk was a laxative and therefore led to gastrointestinal disorders (Green 1951). Milk sugar, i.e. lactose, was found in 1860 to cause diarrhea in dogs, and subsequently, in 1903, it was demonstrated that unhydrolyzed lactose molecule produced a strong osmotic effect (Sahi 1994).

There were two major theories as to the etiology of adult-type hypolactasia. The adaptive theory claimed that lactase activity can be increased by lactose feeding (Bolin, Davis 1970, Murthy, Haworth 1970), while other studies contradicted this conception (Flatz, Rotthauwe 1971) and supported the opinion that the activity of small-intestinal lactase is genetically determined (Bayless, Rosensweig 1966, Simoons 1970, McCracken 1971). The prevalence of the LNP genotype varies considerably throughout the world. The cultural historical hypothesis offers the most appropriate explanation for such differences.

1.4.1 Cultural historical hypothesis of hypolactasia

The cultural historical hypothesis was the first attempt to explain differences in the prevalence of hypolactasia. It was suggested by Simoons (Simoons 1970, Simoons 1969) and elaborated by McCracken (McCracken 1970, McCracken 1971), who believed that the occurrence of this disorder was associated with dairy farming. The assumption was that thousands of years ago humans developed a decline in lactase activity, as did all mammals. However, with the adoption of milk for everyday nutrition those able to tolerate it without suffering from diarrhea had more chance of surviving. Probably such individuals had more children than those with hypolactasia (McCracken 1971). The mutation appearing at this point conferred the ability to retain lactase activity throughout life. It was considered that the advantage was even more obvious in populations where milk was of particular value in the absence of other food sources (Harrison 1975). It was shown that the duration of the dairy farming tradition also contributes to differences in the prevalence of hypolactasia among different ethnicities (Simoons 1970, McCracken 1971).

An amplification of the cultural historical hypothesis was proposed by Flatz and Rotthauwe (Flatz, Rotthauwe 1973). They assumed that the high prevalence of lactose tolerance in European populations was due to the ability of lactose to enhance calcium absorption in an environment with low insolation and a low dietary supply of vitamin D. Those who developed the ability to tolerate lactose had a specific selective advantage over lactose-intolerant individuals. The basis of this hypothesis was the high prevalence of pelvic deformity, rickets and osteomalacia among northern European populations due to a lack of cholecalciferol production as well as a poor nutritional supply of vitamin D. Hence those with the ability to digest lactose absorbed calcium better, had less rickets, less pelvic deformity and more children. However, the assumption of a selection pressure based on lack of vitamin D remains controversial and was excluded in a recent study based on simulation approaches (Itan, Powell et al. 2009). The hypothesis of Cook and al-Torki (Cook, al-Torki 1975) proposed that in a highly arid environment lactose-tolerant individuals have had an increased survival rate due to the active absorption of monosaccharides and water from milk. This advantage helped them to survive cholera epidemics and other severe gastrointestinal diseases common in the tropics.

The cultural historical hypothesis is considered to derive most support from the bulk of research to date (Bell, Draper et al. 1973, Zhvavyi, Kozlov et al. 1991, Sahi 1994, Lember, Tamm et al. 1995, Holden, Mace 1997, Burger, Kirchner et al. 2007).

1.4.2 Prevalence of adult-type hypolactasia

The prevalence of adult-type hypolactasia varies considerably between different populations (Sahi 1994). A map of its distribution among European populations has been created by Sahi (1994) and it clearly shows that the frequency of hypolactasia rises from the North to the South of Europe (Flatz, Rotthauwe et al. 1979). However, before 2002 diverse tests were used in different studies for adult-type hypolactasia diagnostics, which made it difficult to create a clear picture of the overall distribution. The discovery of LCT (Enattah, Sahi et al. 2002) and genotyping implementation (Rasinperä, Savilahti et al. 2004) allowed estimation of the prevalence of the lactase non-persistent genotype. Although the number of genotyped populations is growing, data on some populations are lacking.

The prevalence of adult-type hypolactasia used to be considered lowest in the Danish population. Data were obtained from hospital patients by measurement of disaccharidase activity and showed a prevalence from 1.4 to 6.6% with the assumption of an even lower frequency for the whole population (Gudmand-Hoyer, Dahlqvist et al. 1969, Sahi 1994). It has to be stressed that a prevalence study based on hospital patients can be biased, since a patient group is obviously selective. The finding was not supported by molecular diagnostics, and data on genotype frequency in Denmark are thus lacking. Observations in the Swedish population demonstrate a prevalence of adult-type hypolactasia (C/C₋₁₃₉₁₀ genotype) from 5.1% in adults to 14% in children. In the latter group, however, not all subjects were Caucasian, which may bias the results (Almon, Engfeldt et al. 2007). Previously the frequency of lactose malabsorption among Swedish-speaking students in Finland has been reported to be 7.7%, which must be considered a more precise estimate, since the ethnicity of parents and grandparents were studied and confirmed (Sahi 1974). The frequency of hypolactasia among the Finnish population has been estimated at 17% in a number of studies (Jussila, Isokoski et al. 1970, Sahi 1974, Rasinperä 2006). In Estonians the prevalence of adult-type hypolactasia has been found to be 24% in one phenotypic study (Lember, Tamm et al. 1991) and subsequently the same frequency was confirmed by genotyping (Lember, Torniainen et al. 2006).

The prevalence of lactose malabsorption examined by LTT with BHT test (Czeizel, Flatz et al. 1983) and then the frequency of C/C₋₁₃₉₁₀ genotype among Hungarians was found to be 37% in both studies (Nagy, Bogacsi-Szabo et al. 2009).

In the German population considerable differences in the frequency of lactose malabsorption by region have been shown, with figures from 6-9% in North-West

Germany to 23% in the South-West, caused by intensive migration at the end of World War II (Flatz, Howell et al. 1982). Significant differences in lactose malabsorption frequency were also discovered in Austria, where the Eastern populations have had a 25% frequency and Western 15% frequency of lactose malabsorption. The authors explained this finding by historical data about the distribution of Slavs in the eastern part and a Romanized population in the western part of Austria (Rosenkranz, Hadorn et al. 1982). Among healthy young Polish students the prevalence of C/C-⁻¹³⁹¹⁰ has been reported as 31.5% (Madry, Lisowska et al. 2010), and that of lactase deficiency in France as 23% (Cloarec, Gouilloud et al. 1991). The higher prevalence of adult-type hypolactasia in South Europe in comparison with Northern regions has been clearly demonstrated in the Italian population, where the lowest prevalence ranges from 45-57% (Burgio, Flatz et al. 1984, Sahi 1994), increasing in some regions such as Sardinia up to 90% (Schirru, Corona et al. 2007).

The difference in the prevalence of lactase persistence is influenced by ethnicity, higher frequencies prevailing in those populations whose ancestors traditionally consumed milk (Holden, Mace 1997). However, in the process of migration the genotype prevalence can also be influenced by the gene flow. As an example, the introduction of the -⁻¹³⁹¹⁰ T allele to the Indian subcontinent probably occurred via a small number of migrants (Bersaglieri, Sabeti et al. 2004, Burger, Kirchner et al. 2007, Ingram, Mulcare et al. 2009, Itan, Powell et al. 2009, Romero, Mallick et al. 2012). A similar process can explain the presence of this allele in African populations (Mulcare, Weale et al. 2004, Coelho, Luiselli et al. 2005). The possible role of gene flow in reducing the hypolactasia prevalence in non-milking tribes was first surmised by Sahi (1989) and later confirmed by Kozlov (Kozlov, Lisitsyn 1997).

1.4.3 Russian studies concerning hypolactasia

There is a lack of epidemiological data regarding the prevalence of adult-type hypolactasia among Russians. Sahi (1994) reported that reliable data on the prevalence of hypolactasia in the former Soviet Union were limited. There were no clear details as to study participants and selection criteria, nor on the diagnostic methods used.

In order to gain an overview of data on the prevalence of lactose malabsorption/lactase non-persistence/lactose intolerance we collected all

available materials published either in Russian or in English in Appendix 1, with some comments and remarks in the text.

It is surprising that the prevalence of lactose malabsorption among Russians has been found to be as low as 12.5-16.3% in one study (Valenkevich 1987, Valenkevich, Iakhontova 1989). The method used as well as the inclusion criteria were not clearly reported. Contrary to these results, in 1987 a prevalence of 37% was found in 60 Russian students in Estonia (Labotkin 1987). The study, carried out in Estonia, revealed the prevalence of selective lactose malabsorption among an isolated group of Russians (descendants of so-called “Old Believers”) to be as much as 57% (Lember, Tamm et al. 1991). Using LTT and urine test with galactose Kozlov established a frequency of 51% in Russians from the Ural (Kozlov 1996). However, no description of the sample used was presented. A closely similar prevalence of hypolactasia (40-49%) among Russians from other regions of Russia was subsequently reported by the same author (Kozlov 1998, Kozlov, Vershubskaya et al. 2007). The most recent article giving the prevalence of hypolactasia among Russians based on LTT results was published in 2005 (Valenkevich, Iakhontova 2005). As there was no clear sample description it must be assumed that this was the same data as previously published (Valenkevich 1987).

Recently genotyping has been used for the investigation of the prevalence of the LCT gene in Russians. Studies made of DNA samples taken from subjects from different parts of Russia, mostly central, showed the prevalence of the lactase non-persistent genotype among Russians to range from 36 to 50% (Borinskaia, Rebrikov et al. 2006, Delyagin, Kagramanova et al. 2008).

1.4.4 Hypolactasia in other populations settled in the territory of Russia

A multiplicity of ethnic groups have been settled in the territory of Russia for years. The Uralic language group comprises the Finno-Ugrian and Samoyedic branches (Janhunen 2009). Cattle breeding is reported to have started among Nenets during the Soviet Era, and the first farms were organized in Nenets villages in the 1930s (Khabarova, Grigoryeva et al. 2012). The Slavonic groups, however, have a much longer history of milk drinking. The prevalence of hypolactasia among representatives of these groups differs from the Slavonic group, since they are of different origin (Fig. 3).

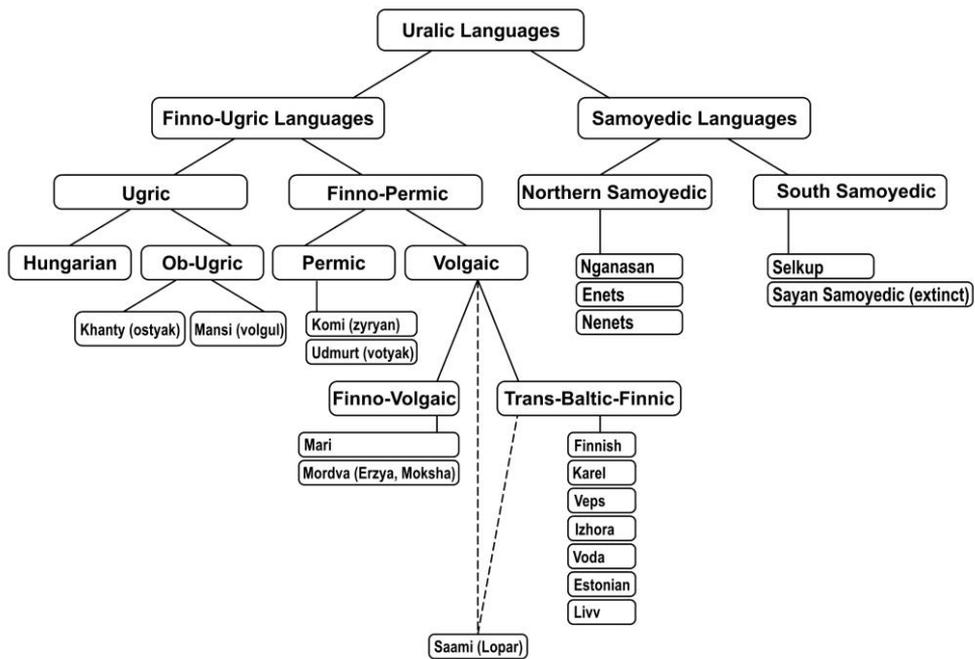


Figure 3. The tree of the Uralic language family (from Great Hungarian Plain 2012 with some modifications)

Data on the prevalence of hypolactasia among the above-mentioned populations are available from different studies.

Among the Khants in West Siberia, the prevalence has been found to be as high as 94% (Lember, Tamm et al. 1995), while according to another study it was 72% in Northern Khanty (Kozlov 1998) and 71% in Northern Mansi. The prevalence of selective lactose malabsorption was found to be 81% among the Maris (Isokoski, Tamm et al. 1990).

Among the indigenous population of Siberia more than 60% have been determined to be milk-intolerant (Zhvavyi, Kozlov et al. 1991). Kozlov has examined the indigenous populations of the polar and related territories of the Russian Federation and established a frequency of hypolactasia of 48% in the Kildin Saami population, 63% in Komi-Izhem, and 88% in West-Siberian Nenets (Kozlov 1998, Kozlov, Vershubskaya et al. 2007).

In small samples from three Finno-Ugric populations: Mordvinians, Karelians and Vepses, the prevalence of hypolactasia has been found to be 11, 11.5 and 11 % respectively (Valenkevich, Iakhontova 1989).

Generally hypolactasia frequencies in indigenous groups in the Arctic and Sub-Arctic territories of Russia are higher than in the "reference" samples of Slavs (Russians, 40-49%) and Permian Finns (Komi-Permiak and Udmurtian, 50-59%) (Kozlov 1998).

The collected data on the prevalence of lactose malabsorption among the Uralic language group are presented in Appendix 2.

1.5 Diagnostics of hypolactasia and lactose intolerance

Hypolactasia can lead to gastrointestinal disorders which remain unspecific. Patients do not always link the appearance of symptoms to milk consumption. Moreover, in some cases symptoms may set in after the indigestion of so-called "hidden" lactose (Eadala, Waud et al. 2009). Simple diagnostic tests have to be used in populations where hypolactasia is considered to be common, i.e. where the prevalence ranges from 15 to 85% (Arola, Tamm 1994).

Nowadays genotyping is the most widely used and appropriate method for the diagnostics of lactase non-persistence (adult-type hypolactasia). The test was proposed by Rasinperä and colleagues (Rasinperä, Savilahti et al. 2004) soon after the LCT gene was discovered (Enattah, Sahi et al. 2002). However, this modern and highly sensitive test cannot be used for the diagnostics of lactose intolerance, and other well-developed tests have thus been adopted.

1.5.1 Measurement of disaccharidase activities in small intestine

Hypolactasia can be diagnosed by enzymatic measurement of lactase activity in small-intestinal biopsy samples (Dahlqvist, Hammond et al. 1968). The method is direct and has been considered the reference method or "gold" standard for any indirect methods (Arola, Tamm 1994, Newcomer, McGill et al. 1975, Heitlinger, Rossi et al. 1991, Dahlqvist, Hammond et al. 1968). The disaccharidase assay gives a direct answer to the question whether a patient is normo- or hypolactic. This is the prominent advantage of this method, while the disadvantage is obviously the invasiveness of the procedure, which renders it unsuitable for primary screening. It

has also been pointed out that the biopsy is unreliable for assessment of lactase activity due to the irregular dissemination of lactase throughout the small-intestinal mucosa (Maiuri, Raia et al. 1991, Hovde, Farup 2009). Disaccharidase values can be affected by such factors as the age and ethnicity of subjects and the intestinal mucosal condition. In cases of mucosal damage the activity of all disaccharidases diminishes, resulting in secondary hypolactasia (Langman, Rowland 1990).

Multiple samples have to be taken from the duodenum, as the level of lactase depends on the biopsy site and there is a gradient of disaccharidase activity in the proximal duodenum. At the inferior duodenal flexure the level of lactase is about 40% less than in the Treitz ligament (Bergoz, Griessen et al. 1981). It has been recommended to use the lactase to sucrase ratio in addition to determination of lactase activity, since this criterion applies almost equally in any part of the small bowel (Newcomer, McGill et al. 1975). The lactase/sucrase cut-off point for hypolactasia is less than 0.3, while that for lactase activity is 10 IU/g protein (Dahlqvist 1984). Recently the Quick Lactase Test has been developed for endoscopic diagnosis of adult-type hypolactasia (Kuokkanen, Myllyniemi et al. 2006, Ojetti, La Mura et al. 2008).

1.5.2 Breath hydrogen tests

The breath hydrogen test with lactose (BTI) represents an indirect test for the diagnosis of lactose malabsorption. Nowadays this test is considered the most reliable, non-invasive and inexpensive technique and accurately reflects the LP/LNP genotype (Marton, Xue et al. 2012). False-positive results may ensue in cases of small bacterial overgrowth (Nucera, Gabrielli et al. 2005). Physical activity prior to the test (Payne, Welsh et al. 1983), use of acetylsalicylic acid (Flatz, Lie 1982) or antibiotics (Gilat, Ben Hur et al. 1978) and smoking (Tadesse, Eastwood 1977) before the test increase the rise in hydrogen concentration.

The sensitivity of the test has been found to range from 69 to 100%, specificity 89–100% (Newcomer, McGill et al. 1975, Buning, Genschel et al. 2005, Szilagyi, Malolepszy et al. 2007). A dosage of 25g of lactose, corresponding to the amount found in 500 ml of milk, has been utilized in recent testing. Recent breath testing consensus proposed a test duration of 4 h, a sample interval of 30 min and a cut-off value of 20 ppm above the baseline (Gasbarrini, Corazza et al. 2009); however, different research groups still use different variables for the breath hydrogen test

(Oberacher, Pohl et al. 2011). There is so far no final agreement as to the lactose dosage and cut-off level, sample interval and duration of testing.

1.5.3 Blood and urine tests

The lactose tolerance test (LTT) is based on measurement of the increase in blood glucose after an oral lactose load. A standard dose of 50g lactose diluted in water is given to patients prior to the test and capillary blood samples are taken every 15, 20 or 30 min up to 2h. The number of samples depends on the interval between samples and can vary from two to six. A rise in blood glucose of less than 1.1 mmol/L is regarded as hypolactasia, while a rise of more than 1.7 mmol /L is indicative of normolactasia. A cut-off at 1.5 mmol/L is used (Arola, Tamm 1994). The specificity of LTT is 77-96% and sensitivity 76-94% as estimated by using an assay of disaccharidases as reference method (Arola, Tamm 1994). In diabetic persons an abnormal glucose level might alter the results (Lerch, Rieband et al. 1991). Although the lactose tolerance test has long been used it still needs standardization for the dose of lactose, the volume and temperature of the water used and the length of the period in which gastrointestinal symptoms are recorded. This requirement will improve the reliability and comparability of the test results (Peuhkuri, Vapaatalo et al. 2000).

The lactose tolerance test with ethanol (LTTE) uses measurement of galactose together with glucose (Jussila 1969). Ethanol ingestion inhibits the metabolism of galactose into glucose in the liver, rendering the test more sensitive. The method was used by Sahi in his family studies (Sahi, Isokoski et al. 1973, Sahi 1974). Later Isokoski and associates (1972) proposed that a reliable diagnosis can be made from one blood sample taken at 40 min and that only the determination of galactose is necessary. In the case of hypolactasia the blood galactose concentration is less than 0.3 mmol/l at 40 min after lactose and ethanol ingestion (Isokoski, Jussila et al. 1972). Simple LTTE is more reliable than LTT, since only one blood sample is needed, the test is not affected by the elevated glucose level in diabetic patients, and it is less vulnerable to gastric emptying rates compared to LTT. A simplified modification of LTTE is LTTE based on urinary galactose determination (LTTE U). The urinary sample is taken instead of blood at 40 min after intake of 150mg/kg of ethanol together with 50g lactose in 400 ml water. The test has been shown to be reliable (Arola, Koivula et al. 1982). The same technique is used in a

strip test, LTTE (US), which is even more suitable for practical needs (Arola, Koivula et al. 1987).

1.5.4 Genotyping

The identification of SNPs associated with adult-type hypolactasia has made it possible to test whether a patient has the LP or LNP genotype (Enattah, Sahi et al. 2002). A test specificity of 100% and sensitivity of 93% were shown for children over 12 years (Rasinperä, Savilahti et al. 2004). The majority of relevant studies designed to compare the genetic test with other direct and indirect methods of hypolactasia diagnostics have shown high concordance between tests (Ridefelt, Hakansson 2005, Szilagyi, Malolepszy et al. 2007, Kerber, Oberkanins et al. 2007, Krawczyk, Wolska et al. 2008, Di Stefano, Terulla et al. 2009, Marton, Xue et al. 2012).

A significant correlation between lactase enzyme activity and the C/C₋₁₃₉₁₀ genotype implies that genotyping of the C/T₋₁₃₉₁₀ variant can be used as a primary screening test for adult-type hypolactasia in clinical practice in a European population (Jarvela 2005). The genetic test is less inconvenient and time-consuming for patients compared with other tests in hypolactasia diagnostics. It is cost-effective for the health care system in Europe, though it remains expensive for the Russian system. The high specificity and sensitivity and also the need to perform this test only once in a lifetime gives an advantage for genotyping in the process of differential diagnosis of unspecific gastrointestinal symptoms.

1.6 Impact of hypolactasia on individual health

1.6.1 Symptoms of hypolactasia

Unspecific abdominal complaints are one of the common reasons for seeking medical care both for children and for adults. Such symptoms as diarrhea, flatulence, borborygmi, abdominal pain and distension are very often taken to reflect irritable bowel syndrome without differential diagnosis against lactose intolerance (Shaw, Davies 1999, Farup, Monsbakken et al. 2004). On the other hand it has been shown that the most common GI symptoms characterizing

intolerance to lactose are flatulence, bloating, diarrhea, gurgling, abdominal distension and abdominal cramping (Suarez, Savaiano et al. 1995, Casellas, Varela et al. 2009, Savaiano, Boushey et al. 2006, Sahi 1974, Pohl, Savarino et al. 2010, Haberkorn, Ermens et al. 2011, Jellema, Schellevis et al. 2010). Especially for regions where hypolactasia is common among the population the clinical diagnosis of lactose intolerance is challenging for medical practitioners (Villako, Maaros 1994, Gudmand-Hoyer 1994).

The development of symptoms depends on the amount of milk consumed as well as individual sensitivity (Hertzler, Huynh et al. 1996, Suarez, Savaiano et al. 1997). The presence of lactose in the large intestine does not always result in gastrointestinal symptoms. Lactose intolerance itself is diagnosed in case when lactose malabsorption is associated with the most common abdominal symptoms, which are lactose-induced. However, clinical intolerance to lactose may not be synonymous with low lactase activity (Lember 2002). It has been shown that people who identify themselves as lactose-intolerant may mistakenly attribute their unspecific symptoms to this disorder (Suarez, Savaiano et al. 1995), whereas such symptoms may often be unrelated to lactose malabsorption (Casellas, Aparici et al. 2010). Intolerance to lactose is not usually life-threatening in that symptoms can be eliminated by removal of lactose from the diet (Swallow 2003). However, the avoidance of or significant reduction in the amount of milk consumed leads to insufficient calcium intake and may provoke unfavorable health effects (Bannan, Levitt 1996, Carroccio, Montalto et al. 1998, Nicklas, Qu et al. 2011) such as poor bone health and osteoporosis, a higher incidence of colon cancer, and a higher risk of developing diabetes.

The onset of symptoms is clearly explained by the mechanism of lactose utilization. In cases of lack of enzymatic (lactase-phlorizin hydrolase) activity lactose passes to the large intestine. The resultant osmotic effect causes diarrhea, (Launiala 1968), while increased levels of fermentation in the intestine result in the increasing production of gases and therefore flatulence, gurgling and abdominal distension (Dahlqvist, Hammond et al. 1968, Arola, Tamm 1994, Swallow, Poulter et al. 2001).

1.6.2 Tolerance to different amounts of lactose

Lactose is a milk sugar contained in different amounts in all milk products. The concentration of lactose in milk is inversely related to the concentration of lipids

and the concentration of casein. Usually it is mistakenly believed that less fatty milk contains less lactose. However, in fact non-fat milk contains more lactose. Subjects who are lactose-intolerant have to be aware of the lactose content in different types of food to avoid an overload of lactose consumed. As an example, 1 cup (245g) of whole milk contains 11g of lactose, while the same portion of non-fat milk can contain 12–14g of lactose. It is also worthy of note that dry milk has the highest amount of lactose – 37.5g of lactose per 100g of milk powder (Shils, Shike c2006).

The development of symptoms depends on the amount of milk consumed as well as individual sensitivity. It has been shown that subjects with hypolactasia can tolerate moderate quantities of milk, up to 12g of lactose/250 ml of milk (Hertzler, Huynh et al. 1996, Suarez, Savaiano et al. 1997). Moreover, if the daily dose of lactose is consumed in small portions and also with a meal, the probability of symptoms occurring is lower (Suarez, Savaiano et al. 1997).

Some studies have identified a few persons who may be sensitive to very small doses of lactose, even 3–5g (Bedine, Bayless 1973, Gudmand-Hoyer, Simony 1977, Gudmand-Hoyer, Simony 1977). One recent work has speculated that many drugs used in the treatment of common gastrointestinal conditions such as dyspepsia, symptoms of irritable bowel diseases and inflammatory bowel diseases contain lactose as an excipient (Eadala, Waud et al. 2009). As the study in question shows no link between the use of such drugs and the appearance of lactose intolerance symptoms, this supposition would seem unjustified. Other findings show that even very small amounts of lactose, including lactose from “hidden” sources, can cause severe symptoms and so-called “systemic lactose intolerance” with many abdominal and non-abdominal symptoms. In most of these cases the diagnosis remains open because lactose intolerance has not been suspected (Matthews, Waud et al. 2005). This finding remains somewhat controversial in that there is no physiological basis for such a systemic effect from lactose.

It is important to mention here that in the case of small doses of lactose consumed by lactose-intolerant persons, GI symptoms could appear for reasons other than lactose (Vesa, Korpela et al. 1996).

1.7 Basis for aims of study

With the exception of two recent studies of C/T₋₁₃₉₁₀ genotype frequency, made among a group of Russians settled in different (mostly central) parts of Russia, there are no data related to the lactase-persistent genotype in Russians, especially in Northern Russians. Moreover, no genetic studies of LCT have previously been made in the Nenets population.

A number of unspecific gastrointestinal problems have presented a challenge to everyday medical practice. In Russia the lack of knowledge of adult-type hypolactasia among medical practitioners makes the diagnosis even more difficult. Another issue is that some patients associate their unspecific abdominal symptoms with milk consumption, making a self-diagnosis of lactose intolerance and excluding milk products from their diet.

Since the genotyping method is nowadays well developed, every discovery of a novel genotype becomes less unique. However, determination of the significance of every new genotype might be more challenging. The novel G/A₋₁₃₉₁₄ was previously found and reported in two persons in Europe, but its possible significance was neither suggested nor investigated.

The gene flow between neighboring populations influences the frequency of LP/LNP genotype and alters the prevalence of adult-type hypolactasia. There are no relevant data in the literature regarding the gene flow between the Nenets and the Russian populations.

2 Aims of the study

The aims of this study were:

- 1) to investigate the prevalence of adult-type hypolactasia (lactase non-persistence, C/C-13910 genotype) among Northern Russians and nomadic Nenets
- 2) to establish the frequency of various gastrointestinal symptoms and consumption of dairy products in the young Northern population with adult-type hypolactasia
- 3) to study the family history of carriers of the novel G/A-13914 genotype variant for lactase persistence/non-persistence found in gastroenterological practice*
- 4) to demonstrate the influence of gene flow from neighboring ethnic groups on the prevalence of lactase non-persistence

*The original study plan was completed because of finding of a novel G/A-13914 genotype variant in author's gastroenterological practice.

3 Subjects and methods

3.1 Study subjects

Several study groups were used in the investigations seeking to answer the study questions. The various groups together with their age, sex distribution and type of samples taken are presented in Table 1.

Table 1. Study groups (I, II, III, IV)

Groups	Article	Number of participants	Mean age/years	Female %	Genotyping	Questionnaire
Students in total:	II	518	19.8 (17–26)	78.2	+	+
group 1	I	241	20.5 (17–26)		+	+
group 2	II	277	19.3 (17–26)		+	+
Family study	III	9	42.4 (6–86)	70.0	+	+
Nenets	IV	181	43.5 (19–80)	91.0	+	+

3.1.1 Students from North West Russia

The students, aged 17 to 26 years, were randomly selected for the investigation from different departments of the Northern State Medical University (NSMU), Arkhangelsk, Russia during the period from 2006 to 2008. Blood samples (n=241) or buccal samples (n=277) were taken to genotype for lactase activity, defining the C/T₋₁₃₉₁₀ variant in 518 subjects in total. All subjects filled in a questionnaire covering personal data, self-reported health status, milk consumption habits, ethnicity and place of birth as well as their parents' and grandparents' ethnicity and place of birth. All students selected for the investigation were healthy at time of study, i.e. they had no acute diseases.

3.1.2 Students with Russian background (I)

Among the first group of students (241 subjects) 231 reported their origin as Russian. However, according to the given origins of the grandparents (at least three out of four grandparents) only 149 were considered ethnically Russian. Of the remainder, 61 gave no data on grandparents and were categorized under “missing information” as to ethnicity. Twenty-one of those who reported their origin as Russian had at least 2 grandparents of another origin, mostly Ukrainian, but also Byelorussians, Komi, Mordvinian and other.

3.1.3 Students from the Arkhangelsk region (II)

For the estimation of milk consumption and GI symptoms among LNP genotype carriers the whole randomized group of students was taken (n=518, comprising first intake of 241 students and the second intake of 277 students). The majority of participants were born in the Arkhangelsk region, the rest in different regions of north-west Russia. The same analysis of ethnicity was applied for these 277 students. However, in the final analysis of milk consumption and GI symptoms among LP/LNP carriers ethnicity was not taken as a variable.

The mean age for the whole group was 19.8 ± 1.7 . Altogether 78.2% of all participants were female.

3.1.4 Family analysis of the G/A-13914 variant (III)

Blood samples for the assessment of the LP/LNP genotype were collected from 148 patients aged from 23 to 60 years referred for medical consultation with a gastroenterologist (the author) from the Northern State Medical University (NSMU), Arkhangelsk, due to various abdominal complaints. There were patients either with self-estimated lactase intolerance or without any complaints of intolerance to milk products. All patients completed the same above-mentioned questionnaire and blood samples for genotyping were taken from all of them.

Among this patient group one subject was found to have a novel genotype in G to A position. Study was then made of all available members of this person's family. Genotyping of eight family members was done to determine the inheritance of the

new variant of LP/LNP genotype, G/A-13914 (Appendix 3). All were relatives of this female 58-year-old patient, who was referred to the gastroenterologist because of stomach pain. All adult family members filled in the questionnaire and gave informed consent. It was not permitted to take blood for genotyping from the children of this family. The patient was referred for endoscopic investigation of the stomach because of ulcer anamnesis and abdominal complaints. After informed consent, two biopsy specimens were taken from the distal duodenum for the measurement of disaccharidase activities. These samples were immediately wrapped in parafilm, placed in air-tight tubes and frozen to -60°C . The tubes were then transferred in dry ice to the scientific laboratory of the Hospital for Children and Adolescents, University of Helsinki.

3.1.5 Lactase non-persistence among Nomadic Nenets (IV)

The material for this study was collected in four settlements of the Nenets Autonomous Okrug (NAO), Archangelsk Region, where the indigenous Nenets live (map in IV). The study included a total of 181 native Nenets.

Every third member of the whole Nenets population of this region was invited to answer a questionnaire and to undergo buccal sampling for genotyping by a doctor from a rotational team. If a selected person was out of the settlement when the study was conducted, the next on the list was invited to participate in the study. Men were absent more often than women.

We used the same questionnaire for other study groups and took buccal samples from all contactable adult participants. All 181 persons involved considered themselves as Nenets. However, according to grandparents' ethnicity only 91 (50.3%) were accepted as native Nenets, having all four grandparents of Nenets origin. Those with one, two or three Nenets grandparents (90 in total) were also taken for comparison.

In the process of genotyping, four buccal samples remained undetermined. Therefore, 89 subjects of clear Nenets origin aged 19-80 (mean 45.8), of whom 91.0% were female and 9.0% male, were selected for the LNP genotype frequency analysis.

All study participants gave their informed consent to participate in the study.

3.2 Methods

3.2.1 Genotyping method (I, II, III, IV)

DNA was amplified by polymerase chain reaction (PCR). We used Taq polymerase (Dynazyme, Finnzymes, Espoo, Finland) with the conditions described elsewhere. The forward primer used was 5'-CCTCGTTAATACCCACTGACCTA -3' and the reverse primer 5'- GTCACITTTGATATGATGAGAGCA-3', which cover approximately 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 products were purified using Shrimp Alkaline Phosphatase (USB) and Exonuclease I (New England Biolabs) at 37°C for 60 min and at 80°C for 15 min.

In sequencing the 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 10 s, at 55°C for 5 s and at 60°C for 4 min. The sequencing reaction followed purification on 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 sequence obtained was analyzed using Sequencher 4.6. software (Gene Codes, USA).

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

For genotyping, either blood or buccal samples were taken. The blood samples were delivered and genotyped at the Department of Medical Genetics, University of Helsinki. The buccal samples were genotyped in the Forensic Laboratory in the University of Tampere.

3.2.2 Assay of intestinal disaccharidases for family study (III)

Upon informed consent, two biopsy specimens were taken from the distal duodenum of the index person for the measurement of disaccharidase activities.

These samples were immediately wrapped in parafilm, placed in air-tight tubes and frozen to -60°C . The tubes were then transferred in dry ice to the scientific laboratory of the Hospital for Children and Adolescents, University of Helsinki. Measurement of disaccharidase activities was made by the method of Dahlqvist (1984), originally described by Launiala K and associates (Launiala 1968, Launiala, Perheentupa et al. 1964). A commercial preparation of disaccharidases (Sigma G-5003, I-4504 and G5160) was used as positive control. The test was modified for smaller volumes compared to the original report and is nowadays made on microtiter plates.

3.2.3 Questionnaire (I, II, III, IV)

The questionnaire used in this series was granted by (Sahi 1974) and previously validated in a Finnish study. It was translated from Finnish into Russian (Appendix 4) and no changes were made either in the order or the content of the questions. The only item added to the end of questionnaire was the table collecting information on ethnicity and place of birth of the respondents, their parents and grandparents. Among the student group there were no unreturned questionnaire forms, since all questionnaires were filled in the presence of the researcher. Only those who were absent on the day of study or refused to participate were not included. An example of the data collection process was shown in Article I. Among the patients the response rate was 100%, since each subject participated of his or her own volition. The response rate among the Nenets was also almost 100%, as the study was made during annual medical examinations when all Nenets attend for check-up by a doctor of the rotational team.

3.2.4 Evaluation of gastrointestinal complaints (II)

Gastrointestinal symptoms were assessed by the question “Have you ever experienced the following symptoms and with what frequency?” The following symptoms were listed: stomach-ache, regurgitation, flatulence, heartburn, nausea and diarrhea. The question was not intended to clarify the connection between milk consumption and symptoms. The various answers were “Every day”, “At least once per week”, “Every second week”, “Sometimes”. If the participant had never had a particular symptom the item was left blank. We combined the answer “Sometimes” together with no symptoms into one group and designated it

“Without GI symptoms”. The second group included the remaining variables and was taken for analyses of differences between genotypes.

The influence of food on the appearance of GI disorders was assessed by the question “Do you have any GI disorders if you consume the following types of food?”, six types of food being included. Subjects were asked to put “Yes” or “No” opposite every type of food.

Comparison was made between the lactase non-persistent group (C/C₋₁₃₉₁₀) and the lactase-persistent group (C/T₋₁₃₉₁₀ and T/T₋₁₃₉₁₀) in the student group for all the above-mentioned questions.

3.2.5 Evaluation of milk consumption by questionnaire (II)

In the last part of the questionnaire subjects were asked to define their everyday milk product consumption from 0 to more than 5 glasses per day. The choices were “Not at all”, “1–2 glasses”, “3–5 glasses” and “More than 5 glasses” of milk /sour milk per day. There was also an option to declare consumption less than 1–2 glasses per day for those who consumed milk very rarely. For the analysis all answers were classified into two groups: less than daily milk consumption or none and daily milk consumption.

Comparison was made between the lactase non-persistent group (C/C₋₁₃₉₁₀) and the lactase-persistent group (C/T₋₁₃₉₁₀ and T/T₋₁₃₉₁₀) among the students for all the above-mentioned questions.

3.2.6 Method of gene flow study (I, IV)

The gene flow was charted by the method of concordance of grandparents’ ethnic origin (Senior, Bhopal 1994). The term ethnicity was used in the biological sense in our study. The ethnicity of a person was defined as Russian if having at least three grandparents of Russian origin. In the case of the Nenets we used more strict selection, defining as a Nenets only those who had all four grandparents of Nenets origin.

3.2.7 Statistical methods (I, II, IV)

We used frequency and cross-tabulation analysis (I, IV). Differences between groups were tested by χ^2 test. $P < 0.05$ was considered statistically significant.

Odds ratios (OR) with 95% confidence interval (CI) for -13910C/C genotype in the logistic regression analysis were calculated for gastrointestinal symptoms in total and symptoms upon consumption of several types of food, and for volume of milk/sour milk consumption (II). Statistical analyses were performed with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA).

3.2.8 Ethical approval (I, II, III, IV)

Every part of the study was approved by the Ethics Committee of NSMU (Northern State Medical University, Arkhangelsk), extract from minutes No 08/06 from 29.11.2006, No 07/08 from 09.06.2008.

Additional approval was obtained from the Health Care Department of NAO (Nenets Autonomous Okrug) for the Nenets study (IV).

4 Results

4.1 Prevalence of adult-type hypolactasia of North Russia (I, IV)

The prevalence of adult-type hypolactasia (C/C₋₁₃₉₁₀) among the 149 young Russians who were born and lived in North-West Russia was found to be 35.6%, while the frequencies of C/T₋₁₃₉₁₀ and T/T₋₁₃₉₁₀ were 51% and 13.4% respectively.

The prevalence of the lactase non-persistence genotype (C/C₋₁₃₉₁₀) among the Nenets having only Nenets in two previous generations was found to be 90% (Table 2).

Table 2. Prevalence of lactase persistent/non-persistent genotype by ethnicity (I, IV)

	C/C ₋₁₃₉₁₀		C/T ₋₁₃₉₁₀ or T/T ₋₁₃₉₁₀		Total	
	n	%	n	%	n	%
Russians	53	35.6	96	64.4	149	100
Nenets	80	90.0	9	10.0	89	100
Total					238	100

4.2 Gastrointestinal symptoms and milk consumption in young Northern population with adult-type hypolactasia (I, II)

Among carriers of the LNP genotype in the student study group (N=518) there were 10 per cent units fewer milk-consumers in comparison with the LP group (46.1 and 56.6%). The differences were statistically significant ($p = 0.03$). Sour milk consumption involved no significant differences in symptoms between LP and LNP subjects.

Milk consumption was associated with GI disorders in 13.3% of subjects with LNP genotype and in 7.1% with LP genotype. Complaints of GI disorders caused by milk consumption were different between genotypes ($p = 0.02$).

Of all types of food analyzed only milk brought out statistically significant differences in symptoms between LP and LNP subjects (OR =1.95, CI 1.03-3.69).

In regression analysis there was no connection between food consumed and the appearance of symptoms besides milk consumption.

4.3 Family history of the index person with rare genotype variant (III)

A rare variant G to A residing 13914 bp upstream of the LCT gene was identified in one subject from the patient group. This woman is a carrier of the most frequent variant of LNP genotype, C/C₋₁₃₉₁₀, and she carries the rare variant G/A₋₁₃₉₁₄. The results of measurement of disaccharidase activities in this subject demonstrate an increased level of lactase in two independent biopsy specimens. In both samples lactase activity was higher than the cut-off for adult-type hypolactasia, 10U/g protein, while the lactase/sucrase ratio was decreased. In this person the disaccharidase activities were within the same range as in the carriers of the lactase-persistent genotype C/T₋₁₃₉₁₀ and T/G₋₁₃₉₁₅ variants. The results of DNA genotyping in members of this family are presented in Appendix 3.

Among eight family members whose DNA was available for genotyping five had the lactase non-persistent genotype C/C₋₁₃₉₁₀. The rest had the C/T₋₁₃₉₁₀ variant. None of the family members carried the T/T₋₁₃₉₁₀ variant. The G>A₋₁₃₉₁₄ variant was also present in the mother (F1) and sister (F5) of the index person. The daughter (F6) of the individual F5 also carried G>A₋₁₃₉₁₄, but she was LNP in C/T variant. No DNA was available from the deceased father.

All investigated family members were of Russian origin, and were born in North West Russia. It is known that the grandparents of the index person were also of Russian origin, except for the grandfather on the mother's side, who originated from Poland.

Members of the subject's family consumed very small amounts of milk daily (less than 1 glass per day) independent of genotype. They reported consuming other dairy products rather than milk itself, but also not more than 2 glasses per day. Of all family members neither the index person nor other carriers of ₋₁₃₉₁₄ G>A reported problems from milk consumption. However, the index person's second sister (F8, Appendix 3) and her son (F9), both of them carriers of the lactase non-persistent genotype ₋₁₃₉₁₀ C/C without ₋₁₃₉₁₄ G>A variant, reported gastrointestinal problems related to milk ingestion.

4.4 Influence of the gene flow from neighboring ethnic groups on the prevalence of lactase non-persistence (IV)

The prevalence of the lactase non-persistent genotype (C/C₋₁₃₉₁₀) among the Nenets having only Nenets in two previous generations was found to be 90%. The prevalence among others who regarded themselves as Nenets and reported three, two or one grandparent of Nenets origin was shown to be 72%, 60% and 28%, respectively (p<0.0001, Table 3).

Of all grandparents 78.5% were of Nenets origin, 11% were Komi, 9.4% were Russians, while others (Ukrainians, Byelorussians and others) comprised less than 1% each.

Table 3. Frequency of C/T₋₁₃₉₁₀ by grandparents' origin

Number of grandparents of Nenets origin	C/C ₋₁₃₉₁₀		C/T ₋₁₃₉₁₀ or T/T ₋₁₃₉₁₀		Total	
	n	%	n	%	n	%
Four grandparents	80	90	9	10	89	100
Three grandparents	13	72	5	28	18	100
Two grandparents	31	60	21	40	52	100
One grandparent	5	28	13	72	18	100
Total	129	73	48	27	177	100

5 Discussion

Healthy young Northern Russians have the lactase non-persistent genotype with a frequency of 36%. The figure can presumably be extrapolated to the whole Northern population, since the student study group was entirely representative of that. The prevalence of the C/C-¹³⁹¹⁰ genotype among indigenous Nenets was discovered to be much higher and was estimated to be as much as 90%. Patients with GI complaints evinced an approximately equivalent frequency of LNP genotype compared to young Northern Russians: of patients examined one in three was identified as a carrier of C/C-¹³⁹¹⁰. The novel genotype variant G/A-¹³⁹¹⁴ was identified in one of the participants in the patient group who carried the C/C genotype at -¹³⁹¹⁰ position (III). In the example from the student group we showed that the C/C-¹³⁹¹⁰ genotype had an influence on the amount of milk consumed as well as on the frequency of symptoms induced by milk consumption.

5.1 The representativity of the study groups

Age and gender have no influences on the LNP genotype frequency. For the estimation of LNP genotype prevalence subjects can thus be taken irrespectively to their age and gender. Since ethnicity plays a significant role in LNP genotype frequency (Sahi 1994) the ethnic background of the study subjects was taken into consideration.

The randomized group of students was recruited from different faculties of Medical University. This cohort may be considered representative of the whole Northern Russian population as far as the vast majority of them were born in North-West Russia and reported themselves as Russians.

The patients who were referred for consultation with a gastroenterologist were taken into the investigation without randomization. All referred patients were informed of the aim of the study and expressed willingness to participate. Patients reporting any unspecific abdominal complaints such as diarrhea, bloating, abdominal cramping, flatulence or audible bowel/borborygmi were asked to

participate in the study. The interest was to establish whether the prevalence of the LNP genotype among subjects with GI disorders differed from that in the healthy student group. The prevalence of C/C-13910 was found here to be almost equal in both healthy students and patients group.

Among the Nenets the prevalence of lactase non-persistence was found to be much higher than among neighboring populations, in concordance with the cultural historical hypotheses (Simoons 1969, Simoons 1970, McCracken 1971). Nenets were taken to our study in randomized order according to the official administrative lists and every third patient was then marked and invited. In as far as the Nenets still follow a nomadic life style it was not always manageable to get every “third” according to the list. Men were out of the settlements quite often. In that case the next on the list was taken for study.

The majority of the participants in this series were women. Since neither gender nor age affects C/C-13910 frequency, we may claim to have found the actual prevalence of adult-type hypolactasia among the study participants. However, gender and age differences can influence participants’ answers on symptoms. In reporting subjectively adults behaved more seriously in filling the questionnaire compared to students. One may assume that the young age of the respondents and the absence of true interest in the results of the investigation may somehow bias the results of questionnaire, but in no way influences the prevalence of the genotype.

5.2 The validity of methods used through the study

The genotyping method used in this study is a direct method of diagnosing genetic changes, with 100% specificity for the estimation of the prevalence of adult-type hypolactasia in a population older than 12 years (Jarvela 2005). It has been clearly shown that the C/C-13910 genotype correlates with low lactase activity, whereas C/T-13910 and T/T-13910 genotypes maintain a higher lactase activity (Rasinperä, Savilahti et al. 2004, Enattah, Sahi et al. 2002). The use of genotyping thus gives a possibility to avoid unnecessary biopsy for lactase non-persistence diagnostics (Enattah, Sahi et al. 2002, Kuokkanen, Enattah et al. 2003, Rasinperä, Savilahti et al. 2004, Jarvela 2005).

The method shows the actual frequency of the LNP genotype and thus makes it possible to estimate the prevalence of adult-type hypolactasia in different ethnic populations. The genotyping for C/C-13910 identification has previously been used

in two Russian studies (Borinskaia, Rebrikov et al. 2006, Kozlov 2004), but has never been used in a Northern Russian population nor in a population of nomadic Nenets. The argument for genotyping is that the method is not confounded by environmental and subjective factors which may affect the results of the other tests (Jarvela 2005). On the other hand, genotyping is not suitable for the estimation of lactose intolerance, since carriers of the LNP genotype are not always lactose-intolerant (Haberborn, Ermens et al. 2011).

In the Family study measurement of disaccharidase activities was undertaken to establish the significance of the novel G/A₋₁₃₉₁₄ variant. The method of Dahlqvist (1984), described by Launiala K and associates (Launiala, Perheentupa et al. 1964) was applied for this purpose. The method has previously been used in many studies (Sahi 1974, Savilahti, Launiala et al. 1983, Heitlinger, Rossi et al. 1991). Here the whole process was conducted in accordance with the instructions of the scientific laboratory of the Hospital for Children and Adolescents in the University of Helsinki.

The present questionnaire has previously been used in other studies (Sahi 1974). All persons here were of active age and therefore capable of understanding the questions, and moreover had the possibility to ask the researcher in cases of doubts regarding meaning. We thus trust the answers to be accurate. However, since it was obvious that patients were more interested in participation than the students, patients' answers might be the more reliable. The questions on milk consumption caused some difficulties in that originally the questionnaire was designed for the population consuming milk in ordinary amounts. According to the results here Northern Russians, especially young persons, consume very small amounts of milk daily, some of them less than one glass per day. Hence they had difficulties in reporting daily amounts of consumed milk.

The ethnicity question was the most challenging especially for young Russians, since some of them were not aware of the ethnicity of their grandparents. In cases of complete unawareness regarding the origins of their ancestors, participants were allowed to leave the box empty. These cases were excluded from the final analysis of lactase non-persistence prevalence among Russians and were kept as a group "others" apart of Russians. Nenets subjects as well as the patient group experienced no challenges with the ethnicity definition. All patients were adults and were aware of the ethnicity of their ancestors. Nenets, who traditionally preserve their ethnic features with particular care, were absolutely sure of their family history.

It is always a challenge to determine Russian ethnicity since there have been many mixtures between neighbors. Moreover, during the Soviet era the term

“ethnicity” was substituted by the term “nationality” (Kozlov, Vershubskaya et al. 2007). Especially young people lack knowledge of their ancestry, so that it was a demanding task to collect information on this aspect. The method of concordance of grandparents’ national origin was chosen to ascribe ethnicity (Senior, Bhopal 1994). This approach gives an opportunity to count the number of grandparents belonging to the same ethnicity. A person was considered to be Russian if having at least three Russian grandparents. For the Nenets study group we enhanced the rule to take all grandparents of Nenets origin into account, which has not been used before. Application of this method brought to light fairly important findings. Although the administrative list includes Nenets only, the self-reported ancestors of the participants were not exclusively Nenets. Moreover, the prevalence of the LNP genotype had a strong correlation with the number of indigenous grandparents. This supports the cultural historical hypothesis of hypolactasia and demonstrates the difference between biological and self-identified ethnicity.

5.3 The prevalence of adult-type hypolactasia

The prevalence of adult-type hypolactasia differs among populations. A map of prevalence was created in 1994 (Sahi 1994) based on studies made by indirect methods such as LTT, LTTE, BHT or measurement of disaccharidase activity in the small intestine. The implementation of the genotyping method soon after the discovery of LCT has allowed estimation of the prevalence of the LNP genotype among different populations. Many European populations have already been genotyped for LNP. In Russia the genotyping method for LNP has been used twice prior to our study (Borinskaia, Rebrikov et al. 2006, Kozlov 2004). Neither Northern Russians nor Nenets were involved in these studies.

The prevalence of lactose malabsorption was shown to be lowest in Northern Europe rising towards the South of Europe. There are a limited number of studies investigating the prevalence of lactose malabsorption among Russians. The available works show a prevalence of lactose malabsorption obtained by different methods from 12.5 to 57% (Valenkevich 1987, Valenkevich, Iakhontova 1989, Lember, Tamm et al. 1991, Kozlov 1996, Kozlov, Vershubskaya et al. 2007). According to Russian studies using the genotyping method (Borinskaia, Rebrikov et al. 2006) the prevalence of adult-type hypolactasia among Russians living in the central part of the territory depended on area of residence and ranged from 36% to 50%. Our study, involving young Russians born in the North West of Russia,

demonstrated a prevalence of adult-type hypolactasia as high as 36%, which is partly in accord with previous data. The novelty of the study was that subjects were carefully analyzed from the point of view of their ethnicity and the method of concordance of grandparents' ethnicity (Senior, Bhopal 1994) was applied for the purpose. Since the study group was representative for the whole Northern Russian population it may be asserted that this is the true prevalence of lactase non-persistence (adult-type hypolactasia, C/C-13910 genotype) among the Northern Russian population.

It was previously known that hypolactasia frequencies in indigenous groups in the Arctic and Sub-Arctic territories of Russia are higher than in the "reference" samples of Slav (Russian, 40-49%) and Permian Finn (Komi-Permiak and Udmurtian, 50-59%) groups (Kozlov 1998). In the studies mentioned the prevalence of lactose malabsorption among Nenets from Siberia was shown to be 78% in a sample of 9 persons. In early studies using other methods apart of genotyping the prevalence of adult-type hypolactasia among the West Siberian Nenets population was shown to be 88% (Kozlov, Vershubskaya et al. 2007). Among all indigenous groups inhabiting different territories of the Russian Federation the highest prevalence was established among the Khants (near the River Ob), with a figure of 94% (Lember, Tamm et al. 1995) and 89% among Chukchi, while the frequency of LNP in Komi-Permyak was estimated as 42% (Kozlov, Vershubskaya et al. 2007).

Differences in earlier histories can explain such genetic differences in neighboring populations. The Nenets who traditionally preserve their ethnic features with great care, are known to be a non-milk consuming people (Mirov 1945). Therefore a high frequency of LNP was assumed to be found among their population. The current study showed that the prevalence of the C/C-13910 genotype among Nenets who had all parents and grandparents of Nenets origin was as high as 90%. The prevalence among others with three, two or one grandparent of Nenets origin was respectively lower. Use of the method of concordance of grandparents' origin (Senior, Bhopal 1994) made it possible to bring to light the actual prevalence of adult-type hypolactasia among the indigenous Nenets.

5.4 Milk consumption and gastrointestinal symptoms

According to the present results milk consumption is very low among the young Russian population. However, our study showed that lactase non-persistent subjects have a higher frequency of GI disorders associated with milk even in the population consuming very small amounts of milk. There has been a tendency over the last two decades to reduce milk consumption in Northern Europe (Weaver 2010). Among our participants the amount of milk consumed was even less than in other countries (Lember, Torniainen et al. 2006).

The study also demonstrated that among LNP carriers there were fewer milk consumers compared to LP subjects. Lower milk consumption among LNP subjects has been demonstrated among other populations (Lember, Torniainen et al. 2006, Kull, Kallikorm et al. 2009, Laaksonen, Mikkil et al. 2009). Interestingly, milk was the only type among six tested foods to show an influence on GI symptoms differently by genotype in our study.

Taking into account that nowadays there is tendency to increase milk consumption in Russia by supplying school children with the essential amount of milk according to the Federal program (The national program "School milk" 2013), it might be assumed that the frequency of GI disorders could rise among LNP subjects. It is therefore important either for people themselves or for health care personnel to be aware of lactase persistence/non-persistence. The manufacture of lactose-free products may be beneficial for the Russian population, since milk is an important and "cost-effective" source of everyday nutrition (Miller, Jarvis et al. 2001, Haug, Høstmark et al. 2007, Weaver 2010, Valenkevich, Iakhontova 2005).

5.5 The novel G/A-13914 variant

The most frequently encountered C/T-13910 genotype variant among Europeans was found to be responsible for the regulation of lactase activity among our study population. Other variants (C/G-13907, T/C-13913, T/G-13915 and G/C-13010) previously associated with the regulation of lactase activity in the southern part of the world (Tishkoff, Reed et al. 2007, Imtiaz, Savilahti et al. 2007, Ingram, Elamin et al. 2007) were not present here. However, we found the variant G/A-13914 upstream of the lactase gene (LCT), which is associated with lactase persistence in its carriers. The fact is that the subject in question had LNP (C/C-13910 genotype) in the main position and therefore should evince lactase activity lower than 10

U/g/protein, which has been shown to be the cut-off point for adult-type hypolactasia. In fact the lactase activity determined in this person was higher in two biopsy samples taken. We therefore assumed that the A₋₁₃₉₁₄ allele is related to an increase in lactase activity. The origin of ₋₁₃₉₁₄ G/A is not at the present time clear and remains to be studied. Previously the variant was described briefly in two Europeans (Tag, Schiffers et al. 2007, Tag, Oberkanins et al. 2008) from different populations. The significance of this genotype has not previously been studied. It is conceivable that this mutation has appeared in different regions of the world.

5.6 Cultural historical hypothesis

The cultural historical hypothesis associates the occurrence of hypolactasia with dairy history (Simoons 1970, McCracken 1970). Other conceptions such as the calcium absorption hypothesis (Flatz, Rothauwe 1973) can be seen as amplifying the cultural historical hypothesis of hypolactasia. Population traditionally keeping cows and consequently using milk products as preferred food had a survival advantage (McCracken 1971). The mutation appearing long ago conferred the ability to retain lactase activity throughout life on a certain number of people (Simoons 1970). However, there are several populations such as the Nenets who are known to be non-milking tribes and who are nomadic and do not keep cows (Mirov 1945). They use other sources of everyday nutrition rather than milk products. These tribes are assumed to be characterized by a high frequency of hypolactasia, and one example may be the Nenets from North-West Russia. In our Nenets study we found support for the cultural historical hypothesis of hypolactasia and demonstrated a frequency of the C/C₋₁₃₉₁₀ genotype as high as 90% among those who had only Nenets among their ancestors. The fact is that the frequency of the LNP genotype diminishes proportionally to the number of ethnicities other than Nenets in a person's pedigree.

The study results support the cultural historical hypothesis. Dairy farming had a leading role in the process of mutation increasing the LCT gene frequency. The gene flow from neighboring populations may alter the prevalence of hypolactasia in non-milking tribes. However, those who had all grandparents of the same (Nenets) origin had the highest frequency of the LCT gene. Mutations take place all the time and all over the world at a certain frequency, but mutation enrichment occurs when it is of advantage to the human being.

5.7 Practical significance of the study

Genotyping is a direct method of diagnosing adult-type hypolactasia and can be used in clinical practice in the process of the differential diagnostics of unspecific abdominal complaints.

There is still a lack of knowledge as to the age of lactose intolerance onset among Russians as well as among Nenets. Research aiming to establish the age of onset of gene down-regulation among these populations could be of great interest.

Taking into account the beneficial properties of milk for humans and the high prevalence of hypolactasia among the Northern population it would be beneficial to supply this region with lactose-free products to provide balanced and useful nutritional support.

6 Conclusions

The prevalence of adult-type hypolactasia in the population of Northern Russia was found to be 36%, while in the Nenets group the figure was found to be 90% among indigenous Nenets having only Nenets ancestors in their pedigree.

Lactase non-persistence has an influence on milk consumption and symptom appearance. Young Northern Russians consume small amounts of milk. However, milk consumption was found to be associated with gastrointestinal symptoms in lactase non-persistent subjects.

The novel G/A₋₁₃₉₁₄ variant was associated with increased lactase activity in one subject with C/C position in LNP genotype, suggesting that the increased lactase activity is most likely to be associated with the G/A₋₁₃₉₁₄ variant.

The results of our study supported the cultural historical hypothesis of hypolactasia. The highest prevalence of adult-type hypolactasia was discovered among a group of indigenous people having only Nenets in their pedigree, while the prevalence of the lactase non-persistent genotype among others having three, two or one Nenets among ancestors was lower. It may therefore be concluded that not only a history of milk drinking but also the gene flow from other populations influences hypolactasia frequency.

Appendixes

Appendix 1. Prevalence of lactose malabsorption/hypolactasia in Russians and other Slavonic tribes

Ethnicity	Study subjects or sample	Methods					Age	Number	Prevalence	Year of publication	Reference
		LTT	Urine test	BHT	Biopsy	Genotyping					
Russians (Leningrad)	Healthy	+			+		25-55	64	12.5	1987	(Valenkevich 1987)
Russians (Karelian ASSR)	Healthy	No information					20-50	104	16.3	1989	(Valenkevich, Iakhontova 1989)
Russians (Estonia)	No information						Students	60	37.0	1987	(Labotkin 1987)
Russians ("Old Believers")	No information	+	+		+		20-65	103	57.0	1991	(Lember, Tamm et al. 1991)
Russians of Ural	No specific information	+	+				17-55	49	51.0	1996	(Kozlov 1996)
Russians of Udmurtian Republic	Healthy	+	Symptoms				18-55	30	40.0	1998	(Kozlov 1998)
Russians of West Siberia	Healthy	+	Symptoms				18-55	47	49.0	1998	(Kozlov 1998)
Russians (North-West Russia)	No specific information	+	+				25-55	108	16.0	2005	(Valenkevich, Iakhontova 2005)
Russians (Kostroma region)	DNA from collection of genome analysis laboratory, Russian Academy of Science					+	No data	102	36.2	2006	(Borinskaia, Rebrikov et al. 2006)
Russians (Kursk)						+	No data	112	53.6	2006	(Borinskaia, Rebrikov et al. 2006)
Russians (Rostov)						+	No data	114	53.5	2006	(Borinskaia, Rebrikov et al. 2006)
Russians (Chukotka)						+	No data	26	46.2	2006	(Borinskaia, Rebrikov et al. 2006)
Russians (Moscow)	Children with atopy					+	12.5*	40	55.0	2008	(Delyagin, Kagramanova et al. 2008)
Belorussian	Healthy	+			+		25-55	38	13.0	1987	(Valenkevich 1987)
Ukrainian	Healthy	+			+		25-55	52	5.8	1987	(Valenkevich 1987)
Belorussian	Healthy	+	+				25-55	74	15.0	2005	(Valenkevich, Iakhontova 2005)
Ukrainian	Healthy	+	+				25-55	88	13.0	2005	
Ukrainian	DNA from collection of genome analysis laboratory, Russian Academy of Science					+	No data	122	41.8	2006	(Borinskaia, Rebrikov et al. 2006)
Belorussian						+		101	39.6	2006	(Borinskaia, Rebrikov et al. 2006)

*The mean age

Appendix 2. Prevalence of lactose malabsorption/hypolactasia in different ethnicities settled in the territory of the former Soviet Union

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Ethnicity	Study subjects or sample	Methods					Age	Number	Prevalence	Year of publication	Reference
		LTT	Urine test	BHT	Biopsy	Genotyping					
Khants	Hunters and fishermen and their families	+	+		+		8-57	80	94	1995	(Lember, Tamm et al. 1995)
Maris	Adult population	+					20-60	204	81	1987	(Tamm, Lember et al. 1987)
Mordvinians	Healthy	No information					20-50	26	11	1989	(Valenkevich, lakhontova 1989)
Karelians	Healthy	No information					20-50	78	11.5	1989	(Valenkevich, lakhontova 1989)
Vepses	Healthy	No information					20-50	9	11	1989	(Valenkevich, lakhontova 1989)
Finns	Healthy	No information					20-50	62	11.3	1989	(Valenkevich, lakhontova 1989)
Komi-permiaks	No specific information	+	+				17-55	112	50.0	1996	(Kozlov 1996)
Komi (izhems)	No specific information	+	+				17-55	56	63.0	1996	(Kozlov 1996)
Mansi	No specific information	+	+				17-55	38	71.0	1996	(Kozlov 1996)
Khants	No specific information	+	+				17-55	76	82.0	1996	(Kozlov 1996)
Mansi	Indigenous	+					31.1*	13	69.2	1991	(Zhvavyi, Kozlov et al. 1991)
Khants	Indigenous	+					31.1*	53	67.9	1991	(Zhvavyi, Kozlov et al. 1991)
Nenets (West Siberia)	Indigenous	+					31.1*	7	71.4	1991	(Zhvavyi, Kozlov et al. 1991)
Buryats	Indigenous	+					23.1*	19	47.0	1991	(Zhvavyi, Kozlov et al. 1991)
Kildin Saami	Healthy	+					18-55	50	48.0	1998	(Kozlov 1998)
Komi- Izhems (Ob)	Healthy	+					18-55	56	63.0	1998	(Kozlov 1998)
Nenets (West Siberia)	Healthy	+					18-55	9	78.0	1998	(Kozlov 1998)
Permian Finn (Komi-Permiak and Udmurtian)	Healthy	+					18-55	112 75	50.0 59.0	1998	(Kozlov 1998)
Finns	Healthy	+	+				25-55	98	22	2005	(Valenkevich, lakhontova 2005)
Karelians	Healthy	+	+				25-55	106	20	2005	(Valenkevich, lakhontova 2005)
Vepses	Healthy	+	+				25-55	44	20	2005	(Valenkevich, lakhontova 2005)
Ishorians	Healthy	+	+				25-55	50	20	2005	(Valenkevich, lakhontova 2005)
Estonians (Leningrad region)	Healthy	+	+				25-55	64	23	2005	(Valenkevich, lakhontova 2005)
Chukchi	DNA from collection of genome analysis laboratory, Russian Academy of Science					+	No data	35	88.6	2006	(Borinskaia, Rebrikov et al. 2006)
Udmurti						+	No data	94	55.3	2006	(Borinskaia, Rebrikov et al. 2006)
Komi-permyaki						+	No data	69	42.0	2006	(Borinskaia, Rebrikov et al. 2006)

*The mean age

Appendix 3. Description of family members (II)

Code	Relation to index person	Age at time of investigation	Genotype	Origin and place of birth	Anamnesis of diseases	Milk / sour milk consumption (glasses per day)	Symptoms caused by milk product consumption	Symptoms caused by other foods
Index person		58	C/C-13910 G/A -13914	Russian Archangelsk	Stomach ulcer (bleeding in 2005), rheumatoid arthritis (since 1993), renal carcinoma (operated in 1993)	0,5/1-2	No	Yes
F1	Mother	86	C/C-13910 G/A -13914	Russian Archangelsk region	Stomach cancer (cause of death in 2008)	0/1-2	No	No
F2	Father			Russian Leningrad region	Died in the year of research from cancer of skin			
F3	Daughter	32	C/C-13910	Russian Archangelsk	No	0/1-2	No	No
F4	Granddaughter	6	Parents have not given permission to investigate					
F5	Sister	56	C/C-13910 G/A -13914	Russian Archangelsk	Gastritis	1/1	No	Yes
F6	Niece (daughter of F5)	29	C/C-13910 G/A -13914	Russian Archangelsk	Gastritis	0/1-2	No	Yes
F7	Nephew (son of F5)	22	C/C-13910 G/A -13914	Russian St Petersburg	No	1/0	No	No
F8	Sister	59	C/C-13910 G/A -13914	Russian Archangelsk	No	Very seldom	Yes	Yes
F9	Nephew (son of F8)	34	C/C-13910 G/A -13914	Russian Archangelsk	Ulcer od duodenum, biliary dyskinesia	0/0	Yes	No

Расстройства/боли появляются:

- сразу после начала приёма пищи
- через час после приёма пищи
- позже (в том числе ночные боли)
- боли купируются едой (голодные боли)

Наиболее частая локализация этих болей?

- в области сердца
- в правом подреберье
- в левом подреберье
- в нижней части живота справа
- в нижней части живота слева
- в околопупочной области
- в эпигастрии

5. Часто ли и, начиная с какого возраста, у вас были следующие симптомы? Обозначьте крестиком (галочкой) в клетке.

	каждый день	по меньшей мере раз в неделю	в среднем каждую вторую неделю	нерегулярно/с разной регулярностью	в возрасте лет
Изжога					
Отрыжка					
Боль в желудке					
Вздутие живота					
Выделение газов из кишечника					
Жидкие испражнения раз в день раз в день			
Водянистый понос раз в день раз в день			
Тошнота					
Рвота					
Недомогание					

Мешают ли вам вышеупомянутые недомогания?

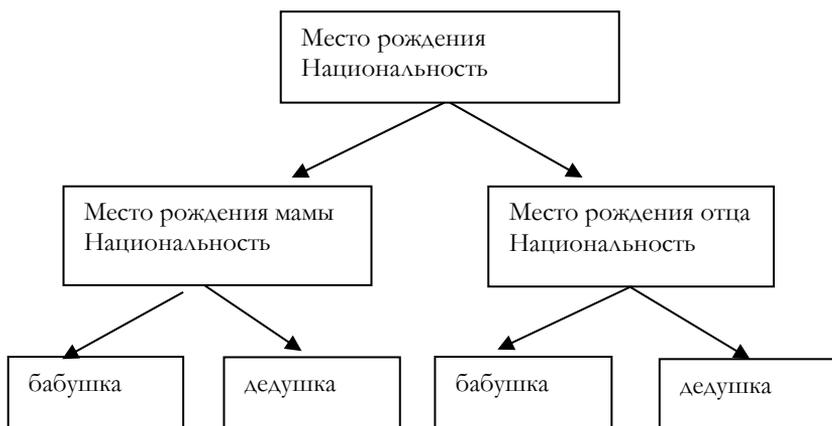
да нет

Если вам они мешают, укажите, какие из них вызывают наибольшее неудобство?

.....

6. Вызывают ли какие-либо желудочно-кишечные расстройства у вас следующие виды пищи?

- | | | | | |
|--|----|-----|-----|--|
| - жирная пища | да | нет | | |
| - жареная пища | да | нет | | |
| - молоко или молочный суп | | да | нет | |
| - простокваша или кисломолочные продукты | | да | нет | |
| - овощи (капуста, брюква) | | да | нет | |
| - фрукты (яблоки) | | да | нет | |
| - какая-либо другая пища | | да | нет | |



Мы благодарим Вас за участие в исследовании. Результаты буду сообщены Вам по тому контактному телефону, который Вы указали в начале анкеты.

Ваша подпись и дата исследования

.....

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References

- AGOSTONI, C. and TURCK, D., 2011. Is cow's milk harmful to a child's health? *Journal of pediatric gastroenterology and nutrition*, **53**(6), pp. 594-600.
- ALMON, R., ENGFELDT, P., TYSK, C., SJOSTROM, M. and NILSSON, T.K., 2007. Prevalence and trends in adult-type hypolactasia in different age cohorts in Central Sweden diagnosed by genotyping for the adult-type hypolactasia-linked LCT - 13910C > T mutation. *Scandinavian Journal of Gastroenterology*, **42**(2), pp. 165-170.
- AROLA, H., KOIVULA, T., JOKELA, H. and ISOKOSKI, M., 1982. Simple urinary test for lactose malabsorption. *Lancet*, **2**(8297), pp. 524-525.
- AROLA, H., KOIVULA, T., JOKELA, H., JAUHAINEN, M., KEYRILAINEN, O., UUSITALO, A. and ISOKOSKI, M., 1987. Strip test is reliable in common prevalences of hypolactasia. *Scandinavian Journal of Gastroenterology*, **22**(4), pp. 509-512.
- AROLA, H. and TAMM, A., 1994. Metabolism of lactose in the human body. *Scandinavian journal of gastroenterology. Supplement*, **202**, pp. 21-25.
- ASP, N.G., DAHLQVIST, A., KUITUNEN, P., LAUNIALA, K. and VISAKORPI, J.K., 1973. Complete deficiency of brush-border lactase in congenital lactose malabsorption. *Lancet*, **2**(7824), pp. 329-330.
- AURICCHIO, S. and MAIURI, L., 1994. Cellular basis of adult-type hypolactasia. *Acta Paediatrica, International Journal of Paediatrics, Supplement*, **83**(395), pp. 14-17.
- BANNAN, P.M. and LEVITT, M.D., 1996. Calcium, dairy products, and osteoporosis: Implications of lactose intolerance. *Primary care update for Ob/Gyns*, **3**(4), pp. 146-151.
- BAYLESS, T.M. and ROSENSWEIG, N.S., 1966. A racial difference in incidence of lactase deficiency. A survey of milk intolerance and lactase deficiency in healthy adult males. *JAMA : the journal of the American Medical Association*, **197**(12), pp. 968-972.
- BEDINE, M.S. and BAYLESS, T.M., 1973. Intolerance of small amounts of lactose by individuals with low lactase levels. *Gastroenterology*, **65**(5), pp. 735-743.
- BEHRENDT, M., KEISER, M., HOCH, M. and NAIM, H.Y., 2009. Impaired trafficking and subcellular localization of a mutant lactase associated with congenital lactase deficiency. *Gastroenterology*, **136**(7), pp. 2295-2303.
- BELL, R.R., DRAPER, H.H. and BERGAN, J.G., 1973. Sucrose, lactose, and glucose tolerance in northern Alaskan Eskimos. *American Journal of Clinical Nutrition*, **26**(11), pp. 1185-1190.
- BERGOZ, R., GRIESEN, M. and INFANTE, F., 1981. Significance of duodenal disaccharidases. A comparative study of duodenal and jejunal values. *Digestion*, **22**(2), pp. 108-112.

- BERSAGLIERI, T., SABETI, P.C., PATTERSON, N., VANDERPLOEG, T., SCHAFFNER, S.F., DRAKE, J.A., RHODES, M., REICH, D.E. and HIRSCHHORN, J.N., 2004. Genetic signatures of strong recent positive selection at the lactase gene. *American Journal of Human Genetics*, **74**(6), pp. 1111-1120.
- BOLIN, T.D. and DAVIS, A.E., 1970. Primary lactase deficiency: genetic or acquired? *The American Journal of Digestive Diseases*, **15**(7), pp. 679-692.
- BOLL, W., WAGNER, P. and MANTEI, N., 1991. Structure of the chromosomal gene and cDNAs coding for lactase-phlorizin hydrolase in humans with adult-type hypolactasia or persistence of lactase. *American Journal of Human Genetics*, **48**(5), pp. 889-902.
- BORINSKAIA, S.A., REBRIKOV, D.V., NEFEDOVA, V.V., KOFIADI, I.A., SOKOLOVA, M.V., KOLCHINA, E.V., KULIKOVA, E.A., CHERNYSHOV, V.N., KUTSEV, S.I., POLONIKOV, A.V., IVANOV, V.P., KOZLOV, A.I. and IANKOVSKII, N.K., 2006. Molecular diagnosis and frequencies of primary hypolactasia in populations of RUSSIA and neighboring countries. *Molekuliarnaia biologiya*, **40**(6), pp. 1031-1036.
- BUNING, C., GENSCHEL, J., JURGA, J., FIEDLER, T., VODERHOLZER, W., FIEDLER, E.M., WORM, M., WELTRICH, R., LOCHS, H., SCHMIDT, H. and OCKENGA, J., 2005. Introducing genetic testing for adult-type hypolactasia. *Digestion*, **71**(4), pp. 245-250.
- BURGER, J., KIRCHNER, M., BRAMANTI, B., HAAK, W. and THOMAS, M.G., 2007. Absence of the lactase-persistence-associated allele in early Neolithic Europeans. *Proceedings of the National Academy of Sciences of the United States of America*, **104**(10), pp. 3736-3741.
- BURGIO, G.R., FLATZ, G., BARBERA, C., PATANE, R., BONER, A., CAJOZZO, C. and FLATZ, S.D., 1984. Prevalence of primary adult lactose malabsorption and awareness of milk intolerance in Italy. *The American Journal of Clinical Nutrition*, **39**(1), pp. 100-104.
- CAMPBELL, A.K., MATTHEWS, S.B., VASSEL, N., COX, C.D., NASEEM, R., CHAICHI, J., HOLLAND, I.B., GREEN, J. and WANN, K.T., 2010. Bacterial metabolic 'toxins': A new mechanism for lactose and food intolerance, and irritable bowel syndrome. *Toxicology*, **278**(3), pp. 268-276.
- CARRERA, E., NESHEIM, M.C. and CROMPTON, D.W.T., 1984. Lactose maldigestion in Ascaris-infected preschool-children. *American Journal of Clinical Nutrition*, **39**(2), pp. 255-264.
- CARROCCIO, A., MONTALTO, G., CAVERA, G. and NOTARBATOLO, A., 1998. Lactose intolerance and self-reported milk intolerance: relationship with lactose maldigestion and nutrient intake. Lactase Deficiency Study Group. *Journal of the American College of Nutrition*, **17**(6), pp. 631-636.
- CASELLAS, F., APARICI, A., CASAUS, M., RODRIGUEZ, P. and MALAGELADA, J.R., 2010. Subjective perception of lactose intolerance does not always indicate lactose malabsorption. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, **8**(7), pp. 581-586.

- CASELLAS, F., VARELA, E., APARICI, A., CASAUS, M. and RODRÍGUEZ, P., 2009. Development, validation, and applicability of a symptoms questionnaire for lactose malabsorption screening. *Digestive diseases and sciences*, **54**(5), pp. 1059-1065.
- CLOAREC, D., GOUILLOUD, S., BORNET, F., BRULEY DES VARANNES, S., BIZAIS, Y. and GALMICHE, J.-., 1991. Lactase deficiency and lactose intolerance-related symptoms in adult healthy subjects from Western France. *Gastroenterologie clinique et biologique*, **15**(8-9), pp. 588-593.
- COELHO, M., LUISELLI, D., BERTORELLE, G., LOPES, A.I., SEIXAS, S., DESTRO-BISOL, G. and ROCHA, J., 2005. Microsatellite variation and evolution of human lactase persistence. *Human genetics*, **117**(4), pp. 329-339.
- COOK, G.C. and AL-TORKI, M.T., 1975. High intestinal lactase concentrations in adult Arabs in Saudi Arabia. *British medical journal*, **3**(5976), pp. 135-136.
- CZEIZEL, A., FLATZ, G. and FLATZ, S.D., 1983. Prevalence of primary adult lactose malabsorption in Hungary. *Human genetics*, **64**(4), pp. 398-401.
- DAHLQVIST, A., 1984. Assay of intestinal disaccharidases. *Scandinavian Journal of Clinical and Laboratory Investigation*, **44**(2), pp. 169-172.
- DAHLQVIST, A., HAMMOND, J.B., CRANE, R.K., DUNPHY, J.V. and LITTMAN, A., 1968. Intestinal lactase deficiency and lactose intolerance in adults. Preliminary report. *Gastroenterology*, **54**(4), pp. Suppl:807-810.
- DELYAGIN, V.M., KAGRAMANOVA, K.G., SHUGURINA, E.G., SICHINAVA, I.V., SOKOLOVA, M.V., BORINSKAYA, S.A. and YANKOVSKY, N.K., 2008. Polymorphism of the lactase gene at children with atopic diseases. *Pediatriya-Moskov*, **87**(4), pp. 15-17.
- DI STEFANO, M., TERULLA, V., TANA, P., MAZZOCCHI, S., ROMERO, E. and CORAZZA, G.R., 2009. Genetic test for lactase non-persistence and hydrogen breath test: is genotype better than phenotype to diagnose lactose malabsorption? *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*, **41**(7), pp. 474-479.
- EADALA, P., WAUD, J.P., MATTHEWS, S.B., GREEN, J.T. and CAMPBELL, A.K., 2009. Quantifying the 'hidden' lactose in drugs used for the treatment of gastrointestinal conditions. *Alimentary Pharmacology and Therapeutics*, **29**(6), pp. 677-687.
- ENATTAH, N.S., JENSEN, T.G., NIELSEN, M., LEWINSKI, R., KUOKKANEN, M., RASINPERA, H., EL-SHANTI, H., SEO, J.K., ALIFRANGIS, M., KHALIL, I.F., NATAH, A., ALI, A., NATAH, S., COMAS, D., MEHDI, S.Q., GROOP, L., VESTERGAARD, E.M., IMTIAZ, F., RASHED, M.S., MEYER, B., TROELSEN, J. and PELTONEN, L., 2008. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *American Journal of Human Genetics*, **82**(1), pp. 57-72.
- ENATTAH, N.S., SAHI, T., SAVILAHTI, E., TERWILLIGER, J.D., PELTONEN, L. and JARVELA, I., 2002. Identification of a variant associated with adult-type hypolactasia. *Nature genetics*, **30**(2), pp. 233-237.
- ENATTAH, N.S., TRUDEAU, A., PIMENOFF, V., MAIURI, L., AURICCHIO, S., GRECO, L., ROSSI, M., LENTZE, M., SEO, J.K., RAHGOZAR, S., KHALIL, I.,

- ALIFRANGIS, M., NATAH, S., GROOP, L., SHAAT, N., KOZLOV, A., VERSCHUBSKAYA, G., COMAS, D., BULAYEVA, K., MEHDI, S.Q., TERWILLIGER, J.D., SAHI, T., SAVILAHTI, E., PEROLA, M., SAJANTILA, A., JÄRVELÄ, I. and PELTONEN, L., 2007. Evidence of still-ongoing convergence evolution of the lactase persistence T-13910 alleles in humans. *American Journal of Human Genetics*, **81**(3), pp. 615-625.
- FARUP, P.G., MONSBAKKEN, K.W. and VANDVIK, P.O., 2004. Lactose malabsorption in a population with irritable bowel syndrome: Prevalence and symptoms. A case-control study. *Scandinavian Journal of Gastroenterology*, **39**(7), pp. 645-649.
- FLATZ, G., HOWELL, J.N., DOENCH, J. and FLATZ, S.D., 1982. Distribution of physiological adult lactase phenotypes, lactose absorber and malabsorber, in Germany. *Human genetics*, **62**(2), pp. 152-157.
- FLATZ, G. and LIE, G.H., 1982. Effect of acetylsalicylic acid on symptoms and hydrogen excretion in the disaccharide tolerance test with lactose or lactulose. *American Journal of Clinical Nutrition*, **35**(2), pp. 273-276.
- FLATZ, G. and ROTTHAUWE, H.W., 1973. Lactose nutrition and natural selection. *Lancet*, **2**(7820), pp. 76-77.
- FLATZ, G. and ROTTHAUWE, H.W., 1971. Evidence against nutritional adaption of tolerance to lactose. *Humangenetik*, **13**(2), pp. 118-125.
- FLATZ, G., ROTTHAUWE, H.W. and SIMOONS, F.J., 1979. Geography and lactose malabsorption. *Digestive diseases and sciences*, **24**(6), pp. 492-494.
- GASBARRINI, A., CORAZZA, G.R., GASBARRINI, G., MONTALTO, M., STEFANO, M.D.I., BASILISCO, G., PARODI, A., SATTA, P.U., VERNIA, P., ANANIA, C., ASTEGIANO, M., BARBARA, G., BENINI, L., BONAZZI, P., CAPURSO, G., CERTO, M., COLECCHIA, A., CUOCO, L., SARIO, A.D.I., FESTI, D., LAURITANO, C., MICELI, E., NARDONE, G., PERRI, F., PORTINCASA, P., RISICATO, R., SORGE, M. and TURSÌ, A., 2009. Methodology and indications of H₂-breath testing in gastrointestinal diseases: The Rome consensus conference. *Alimentary Pharmacology and Therapeutics*, **29**(SUPPL. 1), pp. 1-3.
- GERBAULT, P., LIEBERT, A., ITAN, Y., POWELL, A., CURRAT, M., BURGER, J., SWALLOW, D.M. and THOMAS, M.G., 2011. Evolution of lactase persistence: an example of human niche construction. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **366**(1566), pp. 863-877.
- GILAT, T., BEN HUR, H. and GELMAN MALACHI, E., 1978. Alterations of the colonic flora and their effect on the hydrogen breath test. *Gut*, **19**(7), pp. 602-605.
- GREEN, R.M., 1951. *A translation of Galen's Hygiene (De sanitate tuenda)*. Springfield (Ill.): Charles C Thomas.
- Great Hungarian Plain, online image, viewed 2 February 2012, <http://greathungarianplain.wordpress.com/2012/02/26/>
- GUDMAND-HOYER, E., 1994. The clinical significance of disaccharide maldigestion. *The American Journal of Clinical Nutrition*, **59**(3 Suppl), pp. 735S-741S.

- GUDMAND-HOYER, E., DAHLQVIST, A. and JARNUM, S., 1969. Specific small-intestinal lactase deficiency in adults. *Scandinavian Journal of Gastroenterology*, **4**(4), pp. 377-386.
- GUDMAND-HOYER, E. and SIMONY, K., 1977. Individual sensitivity to lactose in lactose malabsorption. *The American Journal of Digestive Diseases*, **22**(3), pp. 177-181.
- HABERKORN, B.C., ERMENS, A.A., KOEKEN, A., COBBAERT, C.M. and VAN GULDENER, C., 2011. Improving diagnosis of adult-type hypolactasia in patients with abdominal complaints. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, **50**(1), pp. 119-123.
- HARRINGTON, L.K. and MAYBERRY, J.F., 2008. A re-appraisal of lactose intolerance. *International journal of clinical practice*, **62**(10), pp. 1541-1546.
- HARRISON, G.G., 1975. Primary Adult Lactase Deficiency: A Problem in Anthropological Genetics. *American Anthropologist*, **77**(4), pp. 812-835.
- HARVEY, C.B., FOX, M.F., JEGGO, P.A., MANTEI, N., POVEY, S. and SWALLOW, D.M., 1993. Regional localization of the lactase-phlorizin hydrolase gene, LCT, to chromosome 2q21. *Annals of Human Genetics*, **57**(Pt 3), pp. 179-185.
- HAUG, A., HØSTMARK, A.T. and HARSTAD, O.M., 2007. Bovine milk in human nutrition - A review. *Lipids in Health and Disease*, **6**.
- HE, T., VENEMA, K., PRIEBE, M.G., WELLING, G.W., BRUMMER, R.-M. and VONK, R.J., 2008. The role of colonic metabolism in lactose intolerance. *European journal of clinical investigation*, **38**(8), pp. 541-547.
- HEITLINGER, L.A., ROSSI, T.M., LEE, P.C. and LEBENTHAL, E., 1991. Human intestinal disaccharidase activities: correlations with age, biopsy technique, and degree of villus atrophy. *Journal of pediatric gastroenterology and nutrition*, **12**(2), pp. 204-208.
- HERTZLER, S.R., HUYNH, B.-L. and SAVAIANO, D.A., 1996. How much lactose is low lactose? *Journal of the American Dietetic Association*, **96**(3), pp. 243-246.
- HEYMAN, M.B., 2006. Lactose intolerance in infants, children, and adolescents. *Pediatrics*, **118**(3), pp. 1279-1286.
- HOGENAUER, C., HAMMER, H.F., MELLITZER, K., RENNER, W., KREJS, G.J. and TOPLAK, H., 2005. Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. *European journal of gastroenterology & hepatology*, **17**(3), pp. 371-376.
- HOLDEN, C. and MACE, R., 1997. Phylogenetic analysis of the evolution of lactose digestion in adults. *Human Biology*, **69**(5), pp. 605-628.
- HOLLOX, E., 2005. Genetics of lactase persistence - Fresh lessons in the history of milk drinking. *European Journal of Human Genetics*, **13**(3), pp. 267-269.
- HOVDE, O. and FARUP, P.G., 2009. A comparison of diagnostic tests for lactose malabsorption--which one is the best? *BMC gastroenterology*, **9**, pp. 82.
- IMTIAZ, F., SAVILAHTI, E., SARNESTO, A., TRABZUNI, D., AL-KAHTANI, K., KAGEVI, I., RASHED, M.S., MEYER, B.F. and JARVELA, I., 2007. The T/G 13915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population. *Journal of medical genetics*, **44**(10), pp. e89.

- INGRAM, C.J., ELAMIN, M.F., MULCARE, C.A., WEALE, M.E., TAREKEGN, A., RAGA, T.O., BEKELE, E., ELAMIN, F.M., THOMAS, M.G., BRADMAN, N. and SWALLOW, D.M., 2007. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Human genetics*, **120**(6), pp. 779-788.
- INGRAM, C.J.E., MULCARE, C.A., ITAN, Y., THOMAS, M.G. and SWALLOW, D.M., 2009. Lactose digestion and the evolutionary genetics of lactase persistence. *Human genetics*, **124**(6), pp. 579-591.
- ISOKOSKI, M., TAMM, A., SAHI, T., TAMMUR, R., LEMBER, M., KUUSK, L., REIMAND, K., AROLA, H., SUURMAA, K. and VILLAKO, K., 1990. Prevalence of lactose malabsorption among Finno-Ugric nations and cultural historical hypothesis. *The World Congresses of Gastroenterology, 9th, Sydney, Australia*, , pp. PD 540.
- ISOKOSKI, M., JUSSILA, J. and SARNA, S., 1972. A simple screening method for lactose malabsorption. *Gastroenterology*, **62**(1), pp. 28-32.
- ISOKOSKI, M., SAHI, T., VILLAKO, K. and TAMM, A., 1981. Epidemiology and genetics of lactose malabsorption. *Annals of Clinical Research*, **13**(3), pp. 164-168.
- ITAN, Y., POWELL, A., BEAUMONT, M.A., BURGER, J. and THOMAS, M.G., 2009. The origins of lactase persistence in Europe. *PLoS Computational Biology*, **5**(8),
- JANHUNEN, J., 2009. Proto-Uralic—what, where, and when? *The Quasiquicentennial of the Finno-Ugrian Society, Helsinki*, , pp. 57-78.
- JARVELA, I.E., 2005. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). *Scandinavian Journal of Clinical and Laboratory Investigation*, **65**(7), pp. 535-539.
- JARVELA, I.E., 2005. Molecular genetics of adult-type hypolactasia. *Annals of Medicine*, **37**(3), pp. 179-185.
- JELLEMA, P., SCHELLEVIS, F.G., VAN DER WINDT, D.A.W.M., KNEEPKENS, C.M.F. and VAN DER HORST, H.E., 2010. Lactose malabsorption and intolerance: A systematic review on the diagnostic value of gastrointestinal symptoms and self-reported milk intolerance. *QJM*, **103**(8), pp. 555-572.
- JENNESS, R., 1979. The composition of human milk. *Seminars in perinatology*, **3**(3), pp. 225-239.
- JENSEN, T.G.K., LIEBERT, A., LEWINSKY, R., SWALLOW, D.M., OLSEN, J. and TROELSEN, J.T., 2011. The -14010*C variant associated with lactase persistence is located between an Oct-1 and HNF1 α binding site and increases lactase promoter activity. *Human genetics*, **130**(4), pp. 483-493.
- JUSSILA, J., 1969. Diagnosis of lactose malabsorption by the lactose tolerance test with peroral ethanol administration. *Scandinavian Journal of Gastroenterology*, **4**(4), pp. 361-368.
- JUSSILA, J., 1969. Milk intolerance and lactose malabsorption in hospital patients and young servicemen in Finland. *Annals of Clinical Research*, **1**(3), pp. 199-207.
- JUSSILA, J., ISOKOSKI, M. and LAUNIALA, K., 1970. Prevalence of lactose malabsorption in a Finnish rural population. *Scandinavian Journal of Gastroenterology*, **5**(1), pp. 49-56.

- KERBER, M., OBERKANINS, C., KRIEGSHÄUSER, G., KOLLERITS, B., DOSENBAACH-GLANINGER, A., FUCHS, D. and LEDOCHOWSKI, M., 2007. Hydrogen breath testing versus LCT genotyping for the diagnosis of lactose intolerance: A matter of age? *Clinica Chimica Acta*, **383**(1-2), pp. 91-96.
- KHABAROVA, Y., GRIGORYEVA, V., TUOMISTO, S., KARHUNEN, P.J., MATTILA, K. and ISOKOSKI, M., 2012. High prevalence of lactase non-persistence among indigenous nomadic Nenets, north-west Russia. *International journal of circumpolar health*, **71**, pp. 1-6.
- KOZLOV, A., 2004. *Lactase intolerance (primary hypolactasia) in different population groups of Eurasia (dissertation)*. Moscow: Moscow State Pedagogical University.
- KOZLOV, A. and LISITSYN, D., 1997. Hypolactasia in Saami subpopulations of Russia and Finland. *Anthropologischer Anzeiger; Bericht über die Biologisch-Anthropologische Literatur*, **55**(3-4), pp. 281-287.
- KOZLOV, A.I., 1998. Hypolactasia in the indigenous populations of northern Russia. *International journal of circumpolar health*, **57**(1), pp. 18-21.
- KOZLOV, A.I., 1996. The phenocline of primary hypolactasia in Finno-Ugrian population. *American Journal of Human Biology, Abstracts of the 21st Annual Meeting of the Human Biology Association, Durham, North Carolina April 8-10, 1996*, **8**(1), pp. 136.
- KOZLOV, A., VERSHUBSKAYA, G. and KOZLOVA, M., 2007. *Indigenous peoples of Northern Russia : anthropology and health*. Oulu: International Association of Circumpolar Health Publishers.
- KRAWCZYK, M., WOLSKA, M., SCHWARTZ, S., GRUENHAGE, F., TERJUNG, B., PORTINCASA, P., SAUERBRUCH, T. and LAMMERT, F., 2008. Concordance of genetic and breath tests for lactose intolerance in a tertiary referral centre. *Journal of Gastrointestinal and Liver Diseases*, **17**(2), pp. 135-139.
- KRUSE, T.A., BOLUND, L., GRZESCHIK, K.H., ROPERS, H.H., SJOSTROM, H., NOREN, O., MANTEI, N. and SEMENZA, G., 1988. The human lactase-phlorizin hydrolase gene is located on chromosome 2. *FEBS letters*, **240**(1-2), pp. 123-126.
- KULL, M., KALLIKORM, R. and LEMBER, M., 2009. Impact of molecularly defined hypolactasia, self-perceived milk intolerance and milk consumption on bone mineral density in a population sample in Northern Europe. *Scandinavian Journal of Gastroenterology*, **44**(4), pp. 415-421.
- KUOKKANEN, M., ENATTAH, N.S., OKSANEN, A., SAVILAHTI, E., ORPANA, A. and JARVELA, I., 2003. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. *Gut*, **52**(5), pp. 647-652.
- KUOKKANEN, M., MYLLYNIEMI, M., VAUHKONEN, M., HELSKE, T., KÄÄRIÄINEN, I., KARESVUORI, S., LINNALA, A., HÄRKÖNEN, M., JÄRVELÄ, I. and SIPPONEN, P., 2006. A biopsy-based quick test in the diagnosis of duodenal hypolactasia in upper gastrointestinal endoscopy. *Endoscopy*, **38**(7), pp. 708-712.
- LAAKSONEN, M.M.L., MIKKIL, V., RÄSÄNEN, L., RONTU, R., LEHTIMÄKI, T.J., VIKARI, J.S.A. and RAITAKARI, O.T., 2009. Genetic lactase non-persistence,

- consumption of milk products and intakes of milk nutrients in Finns from childhood to young adulthood. *British Journal of Nutrition*, **102**(1), pp. 8-17.
- LABOTKIN, K., 1987. Prevalence of selective lactose malabsorption in young Estonians and Russians. *Acta Comment Univ Tartu*, **790**, pp. 65-67.
- Lactose Intolerance, online image, viewed 2012,
http://www.uic.edu/classes/phar/phar332/Clinical_Cases/carbo%20metab%20cases/lactose%20intol.htm.
- LADAS, S., PAPANIKOS, J. and ARAPAKIS, G., 1982. Lactose malabsorption in Greek adults: correlation of small bowel transit time with the severity of lactose intolerance. *Gut*, **23**(11), pp. 968-973.
- LANGMAN, J.M. and ROWLAND, R., 1990. Activity of duodenal disaccharidases in relation to normal and abnormal mucosal morphology. *Journal of clinical pathology*, **43**(7), pp. 537-540.
- LAUNIALA, K., 1968. The mechanism of diarrhoea in congenital disaccharide malabsorption. *Acta Paediatrica Scandinavica*, **57**(5), pp. 425-432.
- LAUNIALA, K., PERHEENTUPA, J., VISAKORPI, J. and HALLMAN, N., 1964. Disaccharidases of intestinal mucosa in a patient with sucrose intolerance. *Pediatrics*, **34**, pp. 615-620.
- LEBENTHAL, E., ANTONOWICZ, I. and SHWACHMAN, H., 1975. Correlation of lactase activity, lactose tolerance and milk consumption in different age groups. *American Journal of Clinical Nutrition*, **28**(6), pp. 595-600.
- LEBENTHAL, E. and LEE, P.C., 1980. Glucoamylase and disaccharidase activities in normal subjects and in patients with mucosal injury of the small intestine. *Journal of Pediatrics*, **97**(3), pp. 389-393.
- LEMBER, M., 2002. Assessment of lactose tests. *American Family Physician*, **66**(12), pp. 2206, 2208.
- LEMBER, M., TAMM, A., PIIRSOO, A., SUURMAA, K., KERMES, K., KERMES, R., SAHI, T. and ISOKOSKI, M., 1995. Lactose malabsorption in Khants in western Siberia. *Scandinavian Journal of Gastroenterology*, **30**(3), pp. 225-227.
- LEMBER, M., TAMM, A. and VILLAKO, K., 1991. Lactose malabsorption in Estonians and Russians. *European Journal of Gastroenterology and Hepatology*, **3**(6), pp. 479-481.
- LEMBER, M., TORNIAINEN, S., KULL, M., KALLIKORM, R., SAADLA, P., RAJASALU, T., KOMU, H. and JARVELA, I., 2006. Lactase non-persistence and milk consumption in Estonia. *World journal of gastroenterology : WJG*, **12**(45), pp. 7329-7331.
- LERCH, M.M., RIEBAND, H.-., FELDBERG, W. and MATERN, S., 1991. Concordance of indirect methods for the detection of lactose malabsorption in diabetic and nondiabetic subjects. *Digestion*, **48**(2), pp. 81-88.
- LEVITT, M., WILT, T. and SHAUKAT, A., 2013. Clinical implications of lactose malabsorption versus lactose intolerance. *Journal of clinical gastroenterology*, **47**(6), pp. 471-480.
- LEWINSKY, R.H., JENSEN, T.G., MOLLER, J., STENSALLE, A., OLSEN, J. and TROELSEN, J.T., 2005. T-13910 DNA variant associated with lactase persistence

- interacts with Oct-1 and stimulates lactase promoter activity in vitro. *Human molecular genetics*, **14**(24), pp. 3945-3953.
- LOMER, M.C., PARKES, G.C. and SANDERSON, J.D., 2008. Review article: lactose intolerance in clinical practice--myths and realities. *Alimentary Pharmacology & Therapeutics*, **27**(2), pp. 93-103.
- MAAROOS, H., 1991. Secondary hypolactasia: tactics in differential diagnosis. *Hypolactasia clinical and diagnostic aspects*. Tartu: Tartu University, pp. 46-48.
- MADRY, E., LISOWSKA, A., KWIECIEN, J., MARCINIAK, R., KORZON-BURAKOWSKA, A., DRZYMALA-CZYŻ, S., MOJS, E. and WALKOWIAK, J., 2010. Adult-type hypolactasia and lactose malabsorption in Poland. *Acta Biochimica Polonica*, **57**(4), pp. 585-588.
- MAIURI, L., RAIÀ, V., POTTER, J., SWALLOW, D., HO, M.W., FIOCCA, R., FINZI, G., CORNAGGIA, M., CAPELLA, C. and QUARONI, A., 1991. Mosaic pattern of lactase expression by villous enterocytes in human adult-type hypolactasia. *Gastroenterology*, **100**(2), pp. 359-369.
- MARTON, A., XUE, X. and SZILAGYI, A., 2012. Meta-analysis: the diagnostic accuracy of lactose breath hydrogen or lactose tolerance tests for predicting the North European lactase polymorphism C/T-13910. *Alimentary Pharmacology & Therapeutics*, **35**(4), pp. 429-440.
- MATTHEWS, S.B., WAUD, J.P., ROBERTS, A.G. and CAMPBELL, A.K., 2005. Systemic lactose intolerance: A new perspective on an old problem. *Postgraduate medical journal*, **81**(953), pp. 167-173.
- MCCRACKEN, R.D., 1971. Origins and implications of the distribution of adult lactase deficiency in human populations. *The Journal of tropical pediatrics and environmental child health*, **17**(1), pp. 7-10.
- MCCRACKEN, R.D., 1970. Adult lactose tolerance. *JAMA : the journal of the American Medical Association*, **213**(13), pp. 2257-2260.
- MCSWEENEY, P.L.H. and FOX, P.F., eds, 2009. *Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts and Minor Constituents*. Third edition edn. New York, USA: Springer Science+Business Media.
- MILLER, G.D., JARVIS, J.K. and MCBEAN, L.D., 2001. The importance of meeting calcium needs with foods. *Journal of the American College of Nutrition*, **20**(2), pp. 168S-185S.
- MIROV, N.T., 1945. Notes on the domestication of reindeer. *American Anthropologist*, **47**(3), pp. 393-408.
- MULCARE, C.A., WEALE, M.E., JONES, A.L., CONNELL, B., ZEITLYN, D., TAREKEGN, A., SWALLOW, D.M., BRADMAN, N. and THOMAS, M.G., 2004. The T allele of a single-nucleotide polymorphism 13.9 kb upstream of the lactase gene (LCT) (C - 13.9kbT) does not predict or cause the lactase-persistence phenotype in Africans. *American Journal of Human Genetics*, **74**(6), pp. 1102-1110.
- MURTHY, M.S. and HAWORTH, J.C., 1970. Intestinal lactase deficiency among east Indians. An adaptive rather than a genetically inherited phenomenon? *The American Journal of Gastroenterology*, **53**(3), pp. 246-251.

- NAGY, D., BOGACSI-SZABO, E., VARKONYI, A., CSANYI, B., CZIBULA, A., BEDE, O., TARI, B. and RASKO, I., 2009. Prevalence of adult-type hypolactasia as diagnosed with genetic and lactose hydrogen breath tests in Hungarians. *European journal of clinical nutrition*, **63**(7), pp. 909-912.
- NAIM, H.Y., STERCHI, E.E. and LENTZE, M.J., 1987. Biosynthesis and maturation of lactase-phlorizin hydrolase in the human small intestinal epithelial cells. *The Biochemical journal*, **241**(2), pp. 427-434.
- NEWCOMER, A.D., MCGILL, D.B., THOMAS, P.J. and HOFMANN, A.F., 1975. Prospective comparison of indirect methods for detecting lactase deficiency. *The New England journal of medicine*, **293**(24), pp. 1232-1236.
- NICKLAS, T.A., QU, H., HUGHES, S.O., HE, M., WAGNER, S.E., FOUSHEE, H.R. and SHEWCHUK, R.M., 2011. Self-perceived lactose intolerance results in lower intakes of calcium and dairy foods and is associated with hypertension and diabetes in adults. *The American Journal of Clinical Nutrition*, **94**(1), pp. 191-198.
- NIEMINEN, U., KAHRI, A., SAVILAHTI, E. and FÄRKKILÄ, M.A., 2001. Duodenal disaccharidase activities in the follow-up of villous atrophy in coeliac disease. *Scandinavian Journal of Gastroenterology*, **36**(5), pp. 507-510.
- NUCERA, G., GABRIELLI, M., LUPASCU, A., LAURITANO, E.C., SANTOLIVUDO, A., CREMONINI, F., CAMMAROTA, G., TONDI, P., POLA, P., GASBARRINI, G. and GASBARRINI, A., 2005. Abnormal breath tests to lactose, fructose and sorbitol in irritable bowel syndrome may be explained by small intestinal bacterial overgrowth. *Alimentary Pharmacology and Therapeutics*, **21**(11), pp. 1391-1395.
- OBERACHER, M., POHL, D., VAVRICKA, S.R., FRIED, M. and TUTUIAN, R., 2011. Diagnosing lactase deficiency in three breaths. *European journal of clinical nutrition*, **65**(5), pp. 614-618.
- OJETTI, V., LA MURA, R., ZOCCO, M.A., CESARO, P., DE MASI, E., MAZZA, A.L., CAMMAROTA, G., GASBARRINI, G. and GASBARRINI, A., 2008. Quick test: A new test for the diagnosis of duodenal hypolactasia. *Digestive diseases and sciences*, **53**(6), pp. 1589-1592.
- OJETTI, V., NUCERA, G., MIGNECO, A., GABRIELLI, M., LAURITANO, C., DANESE, S., ZOCCO, M.A., NISTA, E.C., CAMMAROTA, G., DE LORENZO, A., GASBARRINI, G. and GASBARRINI, A., 2005. High prevalence of celiac disease in patients with lactose intolerance. *Digestion*, **71**(2), pp. 106-110.
- PAYNE, D.L., WELSH, J.D. and CLAYPOOL, P.L., 1983. Breath hydrogen (H₂) response to carbohydrate malabsorption after exercise. *Journal of Laboratory and Clinical Medicine*, **102**(1), pp. 147-150.
- PERLOW, W., BARAONA, E. and LIEBER, C.S., 1977. Symptomatic intestinal disaccharidase deficiency in alcoholics. *Gastroenterology*, **72**(4 1), pp. 680-684.
- PEUHKURI, K., VAPAATALO, H. and KORPELA, R., 2000. Wide variations in the testing of lactose tolerance: Results of a questionnaire study in Finnish health care centres. *Scandinavian Journal of Clinical and Laboratory Investigation*, **60**(4), pp. 291-298.
- POHL, D., SAVARINO, E., HERSBERGER, M., BEHLIS, Z., STUTZ, B., GOETZE, O., ECKARDSTEIN, A.V., FRIED, M. and TUTUIAN, R., 2010. Excellent

- agreement between genetic and hydrogen breath tests for lactase deficiency and the role of extended symptom assessment. *British Journal of Nutrition*, **104**(6), pp. 900-907.
- POULTER, M., HOLLOX, E., HARVEY, C.B., MULCARE, C., PEUHKURI, K., KAJANDER, K., SARNER, M., KORPELA, R. and SWALLOW, D.M., 2003. The causal element for the lactase persistence/non-persistence polymorphism is located in a 1 Mb region of linkage disequilibrium in Europeans. *Annals of Human Genetics*, **67**, pp. 298-311.
- PRASAD, K.K., THAPA, B.R., NAIN, C.K., SHARMA, A.K. and SINGH, K., 2008. Brush border enzyme activities in relation to histological lesion in pediatric celiac disease. *Journal of gastroenterology and hepatology*, **23**(8 part2), pp. e348-e352.
- RASINPERÄ, H., SAVILAHTI, E., ENATTAH, N.S., KUOKKANEN, M., TÖTTERMAN, N., LINDAHL, H., JÄRVELÄ, I. and KOLHO, K.-., 2004. A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut*, **53**(11), pp. 1571-1576.
- RASINPERÄ, H., 2006. *Adult-type hypolactasia : genotype-phenotype correlation*. Helsinki: University of Helsinki.
- RIDEFELT, P. and HAKANSSON, L.D., 2005. Lactose intolerance: lactose tolerance test versus genotyping. *Scandinavian Journal of Gastroenterology*, **40**(7), pp. 822-826.
- ROMERO, I.G., MALLICK, C.B., LIEBERT, A., CRIVELLARO, F., CHAUBEY, G., ITAN, Y., METSPALU, M., EAASWARKHANTH, M., PITCHAPPAN, R., VILLEMS, R., REICH, D., SINGH, L., THANGARAJ, K., THOMAS, M.G., SWALLOW, D.M., LAHR, M.M. and KIVISILD, T., 2012. Herders of Indian and European cattle share their predominant allele for lactase persistence. *Molecular biology and evolution*, **29**(1), pp. 249-260.
- ROSENKRANZ, W., HADORN, B., MULLER, W., HEINZ-ERIAN, P., HENSEN, C. and FLATZ, G., 1982. Distribution of human adult lactase phenotypes in the population of Austria. *Human genetics*, **62**(2), pp. 158-161.
- ROSSI, M., MAIURI, L., FUSCO, M.I., SALVATI, V.M., FUCCIO, A., AURICCHIO, S., MANTEI, N., ZECCA, L., GLOOR, S.M. and SEMENZA, G., 1997. Lactase persistence versus decline in human adults: Multifactorial events are involved in down-regulation after weaning. *Gastroenterology*, **112**(5), pp. 1506-1514.
- SAHI, T., 1989. Epidemiological aspects of gastrointestinal diseases with special references to hypolactasia in Skolt Lapps. *Actual problems of gastroenterology, Acta Comment Univ Tartu*, pp. 112-127.
- SAHI, T., 1994. Genetics and epidemiology of adult-type hypolactasia. *Scandinavian journal of gastroenterology. Supplement*, **202**, pp. 7-20.
- SAHI, T., 1994. Hypolactasia and lactase persistence. Historical review and the terminology. *Scandinavian journal of gastroenterology. Supplement*, **202**, pp. 1-6.
- SAHI, T., 1974. Lactose malabsorption in Finnish-speaking and Swedish-speaking populations in Finland. *Scandinavian Journal of Gastroenterology*, **9**(3), pp. 303-308.
- SAHI, T., ISOKOSKI, M., JUSSILA, J., LAUNIALA, K. and PYORALA, K., 1973. Recessive inheritance of adult-type lactose malabsorption. *Lancet*, **2**(7833), pp. 823-826.

- SAHI, T. and LAUNIALA, K., 1977. More evidence for the recessive inheritance of selective adult type lactose malabsorption. *Gastroenterology*, **73**(2), pp. 231-232.
- SAHI, T., 1974. *The inheritance of selective adult-type lactose malabsorption*. Helsinki: University of Helsinki.
- SAVAIANO, D.A., BOUSHEY, C.J. and MCCABE, G.P., 2006. Lactose intolerance symptoms assessed by meta-analysis: a grain of truth that leads to exaggeration. *The Journal of nutrition*, **136**(4), pp. 1107-1113.
- SAVILAHTI, E., LAUNIALA, K. and KUITUNEN, P., 1983. Congenital lactase deficiency. A clinical study on 16 patients. *Archives of Disease in Childhood*, **58**(4), pp. 246-252.
- SCHIRRU, E., CORONA, V., USAI-SATTA, P., SCARPA, M., CUCCA, F., DE VIRGILIIS, S., ROSSINO, R., FRAU, F., MACIS, M.D., JORES, R.-. and CONGIA, M., 2007. Decline of lactase activity and C/T-13910 variant in Sardinian childhood. *Journal of pediatric gastroenterology and nutrition*, **45**(4), pp. 503-506.
- SEBASTIO, G., VILLA, M., SARTORIO, R., GUZZETTA, V., POGGI, V., AURICCHIO, S., BOLL, W., MANTEI, N. and SEMENZA, G., 1989. Control of lactase in human adult-type hypolactasia and in weaning rabbits and rats. *American Journal of Human Genetics*, **45**(4), pp. 489-497.
- SENIOR, P.A. and BHOPAL, R., 1994. Ethnicity as a variable in epidemiological research. *British medical journal*, **309**(6950), pp. 327-330.
- SEPPO, L., TUURE, T., KORPELA, R., JÄRVELÄ, I., RASINPERÄ, H. and SAHI, T., 2008. Can primary hypolactasia manifest itself after the age of 20 years? A two-decade follow-up study. *Scandinavian Journal of Gastroenterology*, **43**(9), pp. 1082-1087.
- SHAW, A.D. and DAVIES, G.J., 1999. Lactose intolerance problems in diagnosis and treatment. *Journal of clinical gastroenterology*, **28**(3), pp. 217-223.
- SHILS, M.E. and SHIKE, M., 2006. *Modern nutrition in health and disease*. 10th ed. edn. Philadelphia: Lippincott Williams & Wilkins.
- SIDDIQUI, Z. and OSAYANDE, A.S., 2011. Selected Disorders of Malabsorption. *Primary Care - Clinics in Office Practice*, **38**(3), pp. 395-414.
- SIMOONS, F.J., 1980. Age of onset of lactose malabsorption. *Pediatrics*, **66**(4), pp. 646-648.
- SIMOONS, F.J., 1978. The geographic hypothesis and lactose malabsorption. A weighing of the evidence. *American Journal of Digestive Diseases*, **23**(11), pp. 963-980.
- SIMOONS, F.J., 1970. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. *The American Journal of Digestive Diseases*, **15**(8), pp. 695-710.
- SIMOONS, F.J., 1969. Primary adult lactose intolerance and the milking habit: a problem in biological and cultural interrelations. I. Review of the medical research. *The American Journal of Digestive Diseases*, **14**(12), pp. 819-836.
- SIMOONS, F.J., JOHNSON, J.D. and KRETCHMER, N., 1977. Perspective on milk drinking and malabsorption of lactose. *Pediatrics*, **59**(1), pp. 98-109.
- SINGH, K.D., BHASIN, D.K., RANA, S.V., VAIPHEI, K., KATYAL, R., VINAYAK, V.K. and SINGH, K., 2000. Effect of Giardia lamblia on duodenal disaccharidase levels in humans. *Tropical gastroenterology : official journal of the Digestive Diseases Foundation*, **21**(4), pp. 174-176.

- SUAREZ, F.L., SAVAIANO, D., ARBISI, P. and LEVITT, M.D., 1997. Tolerance to the daily ingestion of two cups of milk by individuals claiming lactose intolerance. *The American Journal of Clinical Nutrition*, **65**(5), pp. 1502-1506.
- SUAREZ, F.L., SAVAIANO, D.A. and LEVITT, M.D., 1995. A comparison of symptoms after the consumption of milk or lactose- hydrolyzed milk by people with self-reported severe lactose intolerance. *New England Journal of Medicine*, **333**(1), pp. 1-4.
- SWAGERTY, D.L., D.WALLING, A. and KLEIN, R.M., 2002. Lactose intolerance. *American Family Physician*, **65**(9), pp. 1845-1850+1855-1856.
- SWALLOW, D.M., 2003. Genetic influences on carbohydrate digestion. *Nutrition Research Reviews*, **16**(1), pp. 37-43.
- SWALLOW, D.M., 2003. Genetics of lactase persistence and lactose intolerance. *Annual Review of Genetics*. pp. 197-219.
- SWALLOW, D.M., POULTER, M. and HOLLOX, E.J., 2001. Intolerance to lactose and other dietary sugars. *Drug metabolism and disposition: the biological fate of chemicals*, **29**(4 Pt 2), pp. 513-516.
- SZILAGYI, A., MALOLEPSZY, P., HAMARD, E., XUE, X., HILZENRAT, N., PONNIAH, M., MACNAMARA, E. and CHONG, G., 2007. Comparison of a real-time polymerase chain reaction assay for lactase genetic polymorphism with standard indirect tests for lactose maldigestion. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association*, **5**(2), pp. 192-196.
- TADESSE, K. and EASTWOOD, M., 1977. Breath-hydrogen test and smoking. *Lancet*, **2**(8028), pp. 91.
- TAG, C.G., OBERKANINS, C., KRIEGSHAUSER, G., INGRAM, C.J., SWALLOW, D.M., GRESSNER, A.M., LEDOCHOWSKI, M. and WEISKIRCHEN, R., 2008. Evaluation of a novel reverse-hybridization StripAssay for typing DNA variants useful in diagnosis of adult-type hypolactasia. *Clinica chimica acta; international journal of clinical chemistry*, **392**(1-2), pp. 58-62.
- TAG, C.G., SCHIFFLERS, M.C., MOHNEN, M., GRESSNER, A.M. and WEISKIRCHEN, R., 2007. A novel proximal -13914G>A base replacement in the vicinity of the common-13910T/C lactase gene variation results in an atypical LightCycler melting curve in testing with the MutaREAL Lactase test. *Clinical chemistry*, **53**(1), pp. 146-148.
- TAMM, A., LEMBER, M. and SIIGUR, U., 1987. Prevalence of lactose malabsorption (LM) in adult Mari population. *International Epidemiological Association, XI Scientific Meeting*, pp. 104.
- TAMMUR, R., 1991. Lactose intolerance among children, adolescents and in families with hypolactasia. *Hypolactasia. Clinical and Diagnostic aspects. Abstracts of the symposium 21-22.11.1991*, pp.23-24.
- TISHKOFF, S.A., REED, F.A., RANCIARO, A., VOIGHT, B.F., BABBITT, C.C., SILVERMAN, J.S., POWELL, K., MORTENSEN, H.M., HIRBO, J.B., OSMAN, M., IBRAHIM, M., OMAR, S.A., LEMA, G., NYAMBO, T.B., GHORI, J., BUMPSTEAD, S., PRITCHARD, J.K., WRAY, G.A. and DELOUKAS, P., 2007.

- Convergent adaptation of human lactase persistence in Africa and Europe. *Nature genetics*, **39**(1), pp. 31-40.
- The national program "School milk", available: <http://www.schoolmilk.ru/>.
- TORNIAINEN, S., FREDDARA, R., ROUTI, T., GIJSBERS, C., CATASSI, C., HOGLUND, P., SAVILAHTI, E. and JARVELA, I., 2009. Four novel mutations in the lactase gene (LCT) underlying congenital lactase deficiency (CLD). *BMC gastroenterology*, **9**, pp. 8.
- TROELSEN, J.T., 2005. Adult-type hypolactasia and regulation of lactase expression. *Biochimica et biophysica acta*, **1723**(1-3), pp. 19-32.
- VALENKEVICH, L.N., 1987. Current problems of lactase deficiency. *Voprosy pitaniia*, **(4)**(4), pp. 31-34.
- VALENKEVICH, L.N. and IAKHONTOVA, O.I., 2005. *Milk and milk products in doctors' practical work*. St-Petersburg: Politekhnik.
- VALENKEVICH, L.N. and IAKHONTOVA, O.I., 2005. Prevalence of the lactase deficiency among the population of the northwestern region of Russia. *Ekspierimetal'naiia i klinicheskaia gastroenterologija = Experimental & clinical gastroenterology*, **(1)**(1), pp. 97-100, 108.
- VALENKEVICH, L.N. and IAKHONTOVA, O.I., 1989. Lactase deficiency in aboriginal inhabitants of the Mordovian and Karelian ASSR. *Klinicheskaia meditsina*, **67**(7), pp. 56-58.
- VESA, T.H., KORPELA, R.A. and SAHI, T., 1996. Tolerance to small amounts of lactose in lactose maldigesters. *American Journal of Clinical Nutrition*, **64**(2), pp. 197-201.
- VESA, T.H., MARTEAU, P. and KORPELA, R., 2000. Lactose intolerance. *Journal of the American College of Nutrition*, **19**(2 Suppl), pp. 165S-175S.
- VILLAKO, K. and MAAROOS, H., 1994. Clinical picture of hypolactasia and lactose intolerance. *Scandinavian journal of gastroenterology. Supplement*, **202**, pp. 36-54.
- WANG, Y., HARVEY, C.B., HOLLOX, E.J., PHILLIPS, A.D., POULTER, M., CLAY, P., WALKER-SMITH, J.A. and SWALLOW, D.M., 1998. The genetically programmed down-regulation of lactase in children. *Gastroenterology*, **114**(6), pp. 1230-1236.
- WEAVER, C.M., 2010. Role of dairy beverages in the diet. *Physiology and Behavior*, **100**(1), pp. 63-66.
- WILSON, J., 2005. Milk intolerance: lactose intolerance and cow's milk protein allergy. *Newborn & Infant Nursing Reviews*, **5**(4), pp. 203-207.
- WOODING, S.P., 2007. Following the herd. *Nature genetics*, **39**(1), pp. 7-8.
- ZHVAVYI, N.F., KOZLOV, A.I. and KONDIK, V.M., 1991. Lactase deficiency among representatives of various nationalities of Siberia. *Voprosy pitaniia*, **(5)**(5), pp. 32-35.

Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia

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Abstract

AIM: To estimate the prevalence of the lactase non-persistent genotype (C/C-13910) in a northern Russian population in accordance with ethnicity, and to evaluate self-reported milk consumption depending on lactase activity.

METHODS: Blood samples for genotyping lactase activity, defining the C/T-13910 variant by polymerase chain reaction, and direct sequencing were taken from 231 medical students of Russian origin aged 17-26 years. We analyzed milk product consumption by questionnaire which was specially designed for the estimation of milk consumption and abdominal complaints.

RESULTS: We found that the prevalence of the C/C-13190 genotype in the northern Russian population

was 35.6%. The other genotypes nearby C/T-13910 and associated with lactase activity were not present in the study population. The consumption of milk among people with the non-persistent genotype tended to be lower than among the lactose tolerant subjects, but was not statistically significant.

CONCLUSION: An investigation of the lactase persistent genotype in a northern Russian population has not been performed before. The genotype did not affect the consumption of milk products in this population which could be explained by low consumption of milk products among the entire study population.

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Key words: C/C-13910 genotype; Hypolactasia; Lactase persistence/non-persistence; Lactose malabsorption; Milk consumption; North-west Russia

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INTRODUCTION

The prevalence of adult-type hypolactasia (primary lactose malabsorption, lactase non-persistence) varies considerably between different races and populations^[1,2]. Inheritance of primary lactose malabsorption is controlled by a single recessive autosomal gene^[3].

Simoons^[4] and McCracken^[5] suggested the cultural historical hypothesis to explain the differences in prevalence of hypolactasia. Hence, people with lactase persistence could survive better because they could use all nutrients in milk without having diarrhea. It is possible that they had more children than subjects

with hypolactasia. The idea of the calcium absorption hypothesis was proposed by Flatz^[6], who suggested that as lactose is a stimulant of calcium absorption, people who consume milk have less rickets, pelvic deformities and more children. This was genetic selection to the benefit of lactase persistence.

Several laboratory methods are used in the diagnosis of hypolactasia. Previously the prevalence of hypolactasia was detected by a lactose tolerance test (LTT) or a lactose tolerance test with ethanol (LTTE)^[7,8]. The "gold standard" which can be used as a reference method is the direct determination of lactase activity in the small intestinal mucosa, taken by biopsy; however, the invasiveness of the test does not allow its use in everyday practice and screening^[7]. In 2002 the genetic variant associated with adult-type hypolactasia, a one base polymorphism C/T-13910 (rs 4988234) upstream of the lactase coding gene on chromosome 2 was identified. The C/T-13910 variant is located in the OCT-1 binding site and acts as an enhancer^[9]. The variant is inherited recessively so that the C-13910 allele in a homozygous form (the C/C-13910 genotype) is always associated with adult-type hypolactasia and the T-13910 allele (C/T and T/T-13910 genotypes) with persistent lactase activity^[10]. The specificity of the genetic test is 100% with a sensitivity of 93% in subjects older than 12 years^[11]. In addition, several other suggested variants (C/G-13907, T/C-13913, T/G-13915 and G/C-14010) nearby the C/T-13910 variant were identified in African and Middle Eastern populations^[12-14]. Of these variants, 13915 T/G and 14010 G/C are associated with lactase persistence^[12,14].

Data on the prevalence of hypolactasia from studies performed in the territories of Russia are available. The prevalence of lactase malabsorption among Russians has been found to vary from 13% to 57% according to different authors and the different methods used^[15-17]. Many studies were carried out in other populations living in the territories of Russia and the prevalence of lactose malabsorption among them was between 11% and 94%^[16-20].

Genotyping has been used in only two Russian studies^[21,22] which showed that the prevalence of hypolactasia among Russians living in the central part ranged from 36% to 50% and depended on the area of residence^[22].

The main hypothesis of the current study was that people with the C/C-13910 genotype will avoid using milk or their milk consumption will be less compared to those with the lactase persistent genotype.

The objective was to estimate the prevalence of the lactase non-persistent genotype in a northern Russian population of students from the Medical University of Archangelsk. We determined the frequency of the C/T-13910 variants in accordance with ethnicity and estimated self-reported milk consumption and its relationship to the C/T-13910 genotypes.

MATERIALS AND METHODS

Study group

The study was performed in collaboration with the

Department of Family Medicine, Northern State Medical University (NSMU), Archangelsk, Russia, the Department of Medical Genetics, University of Helsinki and the Department of General Practice, University of Tampere. The study was approved by the Ethics Committee of NSMU (No. 08/06 from 29.11.2006). Medical students from different faculties aged 17 to 26 years were enrolled into the investigation. All subjects gave written informed consent and completed a questionnaire on their personal data, self-reported health status, milk consumption habits, ethnicity and their place of birth as well as their parents' and grandparents' place of birth. Blood samples were taken from 241 students. Of these, 231 reported their origin as Russian; but, according to the origins of grandparents (at least three out of four grandparents) only 149 were considered to have Russian ethnicity. Of the others, 61 did not report the data on grandparents and these subjects were classified as "missing information" on ethnicity. Twenty one students who reported their origin as Russian had at least 2 grandparents with another origin mostly Ukrainian, but also Byelorussians, Komi, Mordvinian and others. Final analysis of the prevalence of the C/C-13910 genotype was based on one of three subgroups: Russians, the group with missing data on grandparents and the mixed origin group (Table 1). We included students who were chosen by random methods from four main faculties of the university in the investigation. Randomization in our study was done according to standard rules. The NSMU has 16 faculties and each of them has 10 to 12 groups of students. We took every third group of students according to an official list from every fourth faculty. As a result we received 176 students chosen by a random method. The remaining 65 were taken at an annual medical review. Although that sample was not random, there was no selection because every student who attended medical review during a certain time (while we were collecting the material) was taken. Age and sex do not affect gene frequency.

The number of students and the selection process are presented in Figure 1. The majority of students (155 of 231) who participated in the study were born in the Archangelsk region, the others were born in different regions of north-west Russia (Figure 2).

Questionnaire

We used the questionnaire designed by Sahi^[3] with some modifications in the present study. Milk consumption was estimated using questions about milk and sour milk consumption. For comparison, we divided all subjects into two groups (Table 2). The first group consisted of students who were consumers of milk regardless of amount (answer "Yes" in Table 2) and the second group consisted of those who never consumed milk (answer "No" in Table 2).

Analyses of genotype

DNA was amplified by polymerase chain reaction (PCR). We used Taq polymerase (Dynazyme, Finnzymes, Espoo, Finland) with the conditions described elsewhere. The

Table 1 The distribution of the lactase-persistent/non-persistent genotypes by ethnicity *n* (%)

	CC-13910 ¹	CT-13910 ²	TT-13910 ²	Total
Russian	53 (35.6)	76 (51.0)	20 (13.4)	149 (100)
Others	9 (42.8)	11 (52.4)	1 (4.8)	21 (100)
Information missing	22 (36.1)	26 (42.6)	13 (21.3)	61 (100)
Total	84 (36.5)	112 (48.7)	34 (14.8)	231 (100)

¹Lactase non-persistent genotype; ²Lactase persistent genotype.

Table 2 Consumption of milk products by different C/T-13910 genotype variants *n* (%)

Consumption	CC-13910 ¹	CT-13910 and TT-13910 ²	Total
Milk			
Yes	31 (36.9)	71 (48.3)	102 (44.2)
No	53 (63.1)	76 (51.7)	129 (55.8)
Total	84 (100)	147 (100)	231 (100)
Sour milk			
Yes	41 (48.8)	89 (60.5)	130 (56.3)
No	43 (51.2)	58 (39.5)	101 (43.7)
Total	84 (100)	147 (100)	231 (100)

¹Lactase non-persistent genotype; ²Lactase persistent genotype.

used forward primer was 5'-CCTCGTTAATACCCACT GACCTA-3' and the reverse primer was 5'-GTCACCTT GATATGATGAGAGCA-3' which covered about 400 bp regions 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 60 min and at 80°C for 15 min.

During sequencing, BigDye 3.1 terminator (Applied Biosystems) was used according to the manufacturer's instructions. Sequencing conditions were as follows: 96°C for 1 min, then 25 cycles at 96°C for 10 s, 55°C for 5 s and 60°C for 4 min. The sequencing reaction followed purification by Millipore Multiscreen plates (Millipore, USA) with Sephadex G-50 Superfine sepharose (Amersham Biosciences, Sweden), electrophoresis using the ABI 3730 DNA Analyzer (Applied Biosystems) and base calling by Sequencing Analysis 5.2 software (Applied Biosystems). The obtained sequence was analyzed using Sequencher 4.6. software (Gene Codes, USA)^[23].

Statistical analyses

The Chi-square test was used to test the difference between groups of genotype in milk consumption. *P* < 0.05 was considered statistically significant.

RESULTS

The prevalence of the C/C-genotype was found to be 35.6% among young Russian people who were born and lived in north-west Russia (Table 1). None of the previously identified other variants (C/G-13907, T/C-13913, T/G-13915 and G/C-14010) were present in the study population.

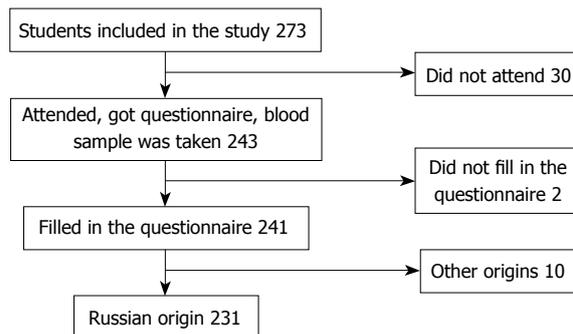


Figure 1 Steps in the data collection process.



Figure 2 Study group by region of birth place in north-west Russia.

The frequency of milk consumption varied among the milk consumers; however, the majority of subjects consumed 1-2 glasses per week or less and only 5 persons of 231 consumed 3 or more glasses per week. The same pattern was found for the consumption of sour milk. Only 3 persons in the entire study population consumed 3 or more glasses of sour milk per week.

Among subjects with lactase non-persistence (C/C-13910 genotype) more than half (63.1%) did not consume milk at all compared with those with the C/T-13910 variant (49.6%). The differences in milk consumption between lactose persistent subjects (C/T and T/T variants together) and those with hypolactasia (C/C-13910 genotype) was not statistically significant (*P* = 0.094).

The consumption of sour milk did not have a significant effect within the group of “non-consumers”; but, subjects with the C/T- and the T/T -variants consumed sour milk relatively more often than subjects with the C/C-13910 genotype (59.3%, 64.7% and 48.8%, respectively). The sour milk consumption among lactose non-persistent subjects (C/C-variant) and lactase persistent subjects (C/T- and T/T- variant together) was not statistically significant (*P* = 0.084).

All 5 persons who consumed milk products in

relatively greater amounts (3 and more glasses per week) had the lactase persistent genotype.

We estimated the gastrointestinal symptoms among subjects with lactase persistent and non-persistent genotypes. However, differences in the frequencies of symptoms were not statistically significant.

DISCUSSION

The prevalence of adult-type hypolactasia among Russians is in accordance with previous investigations and varies from 13% to at least 50%^[15-17,21,22]. The majority of previous studies were performed using the lactose tolerance test, and only two of the recent studies performed in Russia^[21,22], used the same direct genotyping we used in the present investigation. However, these researchers studied the frequency of the lactase persistent/non-persistent genotype among other populations living in the territories of Russia, but did not estimate the milk consumption.

Genetic testing is a direct method of diagnosing adult-type hypolactasia, and is not confounded by environmental factors which may affect the results of the other tests. Therefore the results of this study specify the actual prevalence of adult-type hypolactasia in Russia. An investigation into the lactase persistent genotype among a northern Russian population has not been performed before.

Since the variants (C/G-13907, T/C-13913, T/G-13915 and G/C-14010) nearby C/T-13910 were previously associated with lactase activity in southern parts of the world and were not present in this study, it is possible that these variants are not responsible for the regulation of lactase activity in north-west Russia. This is in agreement with results obtained from other countries in northern Europe^[23,24].

More than half of the study subjects did not drink milk and almost 45% did not drink sour milk at all. The great majority of "milk-consumers" drank very small quantities of milk, 1-2 glasses or less per week. Apparently, this is a habitual feature of the subjects in the study population and it did not depend on genotype. However, those five persons who drank the most milk had the lactase persistent genotype. Our data on the consumption of sour milk confirmed the results of previous studies which showed that people with lactose intolerance are more able to tolerate fermented milk because lactose in these products is reduced by 30%-40%^[25].

It is well-known that people with lactase non-persistence can consume small quantities of milk without any problems^[26]. The results of our study strengthen this theory, even though we did not reach statistical significance in milk product consumption between the lactase persistent and non-persistent subjects. In summary, genotype does not affect the consumption of milk products in a northern Russian population which can be explained by low consumption of milk products among the entire study population.

ACKNOWLEDGMENTS

We thank Timo Sahi for the questionnaire and help in designing the study.

COMMENTS

Background

The prevalence of adult-type hypolactasia (primary lactose malabsorption, lactase non-persistence) varies considerably between different races and populations. Data on the prevalence of hypolactasia from previous studies performed in the territories of Russia are limited especially for northern Russian populations. Moreover, the majority of these studies were performed using other methods. The inheritance of primary lactose malabsorption is controlled by a single recessive autosomal gene and genotyping allows us to determine the true prevalence of the lactase persistent/non-persistent gene. Thus, the prevalence of adult-type hypolactasia among the current population becomes obvious. The hypothesis that the lactase non-persistent genotype can affect milk and sour milk consumption has not been tested before in a northern Russian population.

Research frontiers

This was the first time that genotyping of the lactase persistent gene was performed in a population from north-west Russia and an estimation of milk products consumption according to genotype was performed.

Innovations and breakthroughs

In previous studies, the prevalence of lactase malabsorption among Russians has been found to vary between 13% and 57% according to different authors and the different methods used. Many studies were carried out in other populations living in the territories of Russia where there was great variation in the prevalence of lactose malabsorption, from 11% to 94%. Genotyping has a specificity of 100% and a sensitivity of 93%. Genetic methods have been used before in only two Russian studies which showed the prevalence of hypolactasia among Russians living in the central part to range from 36% to 50% depending on the area of residence. An estimation of the lactase non-persistent genotype affecting milk and dairy milk product consumption in a northern Russian population has not been done before.

Applications

The study results suggest that the prevalence of the lactase non-persistent genotype in a northern Russian population is 35.6% which is more than that in northern Europe and less than that in southern Europe. This prevalence confirms the historical hypothesis of lactase malabsorption. However, the genotype does not affect milk and sour milk consumption among northern Russians which could be a result of relatively low milk product consumption among the entire study population.

Terminology

Hypolactasia means very low activity of lactase in the jejunal mucosa; Lactose malabsorption describes poor lactose hydrolyzing capacity; Adult-type hypolactasia (primary lactose malabsorption, lactase non-persistence) - primary type of hypolactasia which is controlled by a single recessive autosomal gene; CC-13910 genotype defines lactase non-persistence; CT-13910, TT-13910 genotypes define lactase persistence.

Peer review

This manuscript has potential for contribution regarding genetic subtypes and milk intolerance.

REFERENCES

- 1 Sahi T. Genetics and epidemiology of adult-type hypolactasia. *Scand J Gastroenterol Suppl* 1994; **202**: 7-20
- 2 Isokoski M, Sahi T, Villako K, Tamm A. Epidemiology and genetics of lactose malabsorption. *Ann Clin Res* 1981; **13**: 164-168
- 3 Sahi T, Isokoski M, Jussila J, Launiala K, Pyörälä K. Recessive inheritance of adult-type lactose malabsorption. *Lancet* 1973; **2**: 823-826
- 4 Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biologic and cultural interrelations. II. A culture historical hypothesis. *Am J Dig Dis*

- 1970; **15**: 695-710
- 5 **McCracken RD.** Adult lactose tolerance. *JAMA* 1970; **213**: 2257-2260
- 6 **Flatz G.** Genetics of lactose digestion in humans. *Adv Hum Genet* 1987; **16**: 1-77
- 7 **Arola H.** Diagnosis of hypolactasia and lactose malabsorption. *Scand J Gastroenterol Suppl* 1994; **202**: 26-35
- 8 **Jussila J, Isokoski M, Launiala K.** Prevalence of lactose malabsorption in a Finnish rural population. *Scand J Gastroenterol* 1970; **5**: 49-56
- 9 **Lewinsky RH, Jensen TG, Møller J, Stensballe A, Olsen J, Troelsen JT.** T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro. *Hum Mol Genet* 2005; **14**: 3945-3953
- 10 **Rasinpera H.** Adult-Type Hypolactasia: genotype-phenotype correlation [dissertation]. Helsinki Univ 2006. Available from: URL: <http://ethesis.helsinki.fi>
- 11 **Järvelä IE.** Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). *Scand J Clin Lab Invest* 2005; **65**: 535-539
- 12 **Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghorji J, Bumpstead S, Pritchard JK, Wray GA, Deloukas P.** Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet* 2007; **39**: 31-40
- 13 **Imtiaz F, Savilahti E, Sarnesto A, Trabzuni D, Al-Kahtani K, Kagevi I, Rashed MS, Meyer BF, Järvelä I.** The T/G 13915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population. *J Med Genet* 2007; **44**: e89
- 14 **Ingram CJ, Elamin MF, Mulcare CA, Weale ME, Tarekegn A, Raga TO, Bekele E, Elamin FM, Thomas MG, Bradman N, Swallow DM.** A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Hum Genet* 2007; **120**: 779-788
- 15 **Valenkevich LN.** [Current problems of lactase deficiency] *Vopr Pitan* 1987; 31-34
- 16 **Lember M, Tamm A, Villako K.** Lactose-malabsorption in Estonians and russians. *European Journal of Gastroenterology & Hepatology* 1991; **3**: 479-481
- 17 **Kozlov AI.** Hypolactasia in the indigenous populations of northern Russia. *Int J Circumpolar Health* 1998; **57**: 18-21
- 18 **Valenkevich LN, Iakhontova OI.** [Lactase deficiency in aboriginal inhabitants of the Mordovian and Karelian ASSR] *Klin Med (Mosk)* 1989; **67**: 56-58
- 19 **Isokoski M, Tamm A, Sahi T, Tammur R, Lember M, Kuusk L, Reimand K, Arola H, Suurmaa K, Villako K.** Prevalence of lactose malabsorption among Finno-Ugric nations and cultural historical hypothesis [abstract]. In: Abstracts. The World Congresses of Gastroenterology, 9th. 1990: PD 540
- 20 **Zhvavyi NF, Kozlov AI, Kondik VM.** [Lactase deficiency among representatives of various nationalities of Siberia] *Vopr Pitan* 1991; 32-35
- 21 **Kozlov A.** [Lactase intolerance (primary hypolactasia) in different population groups of Eurasia.] [Author's abstract of dissertation] Moscow Pedagogical State Univ 2004 (in Russian)
- 22 **Borinskaia SA, Rebrikova DV, Nefedova VV, Kofiadi IA, Sokolova MV, Kolchina EV, Kulikova EA, Chernyshov VN, Kutsev SI, Polonikov AV, Ivanov VP, Kozlov AI, Iankovskii NK.** [Molecular diagnosis and frequencies of primary hypolactasia in populations of RUSSIA and neighboring countries] *Mol Biol (Mosk)* 2006; **40**: 1031-1036
- 23 **Lember M, Torniainen S, Kull M, Kallikorm R, Saadla P, Rajasalu T, Komu H, Jarvela I.** Lactase non-persistence and milk consumption in Estonia. *World J Gastroenterol* 2006; **12**: 7329-7331
- 24 **Nilsson TK, Johansson CA.** A novel method for diagnosis of adult hypolactasia by genotyping of the -13910 C/T polymorphism with Pyrosequencing technology. *Scand J Gastroenterol* 2004; **39**: 287-290
- 25 **Gudmand-Høyer E.** The clinical significance of disaccharide maldigestion. *Am J Clin Nutr* 1994; **59**: 735S-741S
- 26 **Tamm A.** Management of lactose intolerance. *Scand J Gastroenterol Suppl* 1994; **202**: 55-63

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RESEARCH ARTICLE

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Lactase non-persistent genotype influences milk consumption and gastrointestinal symptoms in Northern Russians

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Abstract

Background: Milk is an important source of nutrients. The consumption of milk, however, may cause abdominal complaints in lactose intolerant individuals. The frequency of -13910C/C genotype is known to be high among Northern Russians, exceeding the prevalence in northern Europe. In our study we tested two hypotheses: 1) subjects with lactase non-persistent genotype (-13910C/C) have more gastrointestinal (GI) 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 in the Northern State Medical University (NSMU) for genotyping the lactase activity-defining -13910C/T variant. All subjects filled in a questionnaire covering their personal data, self-reported GI symptoms and milk consumption habits.

Results: Northern Russians consume very small amounts of milk daily. Among carriers of the lactase non-persistent (LNP) genotype there were 10 percentage units of milk-consumers fewer than among lactase-persistent (LP) subjects ($p = 0.03$). Complaints of GI disorders caused by milk were different between the genotypes ($p = 0.02$). Among all types of food analyzed only milk was associated with increased GI symptoms among subjects with the LNP genotype (OR = 1.95, CI 1.03-3.69)

Conclusions: Subjects with -13910C/C have more GI symptoms from milk. Subjects with lactase non-persistent genotype avoid using milk. In the case of increasing milk consumption symptoms may increase the need for medical consultation. It is thus important either for people themselves or for health care staff to be aware of lactase persistence/non-persistence.

Background

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 as immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides are also present in milk [1,2]. Milk has been considered a major source of calcium as well as the simplest and most “cost-effective” way to ingest calcium [3,4]. In that dairy products also

provide other important nutrients, their consumption improves the nutritional quality of the diet appreciably [4,5].

This notwithstanding, the consumption of milk has decreased over the last decade in Northern Europe [1,5]. Data on milk consumption in Russia in recent years have been very limited and available only from market analyses, which show a decrease in milk consumption in some regions of country [6]. Consumption is low among young Northern Russians [7]. The above-mentioned properties of milk make it important for everyday nutrition, especially for children. Recently a national program, “School milk”, aimed to supply schoolchildren

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with the essential amount of 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, a one-base polymorphism C > T -13910 (rs 4988234) upstream of the lactase encoding gene on chromosome 2, was identified in 2002 [11]. This -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 only suggestive [13-16]. The -13910C/T has been shown to be located in the OCT-1 binding site and acts as an enhancer. Nowadays the genotyping can be used as the test for adult-type hypolactasia with 100% specificity in cases of unspecific abdominal complaints [17,18].

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

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

The prevalence of adult-type hypolactasia (lactase non-persistence, -13910C/C) among Northern Russians has been found to be 35% [7]. It has also been shown that 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 many times higher compared with other Scandinavian countries [30-33]. It also exceeds the prevalence of the -13910C/C genotype among Estonians [34].

Recent studies have shown that subjects with intolerance to lactose tend to reduce their consumption of milk, which is predictable as far as they suffer from symptoms after milk consumption [31,35-38]. In our previous study [7] subjects showed no differences between genotypes in the frequency of GI complaints during life. We found, however, that the consumption of milk is extremely low among the Northern Russian population compared with other Northern populations. Does milk consumption influence the appearance of GI symptoms in a population with a high frequency of the lactase non-persistent genotype but with very low consumption of milk on the whole?

In the present study we sought to test two hypotheses: 1) subjects with lactase non-persistent genotype (-13910C/C) possibly have more gastrointestinal symptoms associated with milk 2) subjects with lactase non-persistence avoid using milk.

Methods

Study population

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

The first group were recruited during the period 2006-2007 and blood samples were drawn for the genotyping. The second group were recruited during the period 2007-2008 and buccal samples were taken for the same purpose. The mean age for whole group was 19.8 ± 1.72 . Women represented 78.2% of all participants. Both groups of students were similar with respect to distributions of age and gender.

For randomization we used the official list of students of the NSMU. Since there are 16 faculties in the University and each has 10 to 12 groups of students we took for our study every third group from every fourth faculty.

The study was approved by the Ethics Committee of the NSMU (No 08/06 from 29.11.2006). All subjects gave their informed consent to participate.

Analyses of genotype

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

Genotyping of blood samples was performed as previously described [11]. DNA was amplified by polymerase chain reaction (PCR). We used Taq polymerase (Dynazyme, Finnzymes, Espoo, Finland) with the

conditions described elsewhere. The forward primer used was 5'-CCTCGTTAATACCCACTGACCTA-3' and the reverse primer was 5'-GTCACCTTTGATATGATGAGAGCA-3', which cover about 400 bp regions 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 60 min and at 80°C for 15 min.

In sequencing, a BigDye 3.1 terminator (Applied Biosystems) was used according to the manufacturer's instructions. Sequencing conditions were as follows: at 96°C for 1 min, then 25 cycles at 96°C for 10 s, at 55°C for 5 s and at 60°C for 4 min. The sequencing reaction followed purification on 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 sequence obtained was analyzed using Sequencher 4.6. software (Gene Codes, USA).

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

Questionnaire

All subjects gave written informed consent and filled in a questionnaire covering their personal data, self-reported condition of health, GI symptoms and milk consumption habits. We used the questionnaire with permission from Sahi [10].

Gastrointestinal symptoms were estimated by the question "Have you ever experienced the following symptoms and in what frequency?" The question was not intended to clarify the connection between milk consumption and symptoms. The various answers were "Every day", "At least once per week", "Every second week", "Sometimes". If the participant had never had the symptom the item was left blank. We classified the answer "Sometimes" together with no symptoms into one group and marked it "Without GI symptoms". The second group included the remaining variables and was taken for analyses of differences between genotypes.

The influence of food on the appearance of GI disorders was estimated by the question "Do you have any GI disorders if you consume the following types of food?" where six types of food being included. Subjects were asked to put "Yes" or "No" opposite every type of food.

In the last part of the questionnaire subjects were asked to define their everyday milk product consumption from 0 to more than 5 glasses per day. The choices were "Not at all", "1-2 glasses", "3-5 glasses" and "More than 5 glasses" of milk/sour milk per day. There was also an option to declare consumption less than 1-2 glasses per day for those who consumed milk very rarely. For the analysis we classified all answers into two groups: less than daily milk consumption or none and daily milk consumption.

Comparison was made between the lactase non-persistent group (-13910C/C) and the lactase persistent group (-13910C/T and -13910 T/T) for all the above-mentioned questions.

Statistical analysis

The difference between genotype groups in milk consumption and others were tested by χ^2 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 and symptoms after several types of food, and for volume of milk/sour milk consumption. Statistical analyses were performed with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA).

Results

The prevalence of the -13910C/C genotype (lactase non-persistence, LNP) among 518 young students from North-West Russia was found to be 35% (n = 180) while the -13910C/T genotype was found in 46% and -13910T/T in 19% of participants.

About sixty percent of all subjects reported GI symptoms (Table 1). The most frequent symptom was stomach-ache. However, no statistically significant

Table 1 The frequency of gastrointestinal symptoms (GI) among young people with lactase-persistent/non-persistent genotype (Question "Have you ever experienced the following symptoms?")

GI symptoms	Lactase non-persistence C/C-13910 (n = 180)		Lactase persistence C/T and T/T- 13910 (n = 338)		p
	n	%	n	%	
Stomach-ache	93	51.7	176	51.9	1.00
Regurgitation	23	12.8	42	12.4	0.89
Flatulence	17	9.4	38	11.2	0.65
Heartburn	14	7.8	23	6.8	0.72
Nausea	13	7.2	25	7.4	1.00
Diarrhea	3	1.7	12	3.5	0.28
Without GI symptoms	74	41.1	142	42.2	0.71

differences were revealed in the frequency of gastrointestinal symptoms in total between the LP and LNP genotype groups. The study brought out no statistically significant differences in symptom appearance between male and female participants.

The majority of subjects consumed very small amounts of milk daily. Only about 40% of all participants used milk every day. Almost 50% did not use milk at all and 13% reported consumption of milk more seldom than daily (Table 2). Among carriers of the LNP genotype there were 10 percentage units fewer milk-consumers in comparison with the LP group (32.2 and 42.0%). The differences were statistically significant ($p = 0.03$). Sour milk consumption did not show significant differences in symptoms between LP and LNP subjects.

Milk consumption was associated with GI disorders in 13.3% of subjects with the LNP genotype and in 7.1% of subjects with the LP genotype (Table 3). Complaints of GI disorders caused by milk consumption were different between the genotypes ($p = 0.02$).

Among all types of food analyzed 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 no connection between consuming food and the appearance of symptoms except in milk consumption.

Discussion

Young Northern Russians consume very small amounts of milk daily. However, among LNP genotype carriers there are even less milk-consumers than among LP subjects. Our findings demonstrate that milk is the only type of food having an influence upon the appearance of GI disorders differently by genotype. We may conclude that subjects with lactase non-persistence (-13910C/C) have more symptoms from milk. Subjects with lactase non-persistent genotype avoid using milk.

Sour milk consumption brought out no differences between the genotypes. This is in accord with the previous proved finding that sour milk is better tolerated than milk by persons with lactose intolerance [21,39]. Sour milk contains less lactose as a result of bacterial fermentation.

Table 2 Milk consumption by genotype

Consumption of milk	Lactase non-persistence -13910C/C (n = 180)		Lactase persistence -13910C/T and -13910T/T (n = 338)	
	n	%	n	%
Less than daily or none	122	67.8	196	58.0
Daily	58	32.2	142	42.0
Total	180	100	338	100

Table 3 The frequency of GI disorders connected to different food stuffs (Question "Do you have any GI disorders if you consume the following types of food?")

Type of food	Lactase non-persistence -13910C/C (n = 180)		Lactase persistence -13910C/T and -13910T/T (n = 338)		p
	n	%	n	%	
Fatty	56	31.1	120	35.5	0.33
Fried	43	23.9	69	20.4	0.37
Milk	24	13.3	24	7.1	0.02
Sour milk and kefir	16	8.9	17	5.0	0.09
Vegetables	10	5.6	14	4.1	0.51
Fruits	7	3.9	19	5.6	0.53
Any other food	28	15.6	52	15.4	1.00
Not any food	88	47.6	174	51.6	0.74

All students recruited for this study were considered healthy. However, only forty per cent of them did not report any GI symptoms and half of them reported stomach-ache, which was the most frequent symptom among all subjects without links to genotype. Possibly this finding requires further investigation of student health to clarify the causes of the frequent GI disorders among a healthy young population.

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.

GI symptoms	OR (95% CI)
Diarrhea	2.12 (0.58 - 7.76)
Flatulence	1.17 (0.62 - 2.21)
Nausea	1.04 (0.50 - 2.14)
Stomach pain	1.00 (0.68 - 1.46)
Regurgitation	0.98 (0.53 - 1.76)
Heartburn	0.82 (0.39 - 1.71)
Symptoms after some food	
Milk	1.95 (1.03 - 3.69)
Sour milk and kefir	1.61 (0.75 - 3.43)
Fried	1.61 (0.97 - 2.67)
Vegetables	1.25 (0.52 - 3.00)
Any other food	1.02 (0.59 - 1.75)
Fatty	0.68 (0.43 - 1.07)
Fruits	0.52 (0.20 - 1.31)
Milk product consumption	
Milk	1.51 (1.04 - 2.17)
Sour milk	1.10 (0.76 - 1.60)

Statistically significant differences are presented in bold face.

Regarding other populations it was shown that only flatulence occurs significantly more frequently among LNP subjects [39,40]. Jussila and associates [41] showed that loose stool was a more frequent symptom in subjects with selective lactose malabsorption.

The northern Russians use very small amount of milk daily. However LNP subjects use less milk than people with LP genotype. Such a self-prescribed reduction is in agreement with a study in Finnish children with the -13910C/C genotype, who naturally diminished their usage of milk [31] and also with a study in children of other ethnicities [42]. It was also shown that other age groups also tend to reduce their milk consumption because of the appearance of symptoms after milk [24,38].

It is possible to assume that the low rate of symptoms was partly related to the low milk intake in our study population. It is likely even if the prevalence of adult type hypolactasia in the study population is as much as 35% and low milk consumption is reported symptoms appear less probably than in other populations. Another explanation for the low rate of symptoms may be the age of lactase activity down-regulation. Since there are no studies estimating the age of decline of enzymatic activity in Russians there is a slight possibility that this group was too young to demonstrate differences in GI disorders.

Vast majority of our randomly selected students were from North-West Russia [7]. Based on the farming history, this area was determined in Soviet Union as milk production area. The region has still been considered as one of the country's milk and meat production areas. The gene-culture co-evolution between cattle milk genes and humans lactose tolerance has been verified for North Central Europe [43] and it seems to be also in north-western part of Russia.

Since milk is an important source of nutrients there are numerous studies aimed to demonstrate the advantages and disadvantages to milk restriction or elimination from the diet. The lack of milk in nutrition in the young population is strongly associated with decreased bone mineral density and leads to osteoporosis in older age [42]. Precise statistic data of prevalence of osteoporosis among the Russian population are lacking. However, it is obvious that osteoporosis as well as a higher risk of bone fractures connected with it is an actual problem for Russia [44]. Building peak bone mass during childhood and adolescent can be the best means of preventing osteoporosis in older age.

Conclusions

It is surely warranted to increase milk consumption among the Russian population by providing a federal program. The benefits from an increase in milk

consumption are obvious. At the same time our study has shown that lactase non-persistent subjects have higher frequency of GI disorders even in population with very small amount of milk consumed. It may be predicted that in the case of increasing milk consumption symptoms may appear more frequently and may increase the need for medical consultation and care. It is therefore important either for people themselves or for health care staff to be alert to lactase persistence/non-persistence.

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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 data processing, and drafted the manuscript.

All authors read and approved the final manuscript.

Competing interests

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References

1. Haug A, Høstmark AT, Harstad OM: Bovine milk in human nutrition - a review. *Lipids in Health and Disease* 2007, **6**:25.
2. Valenkevich LN, Yachontova OI: *Milk and milk products in doctor's practical work* St-Petersburg, Prosveshenie; 2005, (book in Russian).
3. Bannan PM, Levitt MD: Calcium, dairy products, and osteoporosis: Implications of lactose intolerance. *Primary Care Update for OB/GYNS* 1996, **3**(4):146-151.
4. Miller GD, Jarvis JK, McBean LD: The importance of meeting calcium needs with foods. *J Am Coll Nutr* 2001, **20**(2 Suppl):168S-185S.
5. Weaver CM: Role of dairy beverages in the diet. *Physiol Behav* 2010, **100**(1):63-66.
6. Review of Russian Dairy Market. Russian food & drink magazine. 2008, 6 [http://www.foodmarket.spb.ru/eng/archive.php?year=2010&article=653§ion].
7. Khabarova Y, Tornainen S, Nurmi H, Järvelä I, Isokoski M, Mattila K: Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia. *World J Gastroenterol* 2009, **15**(15):1849-1853.
8. The national program "School milk". [http://www.schoolmilk.ru].
9. Sahi T, Isokoski M, Jussila J, Launiala K, Pyöralä K: Recessive inheritance of adult-type lactose malabsorption. *Lancet* 1973, **13**:823-826.

10. Sahi T: **The inheritance of selective adult-type lactose malabsorption.** *Scand J Gastroenterol* 1974, **9**(Suppl 30):1-73.
11. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Järvelä IE: **Identification of a variant associated with adult-type hypolactasia.** *Nat Genet* 2002, **30**:233-237.
12. Enattah NS, Kuokkanen M, Forsblom C, Natak S, Oksanen A, Järvelä I, Peltonen L, Savilahti E: **Correlation of intestinal disaccharidase activities with the -13910C/T variant and age.** *World J Gastroenterol* 2007, **13**(25):3508-3512.
13. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghorji J, Thomas MG, Bradman N, Swallow DM: **Convergent adaptation of human lactase persistence in Africa and Europe.** *Nat Genet* 2007, **39**(1):31-40.
14. Imtiaz F, Savilahti E, Sarnesto A, Trabzuni D, Al-Kahtani K, Kagevi I, Rashid MS, Meyer BF, Järvelä I: **The T/G 13915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population.** *J Med Genet* 2007, **44**(10):e89.
15. Ingram CJ, Elamin MF, Mulcare CA, Weale ME, Tarekegn A, Raga TO, Bekele E, Elamin FM, Thomas MG, Bradman N, Swallow DM: **A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence?** *Hum Genet* 2007, **120**:779-788.
16. Tornaiainen S, Parker MI, Holmberg V, Lahtela E, Dandara C, Järvelä IE: **Screening of variants for lactase persistence/non-persistence in populations from South Africa and Ghana.** *BMC Genet* 2009, **5**:10-31.
17. Järvelä IE: **Molecular diagnosis of adult-type hypolactasia (lactase non-persistence).** *Scand J Clin Lab Invest* 2005, **65**(7):535-539.
18. Büning C, Genschel J, Jurga J, Fiedler T, Voderholzer W, Fiedler EM, Worm M, Weltrich R, Lochs H, Schmidt H, Ockenga J: **Introducing genetic testing for adult-type hypolactasia.** *Digestion* 2005, **71**(4):245-250.
19. Hollox E: **Genetics of lactase persistence - fresh lessons in the history of milk drinking.** *Eur J Hum Genet* 2005, **13**(3):267-269.
20. Villako K, Maaros H: **Clinical Picture of Hypolactasia and Lactose Intolerance.** *Scand J Gastroenterol* 1994, **202**(Suppl):26-35.
21. Gudmand-Hoyer E: **The clinical significance of disaccharide maldigestion.** *Am J Clin Nutr* 1994, **59**(Suppl 3):735S-741S.
22. Suarez FL, Savaiano DA, Levitt MD: **A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance.** *N Engl J Med* 1995, **333**(1):1-4.
23. Casellas F, Varela E, Aparici A, Casaus M, Rodríguez P: **Development, validation, and applicability of a symptoms questionnaire for lactose malabsorption screening.** *Dig Dis Sci* 2009, **54**:1059-1065.
24. Savaiano DA, Boushey CJ, McCabe GP: **Lactose intolerance symptoms assessed by meta-analysis: a grain of truth that leads to exaggeration.** *J Nutr* 2006, **136**(4):1107-1113.
25. Launiala K: **The mechanism of diarrhea in congenital disaccharide malabsorption.** *Acta Paediatr Scand* 1968, **57**(5):425-432.
26. Arola H, Tamm A: **Metabolism of lactose in human body.** *Scand J Gastroenterol Suppl* 1994, **202**:21-25.
27. Swallow DM, Poulter M, Hollox EJ: **Intolerance to lactose and other dietary sugars.** *Drug metabolism and disposition* 2001, **29**(4):513-516.
28. Borinskaia SA, Kal'ina NR, Sanina ED, Kozhekbaeva ZhM, Gupalo Elu, Garmash IV, Ogurtsov PP, Parshukova ON, Boiko SG, Veselovskii EM, Vershubskaia GG, Kozlov AI, Rogaev EI, Iankovskii NK: **Molecular diagnosis and frequencies of primary hypolactasia in population of Russia and neighboring country.** *Mol Biol (Mosk)* 2006, **40**(6):1031-1036. (In Russian, abstract in English).
29. Kozlov A: **Lactase intolerance (primary hypolactasia) in different population groups of Eurasia.** *Doctoral thesis* Moscow; 2004, (in Russian).
30. Sahi T: **Genetics and epidemiology of adult-type hypolactasia.** *Scand J Gastroenterol* 1994, **202**(Suppl):7-20.
31. Rasinpera H: **Adult-Type Hypolactasia: genotype-phenotype correlation.** *Academic Dissertation* University of Helsinki, Helsinki; 2006 [https://oa.doria.fi/bitstream/handle/10024/1389/adulttyp.pdf?sequence=1].
32. Anthoni SR, Rasinperä HA, Kotamies AJ, Komu HA, Pihlajamäki HK, Kolho KL, Järvelä IE: **Molecularly defined adult-type hypolactasia among working age people with reference to milk consumption and gastrointestinal symptoms.** *World J Gastroenterol* 2007, **13**(8):1230-1235.
33. Almon R, Engfeldt P, Tysk C, Sjöström M, Nilsson TK: **Prevalence and trends in adult-type hypolactasia in different age cohorts in Central Sweden diagnosed by genotyping for the adult-type hypolactasia-linked LCT -13910C > T mutation.** *Scand J Gastroenterol* 2007, **42**(2):165-170.
34. Lember M, Tornainen S, Kull M, Kallikorm R, Saadla P, Rajasalu T, Komu H, Järvelä I: **Lactase non-persistence and milk consumption in Estonia.** *World J Gastroenterol* 2006, **12**:7329-7331.
35. Carroccio A, Montalto G, Cavera G, Notarbatolo A: **Lactose intolerance and self-reported milk intolerance: relationship with lactose maldigestion and nutrient intake.** Lactase Deficiency Study Group. *J Am Coll Nutr* 1998, **17**(6):631-636.
36. Obermayer-Pietsch BM, Bonelli CM, Walter DE, Kuhn RJ, Fahrleitner-Pammer A, Berghold A, Goessler W, Stepan V, Dobnig H, Leb G, Renner W: **Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures.** *J Bone Miner Res* 2004, **19**(1):42-47.
37. Lovelace HY, Barr SI: **Diagnosis, symptoms, and calcium intakes of individuals with self-reported lactose intolerance.** *J Am Coll Nutr* 2005, **24**(1):51-57.
38. Kull M, Kallikorm R, Lember M: **Impact of molecularly defined hypolactasia, self-perceived milk intolerance and milk consumption on bone mineral density in a population sample in Northern Europe.** *Scand J Gastroenterol* 2009, **44**(4):415-21.
39. Anthoni S: **Cow's milk related gastrointestinal symptoms in adults.** *Academic Dissertation* University of Helsinki; 2009 [https://oa.doria.fi/bitstream/handle/10024/45470/cowsmilk.pdf?sequence=3].
40. Tamm A: **Management of lactose intolerance.** *Scand J Gastroenterol* 1994, **202**(Suppl):26-35.
41. Jussila J, Isokoski M, Launiala K: **Prevalence of lactose malabsorption in Finnish rural population.** *Scand J Gastroenterol* 1970, **5**(1):49-56.
42. Matlik L, Savaiano D, McCabe G, VanLoan M, Blue CL, Boushey CJ: **Perceived milk intolerance is related to bone mineral content in 10- to 13-year-old female adolescents.** *Pediatrics* 2007, **120**(3):e669-77.
43. Beja-Pereira A, Luikart G, England PR, Bradley DG, Jann OC, Bertorelle G, Chamberlain AT, Nunes TP, Metodievs S, Ferrand N, Erhardt G: **Gene-culture coevolution between cattle milk protein genes and human lactase genes.** *Nat Genet* 2003, **35**(4):311-3.
44. Benevolenskaia LI: **[Issues of osteoporosis in present-day medicine].** *Vestn Ross Akad Med Nauk* 2003, **7**: 15-9, Russian.

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ORIGINAL ARTICLE

The -13914G>A variant upstream of the lactase gene (LCT) is associated with lactase persistence/non-persistenceYULIA KHABAROVA¹, SUVI TORNIAINEN², ERKKI SAVILAHTI³, MAURI ISOKOSKI⁴, KARI MATTILA⁵ & IRMA JÄRVELÄ²

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Abstract

Background. Adult-type hypolactasia (lactase non-persistence) is a common cause of gastrointestinal symptoms. Several DNA sequence variants have been identified for the lactase-persistence/non-persistence (LP/LNP), the most common being the C to T residing -13910 bp upstream of the lactase gene (LCT). We have analysed sequence variants of LP/LNP in subjects originating from Northern Russia. **Methods.** A total of 148 subjects with gastrointestinal complaints were genotyped covering about 400 bp around the -13910C/T variant using direct PCR-sequencing. All patients were interviewed about milk-related symptoms using the questionnaire. Disaccharidase activities were measured from intestinal biopsy specimens of the index person. **Results.** The prevalence of the -13910C/C genotype among 148 patients was 28.4%. A G to A variant residing 13914 bp upstream from the LCT gene (-13914G>A) was identified in one participant carrying the -13910C/C genotype. In two biopsy specimens her lactase activity was above the generally accepted cut off level for adult-type hypolactasia, 10U/g protein. Three other family members also carried the -13914G>A genotype. Among eight family members five had the LNP genotype -13910C/C. **Conclusion.** A rare variant G to A residing 13914 bp upstream of the LCT gene was identified in a subject carrying the more frequent variant -13910C/C. The -13914G>A variant in heterozygous state was associated with increased lactase activity, suggesting that the increased lactase activity is most likely to be associated with the -13914G>A variant. Further studies need to be done to confirm the functional role of this variant.

Key Words: Disaccharidase activity, family study, genotype, hypolactasia, lactose intolerance

Introduction

Adult-type hypolactasia is a normal condition inherited as an autosomal recessive trait [1]. The prevalence of this normal phenomenon varies between ethnic groups and populations [2–4].

Different methods can be used for the diagnosis of adult-type hypolactasia but the lactose tolerance test (LTT) and breath hydrogen test (BHT) have been most commonly used. The disadvantages of these tests are that they are inaccurate [5,6] and time-consuming both for the patient and for the laboratory personnel. As a reference method the direct determination of lactase activity in the small intestinal mucosa, taken by biopsy, has been the

method of choice [5]. But the invasiveness of this method restricts its application in everyday practice and screening.

In 2002 the genetic variant associated with adult-type hypolactasia, a one base pair polymorphism -13910C/T (rs 4988234) upstream of the lactase coding gene on chromosome 2 was identified [7]. The -13910C/T variant is located in the OCT-1 binding site and acts as an enhancer [8]. Whereas the variant is inherited recessively the C-13910 allele in a homozygous form (-13910C/C genotype) is associated with adult-type hypolactasia and the T-13910 allele (-13910C/T and T/T genotypes) with persistent lactase activity [7,9,10,11]. Recently other

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variants (-13907C>G, -13908C>T (rs4988236), -13913T>C, -13915T>G and -14010G>C) nearby to -13910C>T were identified in African and Arab populations [12,13–15]. Of them -13915T>G has been shown to correlate with lactase activity [14] and -14010G/C with lactase persistence [13].

The -13914G>A variant has been described twice [16,17]. The first case originated from Germany although ethnicity of this male was not clear from the report. The patient was investigated because of symptoms of hypolactasia. Another one was an Austrian man. In both cases no other tests to determine lactase persistence/non-persistence (LP/LNP) were carried out.

Only one variant of the LP/LNP genotype -13910C/T has been identified in European populations. We have previously shown that this variant is also the most common among Northern Russian populations [4].

Here we report that the rare G to A nucleotide variant residing 13914 base pairs upstream of the lactase gene is correlated with disaccharidase activities.

Materials and methods

Study material

Blood samples for the assessment of prevalence of LP/LNP genotype were collected from 148 patients referred to medical consultation with the gastroenterologist of the Northern State Medical University (NSMU), Archangelsk, due to various abdominal complaints. All subjects completed a questionnaire on their personal data, self-reported health status, milk consumption habits, ethnicity and their place of birth as well as their parents' and grandparents' place of birth. The study was approved by the Ethics Committee of NSMU (No. 08/06 from 29 November 2006 and additional approval for family study No. 07/08 from 9 June 2008).

Case report

The index case is a 58-year-old female who was referred to the gastroenterologist because of stomach pain. She had stomach bleeding caused by a stomach ulcer one year before the visit. The patient had been taking anti-inflammatory drugs and corticosteroids for several years because of rheumatoid arthritis. She had been operated on 12 years previously for renal carcinoma.

Methods

We interviewed the patients about milk-related symptoms using the questionnaire designed by Sahi [18,19], having added information about ethnicity.

Genotyping was performed as described previously [7]. DNA was amplified by polymerase chain reaction (PCR). Shortly, a 400 bp region covering the -13910C/T variant was amplified and sequenced on both directions using the BigDye 3.1 terminator (Applied Biosystems) according to the manufacturer's instructions with an ABI 3730 DNA Analyzer (Applied Biosystems) and base calling using the Sequence Analysis 5.2 software (Applied Biosystems). The results were analysed by Sequencher 4.1.4 software (Gene Codes, USA).

Analysis of disaccharidase activities from an intestinal biopsy specimen

The index person was referred for an endoscopy investigation of her stomach because of ulcer anamnesis and abdominal complaints. After the informed consent, two biopsy specimens were taken from the distal duodenum of the index person for the measurement of disaccharidase activities. These samples were immediately wrapped into parafilm, put in an air-tight tube and frozen at -60°C. Then the tubes were transferred in dry ice to the scientific laboratory of the Hospital for Children and Adolescents, University of Helsinki. The measurement of disaccharidase activity was done by the method of Dahlqvist [20], originally described by Launiala et al. [21]. The commercial preparation of disaccharidases (Sigma G-5003, I-4504 and G5160) was used as a positive control. The test was modified for smaller volumes compared to the original report and was done on microtiter plates.

Investigation of the index person's family

After the investigation of the index person, we invited all available family members to the study to determine the inheritance of the new variant of LP/LNP genotype. They all filled in the questionnaire and gave an informed consent. Blood samples for genotyping were taken from eight adult family members. We were not allowed to take blood for genotyping from children (V/19 and V/20 in the Figure 1).

Results

The prevalence of the -13910C/C genotype among 148 patients was 28.4%, and the prevalence of the -13910C/T and the -13910T/T genotypes 52% and 19.6% respectively. In one of the participants (index person) a G to A variant residing 13914 bp upstream from the LCT gene (-13914G>A) was identified. Her genotype at position -13910 was -13910C/C.

The results of disaccharidase activities of the index person are shown in Table I. The -13914G>A variant was associated with increased lactase activity

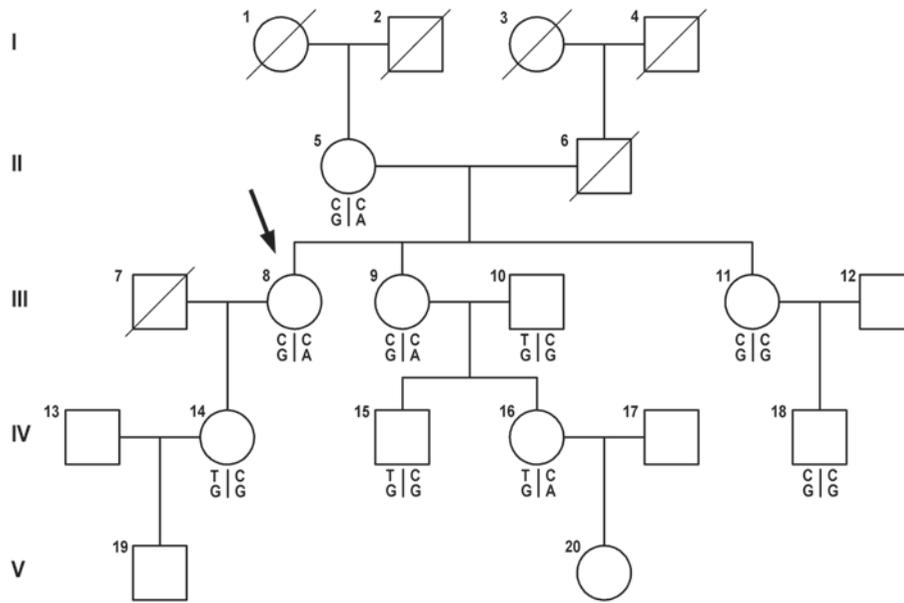


Figure 1. The pedigree of the family and its lactase haplotypes using the -13910C/T and 13914G>A sequence variants. The female index person (III/8) is marked by an arrow.

in the index person's two independent biopsy specimens. In both samples lactase activity was >10U/g/protein, that has been shown to be the cutoff point for adult-type hypolactasia [10,11,14]. In both specimens the lactase/sucrase ratio was decreased, while the absolute value for lactase activity was increased [9,10,11,14]. The disaccharidase activities associated with the -13914G>A are of the same range as in the carriers of the -13910C/T and -13915T/G variants [10,11,14]. As the index person has non-persistent genotype at -13910 locus, it can be supposed that the A-13914 allele is related to the increase in lactase activity.

We then genotyped the DNA obtained from other family members to determine the inheritance of their lactase persistent/non-persistent genotypes (Figure 1). Among eight family members whose DNA was available for genotyping five had the lactase non-persistent genotype -13910C/C. The rest of them had the -13910C/T variant. None of the family members carried the -13910T/T variant. The -13914G>A variant was also present in the mother (II/5 in Figure 1) and sister (III/9) of the index person. The daughter of the individual III/9 also carried -13914G>A. Unfortunately no DNA was available from the deceased father.

All family members consumed very small amounts of milk daily (less than 1 glass per day) independent of the genotypes. They reported consuming other dairy products rather than milk itself, but also in amounts not more than 2 glasses per day. Neither the index person nor another carrier of -13914G>A had reported problems from milk consumption. However, the index person's second sister (III/11) and her son (IV/18), who are both carriers of lactase non-persistent genotype -13910C/C without -13914G>A

variant, reported gastrointestinal problems related to milk ingestion.

All investigated family members were of Russian origin, and were born in North West Russia. It is known that the grandparents of the index person were also of Russian origin except for the grandfather from her mother's side who originated from Poland.

Discussion

The most common -13910C/T variant associated with lactase persistence/non-persistence was also the most common in this study population [4]. Screening of 148 patients with gastrointestinal problems resulted in the identification of a rare variant G to A residing 13914 bp upstream of the LCT gene in one female patient. In two biopsy specimens her lactase activity was above the generally accepted cut off level for adult-type hypolactasia, 10U/g protein.

From eight family members who were available for genotyping, two carried the same genotype (-13910C/C and -13914G>A). These were the mother and sister of the index person. The index person's niece also had the rare variant -13914G>A together with the -13910C/T variant of lactase persistence. While none of them had gastrointestinal symptoms and were all living in other parts of Russia we were not able to take biopsies from them.

The -13914G>A variant has previously been described twice. First, in a 37-year-old male patient who had symptoms of adult-type hypolactasia. He was heterozygous for the -13910C/T variant. The ethnic origin of this patient was not clear from the report, but he was examined in Germany [16]. Another 40-year-old Austrian male had also been

Table I. The results of disaccharidase activities in the intestinal biopsy specimen of the index person.

	1st specimen	2nd specimen
Maltase (U/g/prot)	633	619
Sucrase (U/g/prot)	198	191
Lactase (U/g/prot)	18	26
Lactase/sucrase ratio	0.09	0.13

determined as a carrier of this novel -13914G>A variant [17]. In both cases no other tests to determine lactase persistence/non-persistence were carried out. The index person's grandfather originated from Poland giving a 25% possibility that this new variant came from Central Europe. Interestingly, the other cases with the -13914G>A variant were from Germany and Austria [16,17]. It seems that the -13914G>A is rarely found among European populations but its origin remains to be studied.

All family members reported to consume a maximum of 1–2 glasses of milk products per day. This fact is in accordance with the results of our previous study [4] where we showed that Northern Russians use surprisingly small amounts of milk. In this family, the index person as well as other carriers of the -13914G>A variant belongs to milk consumers whereas other family members with the lactase non-persistent genotype -13910C/C and the absence of the -13914G>A variant do not use milk products at all because of gastroenterological problems. This in fact could contribute to the assumption that the -13914G>A variant is responsible for the rise of lactase activity that allows the subjects to tolerate lactose.

In conclusion, the -13914G>A variant is the third variant of lactase persistence and seems to correlate with lactase enzyme activity. The finding further confirms that the region containing the aforementioned variants is a regulatory region for the lactase gene.

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References

[1] Järvelä IE. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). *Scand J Clin Lab Invest* 2005; 65:535–9.

[2] Sahi T. Genetics and epidemiology of adult-type hypolactasia. *Scand J Gastroenterol Suppl* 1994;202:7–20.

[3] Isokoski M, Sahi T, Villako K, Tamm A. Epidemiology and genetics of lactose malabsorption. *Ann Clin Res* 1981;13: 164–8.

[4] Khabarova Y, Torniaainen S, Nurmi H, Järvelä I, Isokoski M, Mattila K. Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia. *World J Gastroenterol* 2009;15:1849–53.

[5] Arola H. Diagnosis of hypolactasia and lactose malabsorption. *Scand J Gastroenterol Suppl* 1994;202:26–35.

[6] Järvelä I, Torniaainen S, Kolho K-L. Molecular genetics of human lactase deficiencies. *Ann Med* 2009;41:568–75.

[7] Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Järvelä IE Identification of a variant associated with adult-type hypolactasia. *Nat Genet* 2002;30:233–7.

[8] Lewinsky RH, Jensen TG, Møller J, Stensballe A, Olsen J, Troelsen JT. T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro. *Hum Mol Genet* 2005;14:3945–53.

[9] Kuokkanen M, Enattah NS, Oksanen A, Savilahti E, Orpana A, Järvelä I Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. *Gut* 2003;52:647–52.

[10] Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela J, Kolho K-L A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut* 2004;53:1571–6.

[11] Enattah NS, Kuokkanen M, Forsblom C, Natak S, Oksanen A, Järvelä I, Peltonen L, Savilahti E. Correlation of intestinal disaccharidase activities with the -13910C/T variant and age. *World J Gastroenterol* 2007;13:3508–12.

[12] <http://dna-forums.com/index.php?topic/7283-23andme>

[13] Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghorji J, Bumpstead S, Pritchard JK, Wray GA, Deloukas P. Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet* 2007;39:31–40.

[14] Imtiaz F, Savilahti E, Sarnesto A, Trabzuni D, Al-Kahtani K, Kagevi I, Rashed MS, Meyer BF, Järvelä I. The T/G 13915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population. *J Med Genet* 2007;44:e89.

[15] Ingram CJ, Elamin MF, Mulcare CA, Weale ME, Tarekegn A, Raga TO, Bekele E, Elamin FM, Thomas MG, Bradman N, Swallow DM. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Hum Genet* 2007;120:779–88.

[16] Tag CG, Schiffers MC, Mohnen M, Gressner AM, Weiskirchen R. A novel proximal -13914G>A base replacement in the vicinity of the common -13910T/C lactase gene variation results in an atypical LightCycler melting curve in testing with the MutaREAL Lactase test. *Clin Chem* 2007;53:146–8.

[17] Tag CG, Oberkanins C, Kriegshäuser G, Ingram CJ, Swallow DM, Gressner AM, Ledochowski M, Weiskirchen R. Evaluation of a novel reverse-hybridization StripAssay for typing DNA variants useful in diagnosis of adult-type hypolactasia *Clin Chim Acta* 2008;392:58–62.

[18] Sahi T, Isokoski M, Jussila J, Launiala K, Pyoralä K. Recessive inheritance of adult-type lactose malabsorption. *Lancet* 1973;13:823–6.

[19] Sahi T. The inheritance of selective adult-type lactose malabsorption. *Scand J Gastroenterol* 1974;9(Suppl. 30):1–73.

[20] Dahlqvist A. Assay of intestinal disaccharidases. *Scand J Clin Lab Invest* 1984;44:169–72.

[21] Launiala K, Perheentupa J, Visakorpi J, Hallman N. Disaccharidases of intestinal mucosa in a patient with sucrose intolerance. *Pediatrics* 1964;34:615–20.

High prevalence of lactase non-persistence among indigenous nomadic Nenets, north-west Russia

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Objectives. The frequency of adult-type hypolactasia (lactase non-persistence) varies widely among different ethnic groups. The cultural historical hypothesis assumes a link between the occurrence of hypolactasia and the distribution of dairy farming. The nomadic Nenets have been reindeer herders for generations and have therefore not consumed any dairy products. The hypotheses here was that the prevalence of lactase non-persistence (–13910 C/C genotype) among Nenets people having four Nenets grandparents is high, while the prevalence among Nenets originating from ethnically mixed families is lower.

Study design. The material was collected in four typical Nenets settlements in the Nenets Autonomous Okrug in Russia. One-third of the adult Nenets population were invited to answer a questionnaire and to donate buccal samples for genotyping by a doctor from the team of medical professionals who make rounds in this area. The total number of available participants was 177.

Methods. Genotyping was performed with the AbiPrism system. We used the method of concordance of grandparents' national origin to ascribe ethnicity.

Results. The prevalence of adult-type hypolactasia (–13910 C/C) among Nenets who had four Nenets grandparents was found to be 90%. The figures among others reporting three, two and one grandparent of Nenets origin were 72, 60 and 28%, respectively.

Conclusion. The findings are in accord with the cultural historical hypothesis.

Keywords: *adult-type hypolactasia; lactase non-persistence; cultural historical hypothesis; nomadic Nenets; genotyping.*

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Adult-type hypolactasia (lactase non-persistence) is a widespread phenomenon and its prevalence varies considerably in different ethnic groups. The cultural historical hypothesis, suggested by Simoons (1) and elaborated by McCracken (2), associates the occurrence of hypolactasia with dairying history. Populations traditionally keeping dairy cattle and thus favouring milk products in their diet had an advantage to survive. Such populations would presumably have more children than those with lactase non-persistence (3). The mutation appearing here provided an opportunity to lactase activity to persist among a certain number of people. The duration of the dairy farming tradition also contributes to differences in the prevalence of lactose intolerance among different ethnicities. Amplification of

the cultural historical hypothesis was proposed by Flatz and Rotthauwe (4), who assumed that the high prevalence of lactose tolerance in European populations was due to the ability of lactose to enhance calcium absorption in an environment with low insolation and a low dietary supply of vitamin D. Those with the ability to digest lactose absorbed calcium better, had less rickets, less pelvic deformity and more children. The hypothesis was named the calcium absorption hypothesis. Cook (5) proposed that in highly arid environment lactose tolerant individuals have had an increased survival rate due to active absorption of monosaccharides and water from milk. This advantage helped them to survive in cholera epidemics and other severe gastrointestinal diseases common in the tropics. Nowadays the hypothesis that

lactose optimises calcium absorption in regions of lower ultraviolet radiation has been further developed and supported (6). This same hypothesis associates the distribution of the lactase persistence genotype with climatic conditions.

Simoons (1) mentions the reindeer-keeping northern European groups as one of the known still non-milk-drinking tribes. The Nenets are one of such nomadic and reindeer-keeping group, reindeer herding still being regarded as the cultural core of the Nenets identity (7,8). These nomadic groups had no opportunity of selection to benefit from retention of high levels of intestinal lactase, since they had never consumed dairy products. Although a small amount of deer milk might be available to nomadic people, the Nenets are mentioned as one of the tribes who had not practiced milking at all (9). They first had the opportunity to use dairy products in the third decade of the 20th century when they were being assimilated into the general population (7,8) and when the first cattle farms were organised on Nenets lands (10).

The indigenous northern populations have previously been studied to investigate the prevalence of hypolactasia using different laboratory methods, mostly the lactose tolerance test. Findings have shown the frequency of hypolactasia to have been relatively high in indigenous groups in the northern territories of Russia (11,12) compared with other European populations as well as with other ethnic groups in Russia and the Russians themselves (13,14). The prevalence of adult-type hypolactasia among northern Russians has been shown to be 35% (15).

Hypolactasia is inherited recessively (16,17). Since 2002, when the genetic variant associated with adult-type hypolactasia, a one-base polymorphism $-13910\ C/T$ upstream of the lactase-coding gene chromosome 2 was identified (18), the genetic test has been the method of choice for diagnosis. The $C-13910$ allele in homozygous form is always associated with adult-type hypolactasia and the $T-13910$ allele ($-13910\ C/T$ and $-13910\ T/T$ genotype) with lactase persistence (19) in European populations.

There are several terms used in the context of lactase deficiency. *Hypolactasia* implies very low lactase activity in the intestinal mucosa. *Lactose malabsorption* refers to a poor capacity to hydrolyse lactose to glucose and galactose, which can be absorbed in the intestine. *Lactose intolerance* implies the appearance of symptoms after lactose indigestion (20). Genotyping has previously been used in 2 Russian studies of hypolactasia among indigenous populations (14,21).

The prevalence of lactose malabsorption among Nenets from Siberia has been studied and found to be 88% (22), but no genetic study of this population has been performed previously. As the Nenets lifestyle has

remained unchanged over the years, we tested the hypothesis that the prevalence of lactase non-persistence ($-13910\ C/C$ genotype) among Nenets people having four Nenets grandparents is high, while the prevalence among Nenets originating from ethnically mixed families is lower.

This study follows a series of investigations of Finno-Ugrian populations, the aim being to investigate the impact of ethnic background on the prevalence of adult-type hypolactasia among the Nenets.

Material and methods

The concept of ethnicity implies one or more of the following: shared origin or social background; shared culture or traditions which are distinctive, maintained between generations, and lead to a sense of identity and group; and a common language or religious tradition (23). The method of concordance of grandparents' national origin to ascribe ethnicity was used in the present study. The term ethnicity is used in the biological sense.

Material collection

Collection of material was made in four settlements of the Nenets Autonomous Okrug (NAO), Archangelsk Region, where the indigenous Nenets live (Fig. 1, (24)). The Nenets represent 15.2% of the whole population of the NAO, where other ethnicities including Komi and Russians are also settled (25).

The main occupations of the inhabitants of the four settlements are reindeer husbandry, hunting, fishing and also in one case cattle farming. These settlements are thus typical for Nenets living conditions and the sample here can be considered representative for the whole nomadic Nenets population. According to official information, the total number of adult Nenets in these settlements was 493. Of these 42.6% were male and 57.4% female aged from 19 to 80 years old (Table I).

Since there are no primary health care centres in many of the settlements in the NAO a team of medical professionals make rounds in this area. The GP from this team has an official list of Nenets obtained from the local administration. For the present study every third person was selected from this list and requested to answer a questionnaire and donate buccal samples for genotyping. If a selected person was absent from the settlement when the study was performed, the following person on the list was invited to participate. Men were absent more often than women. There were thus 181 participants for the final analysis.

Sample description

All selected subjects were informed of the aim of the study and gave written informed consent before participation and provided information on their ethnicity and place of birth as well as the ethnicity and place of birth of

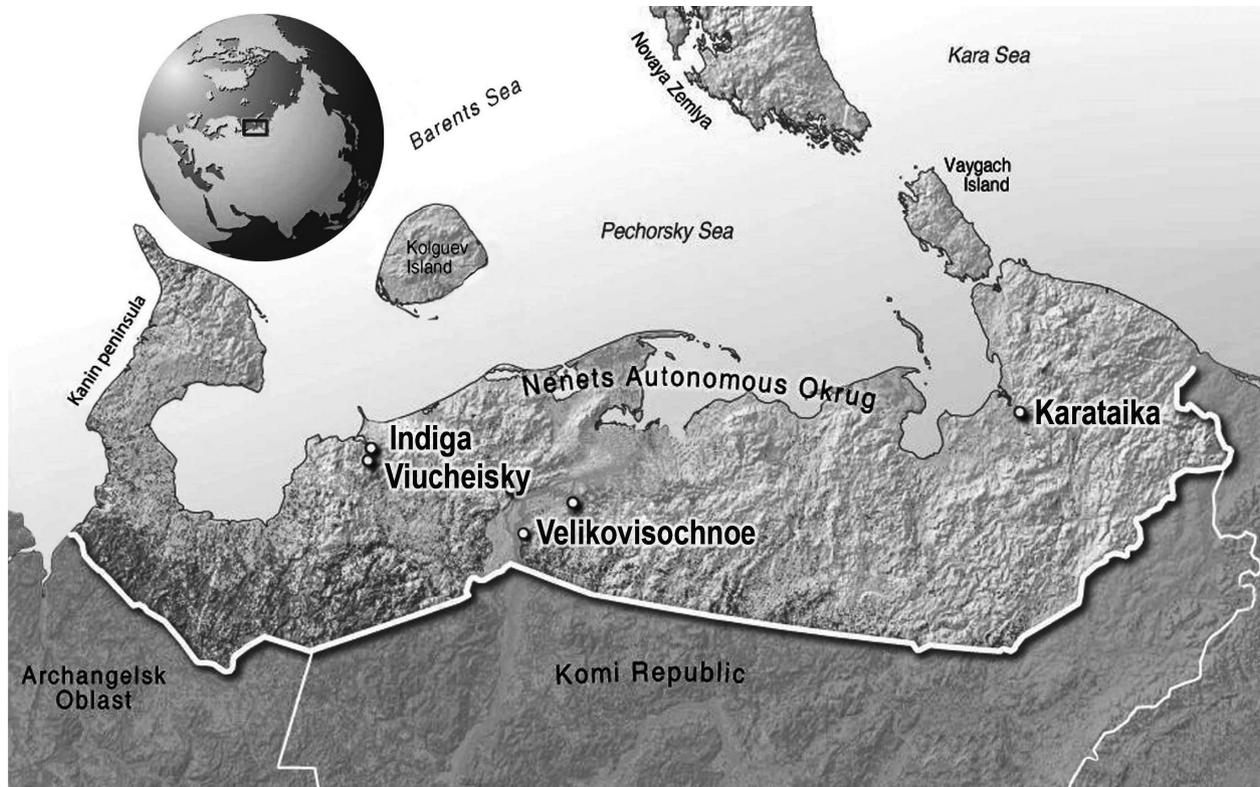


Fig. 1. Map of the Nenets Autonomous Okrug. There are 4 settlements of residence of the study population.

their parents and grandparents. Buccal samples for genotyping were taken from all 181 subjects. However, while all of them considered themselves Nenets, according to the grandparents' ethnicity only 91 (50.3%) were accepted as native Nenets having all four of their grandparents of Nenets origin. Those with 1, 2 or 3 Nenets grandparents (90 in total) were also taken for comparison.

In the process of genotyping the buccal samples, four samples remained undetermined. Three of them were

from females aged from 42 to 61 years old, and one was from a 33-year-old male of mixed ethnicity. Two of the females were of native Nenets origin, having four Nenets grandparents. Thus, 89 people of clear Nenets origin aged 19–80 years old (mean 45.8), of whom 91.0% were female and 9.0% male (Table I), were selected for the frequency analysis. The selection process is shown in Fig. 2.

The study was approved by the Health Care Department of the NAO and the Ethical Committee of the Northern State Medical University, Arkhangelsk, Russia

Table I. Characteristics of the study population (n = 493) and participants (n = 177) by gender and age

	All Nenets inhabitants of particular settlements		Participants			
			4 Nenets grandparents		Less than 4 Nenets grandparents	
	n	%	n	%	n	%
Gender						
Male	210	42.6	8	9	8	9
Female	283	57.4	81	91	80	91
Age distribution						
19–40	221	44.8	35	39	45	51
41–60	200	40.6	39	44	33	38
61–80	72	14.6	15	17	10	11
Total	493	100	89	100	88	100

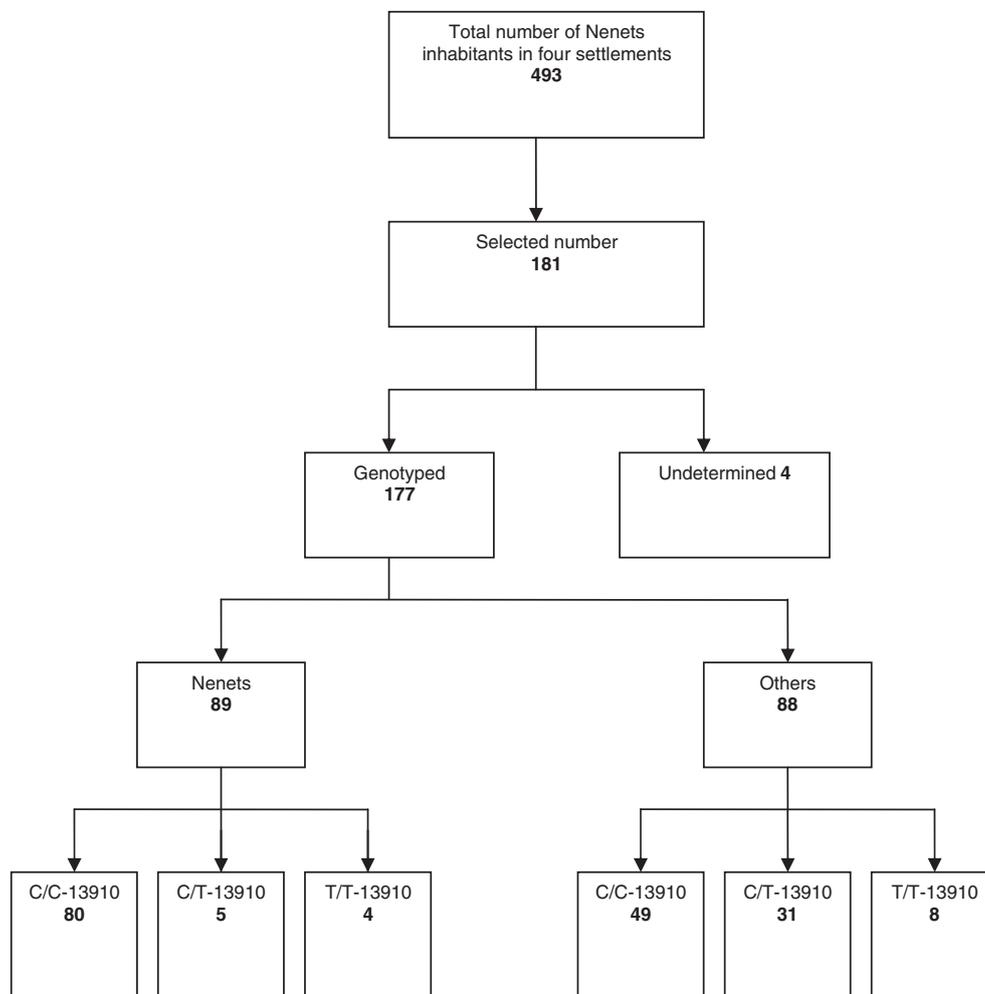


Fig. 2. Flowchart of sample selection.

(No. 09/10, 2010). The study was conducted in accordance with the United Nations Declaration on the rights of indigenous peoples (26).

Genotyping

The buccal samples were genotyped in the Forensic Laboratory of the University of Tampere. The polymorphism of lactase persistence/non-persistence SNP rs4988235 was determined using the TaqMan Human Custom Genotyping Assay from Applied Biosystems. The assay was performed with the AbiPrism 7900 HT sequence detection system (Applied Biosystems, CA, USA) according to the instructions provided with the assay.

Statistical analysis

We used frequency and cross-tabulation analysis. Differences between groups were tested by χ^2 test. Statistical analyses were performed with SPSS version 15.0 (SPSS Inc. Chicago, IL, USA).

Results

The prevalence of lactase non-persistence genotype (–13910 C/C) among Nenets having only Nenets in two previous generations was found to be 90% (Table II). The prevalence among others who regarded themselves as Nenets and reported three, two or one grandparent of Nenets origin was shown to be 72, 60 and 28%, respectively ($p < 0.0001$). Other grandparents were mostly Komi (10.7%) and Russians (8.8%), followed by Ukrainians, Byelorussians and others in single cases.

Discussion

The prevalence of adult-type hypolactasia (–13910 C/C genotype) among the nomadic Nenets inhabiting the Nenets Autonomous Okrug and having all parents and grandparents of Nenets origin was found to be as high as 90%, which is in accord with our hypothesis. The prevalence of the lactase non-persistent genotype among Nenets with three, two or one grandparent of Nenets origin was lower in comparison with those having all Nenets grandparents.

Table II. Frequency of C/T-13910 genotype variants by grandparents' origin

Number of grandparents of Nenets origin	C/C-13910		C/T-13910 or T/T-13910		Total	
	n	%	n	%	n	%
4	80	90	9	10	89	100
3	13	72	5	28	18	100
2	31	60	21	40	52	100
1	5	28	13	72	18	100
Total	129	73	48	27	177	100

The four settlements in which the collection of material was made are typical for Nenets living conditions and can therefore be considered representative for the whole nomadic Nenets population. The distribution by gender in the study group differed from that in the overall population of the area. Compared to Nenets women, Nenets men were absent more often from the settlements due to reindeer keeping, fishing, hunting or for personal reasons. However, since gender does not affect the lactase persistent/non-persistent genotype, this fact caused no biases. The age of participants involves no bias in the genetic method and therefore has no effect on the results.

The findings here bring to light the actual prevalence of adult-type hypolactasia among the indigenous Nenets, since genotyping and the method of concordance of national origin (23) were used for the first time to investigate the frequency of the lactase persistence/non-persistence genotype among this group. Genetic testing is a highly sensitive and highly specific method of adult-type hypolactasia (lactase non-persistence) diagnostics (27) while the method of concordance of national origin allows definition of the ethnicity of investigated persons. We used the term ethnicity in the biological sense in our study. Since all participants were of active age and capable of understanding the questionnaire, we may rely on the accuracy of the answers. Nenets participants had no problems in defining the ethnicity of their grandparents. The Nenets are traditionally careful to preserve their ethnic features and are indubitably aware of family and relatives. Previously published data on lactose malabsorption among the Nenets have presented a sample of 9 persons of Nenets origin from Siberia (13). According to the lactose tolerance test, the prevalence was estimated at 78% (7 of 9 persons). Another study involving 108 Nenets from Siberia showed the prevalence of lactose malabsorption among them to be 88% (22). The prevalence of lactose malabsorption among the Nenets as determined by indirect methods was thus found to be high. Our present results amplify these previously published data and enhance their accuracy in that the genotyping has been confirmed to

correlate closely with disaccharidase activity in the small intestine (27).

The impulse to discover differences in the prevalence of hypolactasia among indigenous peoples derives from the considerable variability in the frequency of lactase non-persistence among them. So far no population with 100% frequency of – 13910 C/C genotype has been discovered. Previously published data on the prevalence of lactose malabsorption among indigenous groups inhabiting different territories in the Russian Federation show the highest prevalence to obtain among the Khants (near the River Ob), with a figure of 94% (11). The frequency of the C/C-13910 genotype in Komi-Permyaks was found to be 42%, while the Chukchi have an 89% frequency of the lactase non-persistent genotype (22). Among other arctic populations a high prevalence of lactose intolerance in 70% investigated by lactose tolerance test was shown among northern Alaskan Eskimos (28). Such genetic differences in neighbouring populations can be explained by different earlier histories.

The Nenets have traditionally never consumed cow milk before the 20th century. Historians believe that this group (formerly Samoyeds) split apart from the Finno-Ugrian speaking group around 3000 BC, and then possibly mixed with the Turkic and Altaic-speaking group around 200 BC. Some groups settled in Siberia, while others remained nomadic and maintained reindeer herding and hunting, traveling long distances (7,29). Cattle breeding is reported to have started among the Nenets during the Soviet era, the first farms being organised in Nenets villages in the 1930s (8,10). The Nenets tribes have thus been isolated and more likely only during the last 70–80 years the gene flow from neighbouring populations has played a role in the prevalence of lactase non-persistence. Those who still have mostly Nenets forebears retain one of the highest prevalences of adult-type hypolactasia.

Our findings support and amplify the cultural historical hypothesis of hypolactasia. Not only the history of milk drinking but also the gene flow from other populations exerts an influence on the frequency of lactase non-persistence.

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I declare that the authors have no competing interests which might be perceived to influence the results and/or discussion reported in this article.

References

1. Simoons FJ. Primary adult lactose intolerance and the milking habit: a problem in biological and cultural interrelations. II. A culture historical hypothesis. *Am J Dig Dis*. 1970;15:695–710.
2. McCracken RD. Adult lactose intolerance. *JAMA*. 1970;213:2257–60.
3. McCracken RD. Lactase deficiency: an example of dietary evolution. *Curr Anthropol*. 1971;12:479–517.
4. Flatz G, Rotthauwe HW. Lactose nutrition and natural selection. *Lancet*. 1973;14:76–7.
5. Cook GC, Al-Torki MT. High intestinal lactase concentrations in adult Arabs in Saudi Arabia. *Br Med J*. 1975;3:135–6.
6. Borinskaia SA, Kozlov AI, Yankovsky AI. Genes, people and traditions of nutrition. *Etnograficheskoe obosrenie* 2009; 3:117–37. [in Russian]
7. Chnirelman VA. Introduction: North Eurasia. In: Lee RB & Daly R, editors. *The Cambridge encyclopedia of hunters and gatherers*. Cambridge: Cambridge University Press; 1999. p. 119–26.
8. Stammler-Gossmann A. “Translating” vulnerability at the community level. Case study from the Russian North. In: Hovelsrud GK & Smit B, editors. *Community adaptation and vulnerability in Arctic region*. Springer, Netherlands; 2010. p. 131–63.
9. Mirov NT. Notes of the domestication of reindeer. *American Anthropologist*. 1945;47:393–408.
10. Information about settlements in the Nenets A.O. In: *Le petit fute* 2003. Nenetskiy avtonomyy okrug. Moscow: Avangard; 2003 [cited]. Available from: <http://ipy-nenets.npolar.no/pdf/20files/Settlements.pdf>
11. Lember M, Tamm A, Piirsoo A, Suurmaa K, Kermes K, Kermes R, et al. Lactose malabsorption in Khants in western Siberia. *Scand J Gastroenterol*. 1995;30:225–7.
12. Zhvavyi NF, Kozlov AI, Kondik VM. Lactase deficiency among representatives of various nationalities of Siberia. *Vopr Pitan*. 1991;5:32–5. [in Russian]
13. Kozlov A. Hypolactasia in the indigenous populations of northern Russia. *Int J Circumpolar Health*. 1998;57:18–21.
14. Kozlov A. Lactase intolerance (primary hypolactasia) in different population groups of Eurasia. [dissertation]. Moscow: Moscow State Pedagogical University; 2004. [in Russian]
15. Khabarova Y, Torniaainen ST, Nurmi HA, Järvelä IE, Isokoski MK, Mattila KJ. Prevalence of lactase persistent/non-persistent genotypes and milk consumption in a young population in north-west Russia. *World J Gastroenterol*. 2009;15:1849–53.
16. Sahi T, Isokoski M, Jussila J, Launiala K, Pyörälä K. Recessive inheritance of adult-type lactose malabsorption. *Lancet*. 1973;2:823–6.
17. Sahi T. The inheritance of selective adult-type lactose malabsorption. *Scand J Gastroenterol*. 1974;30(Suppl):1–73.
18. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Järvelä I. Identification of a variant associated with adult-type hypolactasia. *Nat Genet*. 2002;30:233–7.
19. Rasinperä H, Savilahti E, Enattah NS, Kuokkanen M, Tötterman N, Lindahl H, et al. A genetic test which can be used to diagnose adult-type hypolactasia in children. *Gut*. 2004;53:1571–6.
20. Sahi T. Hypolactasia and lactase persistence. Historical review and the terminology. *Scand J Gastroenterol*. 1994;202(Suppl):1–6.
21. Borinskaia SA, Rebrikov DV, Nefedova VV, Kofiadi IA, Sokolova MV, Kolchina EV et al. Molecular diagnosis and frequencies of primary hypolactasia in population of Russia and neighbouring countries. *Mol Biol (Mosk)*. 2006;40: 1031–6. [In Russian, abstract in English]
22. Kozlov AI, Vershubskya G, Kozlova M. Indigenous peoples of northern Russia: anthropology and health. *Circumpolar Health Supplements No 1*. Oulu: International Association of Circumpolar Health Publishers; 2007. 184 p.
23. Senior PA, Bhopal R. Ethnicity as a variable in epidemiological research. *BMJ*. 1994;309:327–30.
24. Hugo Ahlenius, UNEP/GRID-Arendal. Nenets Autonomous Okrug sites. Arendal: UNEP/GRID-Arendal; 2006 [cited]. Available from: http://maps.grida.no/go/graphic/nenets_autonomous_okrug_sites.
25. The Government of Arkhangelsk Region.: The Government of Arkhangelsk region; [cited]. Available from: <http://www.dvinaland.ru/region/nao/>
26. United Nations. United Nations Declaration on the rights of indigenous peoples. Adopted by the General Assembly 13 September 2007. United Nations; 2008 [cited]. Available from: http://www.un.org/esa/socdev/unpfii/documents/DRIPS_en.pdf.
27. Järvelä IE. Molecular diagnosis of adult-type hypolactasia (lactase non-persistence). *Scand J Clin Lab Invest*. 2005;65:535–9.
28. Bell RR, Draper HH, Bergan JG. Sucrose, lactose, and glucose tolerance in northern Alaskan Eskimos. *Am J Clin Nutr*. 1973;26:1185–90.
29. *New World Encyclopedia*. Nenets. *New World Encyclopedia*; 2010 [cited]. Available from: <http://www.newworldencyclopedia.org/entry/Nenets>.

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