First Bosnian study of LCT -13910C>T and -22018G>A single nucleotide polymorphisms associated with adult-type lactose intolerance

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ABSTRACT

Objectives: It is suggested, that over the last 1x10⁴ years most ancient Europeans, have not been able to digest milk as adults. Lactose tolerance (lactase persistence) is an example of convergent evolution due to strong selective pressure resulting from shared cultural traits-animal domestication and adult milk consumption. This phenotype varies widely in humans, as a function of ethnicity. Recent reports have identified that a genetic polymorphisms -13910C>T and -22018G>A of LCT gene are closely associated with lactase persistence (LP) and lactase non-persistence (LNP) phenotypes. We sought to assess the prevalence of 13910C>T and -22018G>A variants of LCT gene in Bosnian subjects.

Methods: The subjects of the study consisted of 151 unrelated subjects from Bosnia and Herzegovina (60 males and 91 females). The mean age of the study sample was 48.0±16.4) years. PCR-RFLP was used to study of genotype and allele distribution. Data were analyzed using the Stat View computer software version 5.0 (SAS Institute Inc. Cary, NC, USA). Electronic databases including Medline and Embase were searched from 1995 to February 2017.

Results: The genotypes linked to the LP phenotype were found in 74 (41.0%) of the 151 subjects. The frequency of -13910T and -22018A alleles of LCT gene was 24.8% and 24.5%, respectively. The CC/GG genotype, related to LNP was found in 77 (51.0%) individuals.

Conclusion: In studied European populations we observed a linear, gradually increasing trend in the frequency of -13910T allele from South to North (Pearson’s test: 0.5728 , P-value<0.001 ), and Bosnian population perfectly fits into this pattern.

Keywords: LPH-1, lactase persistence, lactase non-persistence, population genetics

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INTRODUCTION

Lactose is a disaccharide from mammalian milk, and human milk contains the highest concentration of it, 7%. Milk sugar, lactose is hydrolyzed into the absorbable monosaccharides, glucose and galactose by lactase (LCT) or more precisely lactase-phlorizin hydrolase-1 (LPH-1) [1].

Lactose intolerance is a worldwide phenomenon that has been widely studied over the years in order to understand the influences of human evolution and migration over the ages. The LCT activity is high during infancy but in most mammals, including most humans decreases after weaning. However, in some humans, especially those from populations with a history of dairy ing, lactase is expressed throughout adulthood. This trait is called lactase persistence (LP) and may have its roots in the Neolithic period [2].

During the Neolithic period, milk consumption became common even among adults, especially in the agriculturally developed areas. In northern Europe, particularly in the populations of the British, Scandinavians and Germans with long history of pastoralism and milking, unprocessed milk were consumed. However, milk consumption in adults was not common in Southern Europe, such as the Mediterranean region. Even nowadays, lactose intolerance is rarely seen in the British, Germans and Scandinavians but is commonly seen in southern European populations. Therefore,
lactase non-persistence (LNP) is the most common phenotype in Europe, except in Northwestern European populations [3]. In lactase non-persistence, known as lactose intolerance, after milk or lactose containing food ingestion lactose reaches the colon, where it is fermented by colonic bacteria. Products of this fermentation and osmotic effects result in abdominal cramps, bloating and diarrhea [4].

Few sources indicate that lactase persistence has evolved independently, in different parts of the world over the last \(1 \times 10^4\) years, and has been subject to strong natural selection as a gene-culture co-evolution [5-7].

The genetic variants -13910C>T and -22018G>A of \(LCT\) gene in introns 13 and 9 of the minichromosome maintenance type 6 gene (\(MCM6\)), respectively, seem to be at the root of this evolved lactose tolerance, ability to digest milk as adults in Europeans [8]. The presence of genotype CC/GG is interpreted as LNP, while the CT or TT genotypes and GA or AA as LP. The long region of haplotype conservation reflects a recent origin, and this, together with high frequencies, may be evidence of positive selection [7].

The genetic structure of the population of Europe attracts great attention, on the one hand from a historical perspective, and on the other, for the interpretation of genetic epidemiological studies. Probably, the genetic variants linked to lactase persistence in European populations were initially undergone positive selection in farmers from the Balkans and Central Europe, and subsequently shaped the distribution of this adaptive trait across Europe [9]. However, not all the pieces of the puzzle were elucidated.

While, the genetic background of LP in a several European populations was examined, in Bosnian population it is still unknown. Therefore, the aim of our study was to establish the first picture of the Bosnian population for the distribution of -13910C>T and -22018G>A polymorphisms of \(LCT\) gene, linked to lactase persistence.

**Results**

\(HhaI\) digestion yields fragments were: 401 bp, 151 bp and 24 bp (the CC genotype); 451 bp, 253 bp, 151 bp, 148 bp and 24 bp (the CT genotype); 253 bp, 151 bp, 148 bp and 24 bp (the TT genotype), while, \(Hhal\): 224 bp (the AA genotype); 224 bp, 116 bp, 108 bp (the GA genotype); 116 bp and 108 bp (the GG genotype) (see Figure 1A and 1B). The restriction analysis fragments were visualized by the electrophoresis in 2-3% agarose gel (Sigma-Aldrich Chemie GmbH, Munich, Germany) stained with DNA-star dye, running at 100 V for 40 min (Lonza Inc., Rockland, ME, USA).

For quality control purposes, approximately 10% of the samples were re-genotyped in a blinded fashion and the same results were obtained.

In our study included 151 subjects (91 female), mean age 48.0±16.4 years. According to their ethnic origin, all were Bosnians from Sarajevo (an estimated population of 369.534 inhabitants, while in Bosnia and Herzegovina 3.867.055 [10]. Samples from volunteers were collected at the Laboratory for Molecular Medicine, Center for Genetics, Faculty of Medicine, University of Sarajevo and from patients on hemodialysis from Clinic for Hemodialysis, Clinical Center, Sarajevo. Written informed consent was obtained from all participants. Twins and subjects with any serious illness (including: hepatic, pulmonary, renal and cancer) were excluded from the study. The present study was conducted according to the standards of the Declaration of Helsinki (1975, revised 2000), and the protocol of the study was approved by the local bioethical committee (decision reference number KB-0012/12/16).

Genomic DNA was extracted from buccal swabs, according to the manufacturer’s instructions (PrepFilerTM Forensic DNA Extraction Kit, Life Technologies, USA) as previously reported by Adler et al. [11]. The \(LCT\)-13910C>T (NM_005915.4:c.1917+326C>T; rs4988235) and -22018G>A polymorphisms were analyzed by PCR-RFLP using relevant primers as follows: forward 5’-CAT GGA GGA TTA CAG TGC GAC AGC-3’ and reverse 5’-CCT TGG TTG AAG CGA AGA TGG GA-3’ and forward 5’-TAAGAAACTTTTACACTC-3’ and reverse 5’-AGAAAATGCGTTTTCGCCATG-3’, respectively. Primers were designed based on database [12], and synthesized by TIB Mol Biol (Poznan, Poland). For each polymorphism, PCRs were performed using the LabCycler (SensoQuest GmbH, Gottingen, Germany) with the following temperature profiles: initial denaturation at 94°C for 5 min; 34 cycles of 20 s at 94°C, 40 s at 58°C and 40 s at 72°C; final extension step at 72°C for 7 min. For the \(LCT\)-13910C>T and -22018G>A polymorphisms, amplification was followed by digestion with 1U \(Faq\) I (BomFl) (5’GGGAC(N)10\(↓\)3’ 3’CC C C T G (N)\(↑\)5’) and 5U \(Hhal\) (5’GGCG\(↓\)C3’3’C\(↑\)GCC5’) restriction enzymes, respectively (Thermo Fisher Scientific).

**Patients and methods**

Our study included 151 subjects (91 female), mean age 48.0±16.4 years. According to their ethnic origin, all were Bosnians from Sarajevo (an estimated population of 369.534 inhabitants, while in Bosnia and Herzegovina 3.867.055 [10]. Samples from volunteers were collected at the Laboratory for Molecular Medicine, Center for Genetics, Faculty of Medicine, University of Sarajevo and from patients on hemodialysis from Clinic for Hemodialysis, Clinical Center, Sarajevo. Written informed consent was obtained from all participants. Twins and subjects with any serious illness (including: hepatic, pulmonary, renal and cancer) were excluded from the study. The present study was conducted according to the standards of the Declaration of Helsinki (1975, revised 2000), and the protocol of the study was approved by the local bioethical committee (decision reference number KB-0012/12/16).
We reviewed the literature data set containing allele frequencies of the population belonging to 18 countries (the average population size was 421 subjects, range 45-1876 subjects for the population). The total number of people in all 18 populations was 7,580, equivalent to 15,160 alleles. When a few studies were available for the same population, the ultimate frequency of alleles was computed as weighted average (Table 2).

### Table 2. Distribution of allele -13910T of LCT gene in European populations

<table>
<thead>
<tr>
<th>Country, abbreviation by UN Statistics Division-Standard Country and Area Codes Classifications</th>
<th>Population, n</th>
<th>No. of alleles</th>
<th>Latitude</th>
<th>Frequency of allele linked to LP [%]</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria, AUT</td>
<td>Austrians, 120</td>
<td>240</td>
<td>48</td>
<td>48.3</td>
<td>[13]</td>
</tr>
<tr>
<td>Belarus, BLR</td>
<td>Belarusians, 92</td>
<td>184</td>
<td>54</td>
<td>85.9</td>
<td>[14]</td>
</tr>
<tr>
<td>Bosnia and Herzegovina, BIH</td>
<td>Bosnians, 151</td>
<td>302</td>
<td>51</td>
<td>24.8</td>
<td>our study</td>
</tr>
<tr>
<td>Denmark, DNK</td>
<td>Danes, 158</td>
<td>316</td>
<td>55</td>
<td>78.8</td>
<td>[15]</td>
</tr>
<tr>
<td>Estonia, EST</td>
<td>Estonians, 355</td>
<td>710</td>
<td>59</td>
<td>51.4</td>
<td>[16]</td>
</tr>
<tr>
<td>Finland, FIN</td>
<td>Finns, 1876</td>
<td>3752</td>
<td>60</td>
<td>58.0</td>
<td>[17]</td>
</tr>
<tr>
<td>France, FRA</td>
<td>French, 310</td>
<td>620</td>
<td>49</td>
<td>58.1</td>
<td>[7,17]</td>
</tr>
<tr>
<td>Germany, DEU</td>
<td>Germans, 434</td>
<td>868</td>
<td>52</td>
<td>56.3</td>
<td>[15]</td>
</tr>
<tr>
<td>Greece, GRC</td>
<td>Greeks, 182</td>
<td>364</td>
<td>38</td>
<td>10.7</td>
<td>[18,19]</td>
</tr>
<tr>
<td>Hungary, HUN</td>
<td>Hungarians, 110</td>
<td>220</td>
<td>47</td>
<td>59.9</td>
<td>[20]</td>
</tr>
<tr>
<td>Ireland, IRL</td>
<td>Irish, 65</td>
<td>130</td>
<td>53</td>
<td>95.0</td>
<td>[9]</td>
</tr>
<tr>
<td>Italy, ITA</td>
<td>Italians, 468</td>
<td>936</td>
<td>42</td>
<td>10.3</td>
<td>[19]</td>
</tr>
<tr>
<td>Poland, POL</td>
<td>Poles, 200</td>
<td>400</td>
<td>52</td>
<td>46.0</td>
<td>[21]</td>
</tr>
<tr>
<td>Russia, RUS</td>
<td>Russians, 233</td>
<td>466</td>
<td>55</td>
<td>31.8</td>
<td>[7,22]</td>
</tr>
<tr>
<td>Spain, ESP</td>
<td>Spaniards, 1643</td>
<td>3286</td>
<td>40</td>
<td>39.4</td>
<td>[15,23,24]</td>
</tr>
<tr>
<td>Sweden, SWE</td>
<td>Swedes, 784</td>
<td>1568</td>
<td>59</td>
<td>74.4</td>
<td>[25]</td>
</tr>
<tr>
<td>The United Kingdom, GBR</td>
<td>British, 354</td>
<td>708</td>
<td>51</td>
<td>70.2</td>
<td>[15]</td>
</tr>
<tr>
<td>The Netherlands, NLD</td>
<td>Dutch, 45</td>
<td>90</td>
<td>52</td>
<td>65.6</td>
<td>[15]</td>
</tr>
</tbody>
</table>
In Northern European populations (n=3592) represented by Danes, Estonians, Finns, Swedes, the British and the Irish, the average frequency of allele T was 63.8% [9,15-17,25]. In Southern European populations (n=2444), such as: Bosnians, Greeks, Italians and Spaniards, the average frequency of allele T was 38.0% [our study, 15,18,19,23,24]. In Eastern European populations (n=635): Belarusians, Hungarians, Poles and Russians and Western European populations (n=909): Austrians, Dutch, French and Germans, the average frequency of allele T was 48.7% and 56.3%, respectively [7,13-15,20-22,26].

About 65% of the World’s population have an ancestral condition, a hereditary decrease in lactase synthesis after weaning, known as primary lactase deficiency or lactase non-persistence, which manifests as lactose intolerance [3,27]. Lactose intolerance symptoms usually do not occur, until there is less than 50% of lactase activity. On the other hand, it is known that, regular lactose intake may have an effect on tolerance, through induction and following adaptation of intestinal flora [28].

The lactase persistence is common in Europids as well as in many Afro-Americans, Asians and Middle Eastern and Southern populations, and again is rare or absent elsewhere in the world [29,30,31,32]. It is assumed that, in human evolution, the lactase persistence developed several times independently, in different parts of the world [30]. It has been proven that the distribution of physiological decline of lactase depends on the geographic region and ethnicity [33,34]. In different populations, arose several separate variants, which have allowed quickly modifying LCT expression and have been conserved in populations consuming milk lifelong. Thus, in Saudis lactase persistence is mediated by allele -13915G, while in Africans by few alleles: -14010G, -13915G and -13907G [30,32,35]. The -13910T allele is about 86% to 98% linked to lactase persistence in Europids. While, alleles -13910T and -22018A are, respectively about 100% and 97% linked to lactase persistence in Finns [36]. It emphasizes the importance of regulatory mutations in recent human evolution [37].

The frequency of allele T is high in Northern European populations and decreases across Southern European populations and the Middle East [29].

In studied populations from 18 countries, we observed a linear increasing trend in the frequency of -13910T allele from South to North (Figure 2.). Statistically significant differences are observed between the populations of Northern Europe (63.8%, n=2592) vs. Southern (38.0%, n=2444) (p=0.003). Furthermore, significant differences are observed between the populations of Eastern Europe (48.7%, n=635) vs. Western (56.3%, n=909), Western (56.3%, n=909) vs. Northern (63.8%, n=2592), Western (56.3%, n=909) vs. Southern (38.0%, n=2444), Eastern (48.7%, n=635) vs. Northern (63.8%, n=2592) and Eastern (48.7%, n=635) vs. Southern (each p<0.001).

The lowest frequency of allele T was observed in Italians and Greeks, 10.3% and 10.7%, respectively [18,19], while the highest in Irish and Belarusians, 95.0% and 85.9%, respectively. However, these results should be

![Figure 2. Linear trend in the frequency of -13910T allele of LCT gene with increase in latitude by 1 unit, frequency increases by 2.19%](image-url)

AUT - Austria, BLR - Belarus, BIH - Bosnia and Herzegovina, DEU - Germany, DNK - Denmark, ESP - Spain, EST - Estonia, FIN - Finland, FRA - French, GBR - the United Kingdom, GRC - Greece, HUN - Hungary, IRL - Irish, ITA - Italy, NLD - the Netherlands, POL - Poland, RUS - Russia, SWE - Sweden (references as in Table 2).
interpreted with caution due to the small sample size of both Irish and Belarusians population, n=65 and n=92, respectively [9,14].

Most studies in European populations focus on the distribution of allele -13910T. However, we found few studies on the distribution of both alleles -13910T and -22018A. Unfortunately, due to lack of genotypes -13910C>T and -22018G>A of the LCT gene, the percentage of LP subjects could not be calculated. It should be noted that the percentage of individuals with -13910T and -22018A mutated alleles was similar and lowest in Greeks and Italians and was 9.0% and 12.0%, 13.4% and 15.7%, respectively [19]. While, in Russians both, -13910T and -22018A allele distribution was 24.0%, and in Austrians and French 48.3% and 49.6% and 53.8% and 54.7%, respectively [7,13]. Distribution of alleles -13910T and -22018A in Bosnian population was similar to those of the Russians, but our sample was three times more numerous (n=50 vs. n=151). It is noteworthy that high compatibility of two variants associated with LP suggests that the LP is a case of parallel selection, or convergent evolution. Unfortunately, we did not find the data for neighboring countries of Croatia and Serbia. It would also be interesting to compare our data with other Balkan countries.

**Conclusion**

Our results are another element of puzzle, which completes the European data on lactase persistence, where Bosnian population perfectly fits into European pattern.

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**Declaration of interest**

The authors declare no conflicts of interest.

**References**


