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Frequency of lactase persistence genotype in a healthy Polish population

Communication

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Abstract: The majority of mammals are unable to digest lactose due to post-weaning deactivation of the *LCT* gene, which is responsible for encoding the enzyme lactase (*i.e.*, adult-type hypolactasia). A substitution of C with T at position –13910 bp upstream of the *LCT* gene has been linked to the lactase persistence phenotype in European populations. We investigated the frequency of LCT-13910C>T polymorphism in 223 blood donors from central Poland. All samples were genotyped by polymerase chain reaction and direct sequencing. The LCT-13910 T allele (lactase persistence) was present in 51% of individuals sampled from the Polish population. We did not find any non-European variants associated with lactase persistence (LCT-13907C>G, LCT-13913T>C, LCT-13915T>G), or any new polymorphisms within the sequenced region. Allele frequencies obtained are in agreement with results from other countries and confirm the unique pattern of distribution of the LCT-13910C>T genotype in Europe.

Keywords: *Hypolactasia • Lactase persistence • Lactose malabsorption • LCT-13910C>T genotype • Milk • Poland*

1. Introduction

Lactose, a disaccharide present in mammalian milk, is enzymatically hydrolysed into glucose and galactose by lactase-phlorizin hydrolase (LPH). LPH is secreted into the lumen of the intestine in a significant proportion of the global human population (lactase persistence) [1]. The rest of the population (65%) displays lactase non-persistence: lactase activity declines after weaning [2], and the consumption of milk or lactose-containing products often results in clinical symptoms such as abdominal pain, flatulence and diarrhoea [3]. The global age of onset of lactase non-persistence ranges from approximately 2 years in the Thai population to approximately 12 years of age among the Finns [4,5]. In Europe, lactase persistence follows a North-South gradient. In Scandinavia, for example, about 90% of adults are milk-tolerant, while in the Italian population this frequency ranges from 11% to 50% [6-8]. The human lactase gene is located on the 2q21-22 chromosome [9]. A single nucleotide polymorphism (SNP) C/T (located -13910 bp upstream from the start codon of the LPH, within intron 13 of *MCM6* (minichromosome maintenance complex component 6), is strongly associated with lactase non-persistence/persistence in Europeans [10]. The homozygous LCT-13910 C variant (the C/C genotype) is associated with hypolactasia, while the LCT-13910 T allele (C/T and T/T genotypes) is responsible for lactase persistence [11]. The transcriptional activation of the lactase promoter and the level of lactase protein production depends on the T/C variant, thus the SNP operates as *cis* regulatory element of the lactase gene [12]. In a study of Caco-2 cells, the LCT-13910 T allele was associated with a 4-fold increase in lactase promoter activity compared to the LCT13910 C allele [13]. The strong correlation between allelic variants LCT-13910C>T and the activity of lactase means that allelic variation can be a very useful diagnostic marker of adult-type hypolactasia [14]. Several variants, including LCT-13907C>G, LCT-13913T>C, LCT-13915T>G, LCT-14010G>C, and LCT-22018G>A, have been identified in other populations (e.g., Africa, Middle East, China). These variants are probably also associated with lactase persistence [15-17].

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It has been suggested that, in some regions, the introduction of milk into the human diet during the Neolithic Revolution (*circa* 6-9 thousand years ago) resulted in a strong, positive selection for the ability to digest lactose [15]. Lactase persistence guaranteed regular access to a rich source of energy, minerals and vitamins, which could act as a major selective factor for the LCT-13910 T allele [18]. Thus, it is hypothesized that the allele spread throughout Europe as farmers brought new technologies from the Near East. The allele was not found in the few European DNA samples obtained from Linear Pottery and Körös Neolithic cultures [19], probably because the individuals studied represented European mtDNA haplogroups rather than those which originated in the region where new farming technologies arose.

With these findings in mind, our objective was to determine the prevalence of lactase non-persistence/persistence in the Polish population, based on the frequency of LCT-13910C>T polymorphism. To date, only one paper has reported the prevalence of adult-type hypolactasia in Polish adults, based on the common clinical symptoms of lactose intolerance. These data indicate that 37.5% of Polish adults are not able to digest lactose [20], but also highlight that the genotype most abundant in Europe (LCT-13910C>T) has not yet been investigated in Poland.

2. Experimental Procedures

2.1 Study population

A random sample (n=223) of individuals aged 20-63 were drawn from the Polish population. Only participants with both parents of Polish descent were selected for the study. DNA was isolated using the MagNA Pure® Compact Nucleic Acid Purification System (Roche, Japan). The study was approved by the Ethics Committee of the Medical University of Łódź.

2.2 Genotyping

The DNA fragment spanning the LCT-13910C>T variant was amplified in a polymerase chain reaction using primer pairs (5'-GCGCTGGCAATACAGATAAGATA-3') and (5'-AATGCAGGGCTCAAAGAACAA-3'), which yielded a PCR product that was 111 bp long. Amplification reactions were performed in 25 µl, with 1 µl of extract, using standard reagents including DFS Taq Polymerase (Bioron), with a profile of 25 cycles and annealing at 55°C. Approximately 4 µl of the amplicons were visualized on silver-stained 10% polyacrylamide gel. PCR products, after cleaning on spin columns (Clean-up, A&A Biotechnology), were extended using the BigDye® 3.1 termination-ready reaction mix. Each sequencing reaction (20 µl) contained 4 µl of BigDye® mix, 30 ng of primer and 50–70 ng of amplicon. Cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 36 cycles at 95°C for 30 seconds, 56°C for 8 seconds, and 60°C for 4 minutes. Extension products were cleaned on spin columns (ExTerminator, A&A Biotechnology), dried in a Speed-Vac system, resuspended in 20 µl of deionized formamide and analyzed with an ABI Prism 310™ Genetic Analyzer. Sequences were edited using BioEdit and MEGA 4: *Molecular Evolutionary Genetics Analysis* [21].

3. Results

Genotype LCT-13910C>T was successfully determined in 223 subjects living in central Poland. The genotype distributions of this SNP showed no deviation from the Hardy-Weinberg equilibrium (χ^2=0.31; P=0.856). Within the 111 bp sequence we did not find any non-European variants or any other polymorphism down- or upstream of the LCT-13910C>T site. The average sequence read length was 85-90 bp, and included fragments from -13917 to -13832 bp upstream of the *LCT* gene. The frequencies of different LCT-13910C>T genotypes are presented in Table 1.

4. Discussion

Frequencies of the LCT-13910C>T genotypes suggest that 49% of Polish adults are lactose-intolerant. However, the study by Ksiazyk demonstrated a somewhat lower frequency of hypolactasia in Poland (37.5%) [20]. The discrepancy observed between genotype and phenotype may be attributed to the indirect methods used in the former study to determine hypolactasia (*i.e.*, using clinical symptoms only). Moreover, some authors have demonstrated that some individuals with the C/C genotype are able to consume milk (especially during meals) without displaying symptoms of lactase non-persistence [22]. Results obtained using genotyping are

Genotype of the LCT-13910C>T variant	Frequency % (n)
CC	49% (109)
CT	43% (96)
TT	8% (18)
	100% (223)

Table 1. Frequency of the LCT-13910C>T variant.

expected to be more precise, because the study subject is not exposed to environmental factors which may confound the results.

Almost half of the subjects involved in the present study were found to be the C/C variant (49%). This value is in agreement with results obtained for other countries and confirms the unique pattern of distribution of the LCT-13910C>T genotype in Europe (see Table 2) [23]. Our results suggest that the LCT-13910C>T variant is probably the only SNP responsible for the regulation of lactase activity in the Polish population. This statement is supported by the lack of polymorphisms (either from this study or previously known) within the sequenced region (LCT-13907C>G, LCT-13913T>C, LCT-13915T>G). However, we have not determined every known lactase persistence genotype variant from other ethnic groups. Further study is therefore needed to confirm our theory.

Daily milk intake is not strictly correlated with the LCT-13910C>T genotype, but some reports proved statistically significant relationships between these two factors [22]. For example, a study conducted by the Agricultural University of Poznań [24], based on a questionnaire about dairy product consumption, showed that almost half of Polish participants (47.6%) reported drinking milk at least 4 out of 7 days of the week. Only 15.6% of subjects declared that they did not drink milk at all. The rest (36.8%) reported rarely drinking milk. These results are in agreement with the genotype frequencies obtained in our study: just over half (51%) of the population examined possessed the lactose-tolerant variant and would therefore be expected to tolerate milk products.

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Ethnic [Reference]	Genotype %					
	n	TT	CT	CC	χ^2	P-value [a]
Irish [25]	47	77	21	2	116.6	4.70×10^{-26}
Swedish [22]	684	46	40	14	159.9	1.93×10^{-35}
Finns [10]	938	34	48	18	114.1	1.66×10^{-25}
Estonian [26]	314	27.7	47.5	24.8	47.9	4.00×10^{-11}
Russian - north-western Russia [27]	149	13.4	51	35.6	7.4	0.025
Hungarian [28]	110	15	48	37	2.9	0.240*
Italian - southern Italy [8]	100	0	11	89	42	$7{,}4 \times 10^{-10}$

Table 2. Genotype distributions of LCT-13910C>T polymorphism in different European countries.

** - no significant difference (P>0.05)*
[a] - compared with Polish control group by Chi-square test

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