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Milk consumption is not affected by C/T-13910 single nucleotide polymorphism in a Tajik population in a southern region of Uzbekistan

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Abstract: Objective: To determine the prevalence of adult-type hypolactasia in the Tajik population living in the south of Uzbekistan by genotyping of allelic variants C/T-13910 and identifying their tolerance to milk. Methods: 100 ethnic Tajik adults were examined for C/T-13910 single nucleotide polymorphisms using polymerase chain reaction/restriction fragment length polymorphism. Milk tolerance was analyzed by a questionnaire developed to assess dyspepsia after milk and dairy consumption. Results: A genetic marker of hypolactasia - C/C-13910 genotype was detected in 83% of the adult Tajik population. However, despite the high prevalence of lactose intolerance in the Tajik population, the majority consume between 100-200 ml of milk per day. Conclusion: The Tajik population is characterized by a high prevalence of hypolactasia. Milk consumption is not affected by the determined genotypes since people of Tajik ethnicity consume milk in small quantities.

Keywords: Hypolactasia, Milk Consumption, Tajik Population

1. Introduction

Single Nucleotide polymorphism C/C -13910 is responsible for the decrease in the lactase activity in the brush border of enterocytes and for the development of adult-type hypolactasia or lactase deficiency [1]. It is one of the most common enzyme deficiencies among mammals, and has a homozygous recessive inheritance trait [2, 3]. Most of the adult human populations have high prevalence of hypolactasia and intolerance to milk [4]. The activity of lactase in the intestinal mucosa progressively decreases after the weaning period [5, 6].

Genetic nucleotide polymorphism alleles C/T, T/T (13910) refer to one of the most ancient and prominent human gene mutations associated with genetic selection. In spite of the fact that this mutation has been studied both historically and genetically, it requires further study [1, 3]. A mutation of the gene responsible for hypolactasia in the adult population took place in the context of domestication of cattle, with the development of animal husbandry and the use of fresh milk by the people in the Neolithic period between 5,000 and 10,000 years BC [7, 8]. In European ancestry, nucleotide polymorphism C/T -13910 originated about 7500 BC in the Balkans and Central Europe (archaeological Linearbandkeramik and Starčevo cultures) and then spread to the east, towards India, and north Africa [8, 9]. Stable lactose absorption in the adult population in the area of sub-Saharan Africa has evolved independently from the European lineage with C/T-13910 mutation associated with the breeding of Arabian camels [10].

The use of fresh milk among the peoples of northern Europe, especially by the tribes inhabited in the Neolithic period Britain, Germany and Scandinavia may be an explanation for the more severe C/T-13910 allele selection and tolerance of milk among indigenous ethnic populations of northern Europe compared to the current southern population such as among Italians. [11]. Intolerance to milk is high in populations living in Southeast Asia and sub-Saharan Africa [12].

Hypolactasia is also common among Turkish populations: the frequency of hypolactasia in the adult population of

Turkey is 71.3% [13], Uzbeks - 81 % [14], Kazakhs - 73.4 % [15]. In China (northeastern China, Hun subgroup) adult-type hypolactasia appears to be 92.3%, and among Mongols it is 87.9% [16].

The prevalence of hypolactasia in the Indo-Iranian groups of the population has been found to be 20% in India [17] and 86% among Iranian adults [18].

Clinically, in people with hypolactasia, milk consumption can lead to symptoms of lactose intolerance such as abdominal pain, borborygmi, flatulence and diarrhea [5]. Lactose intolerance is the most common reason for milk intolerance in adults.

There are no publications regarding adult-type hypolactasia, nor on milk intolerance among these ethnic Tajik isolated populations. The Tajik population is traditionally agrarian, with a tradition of cattle-breeding. Milk belongs to a traditional Tajik diet, but usually in limited amounts. Tajiks give fresh milk mostly to children. In adults "Shirchoy" or tea with milk is used mostly in the winter. Sour milk "Dzhugorot" and cheese “Chakka” are widely used, but these are fermented dairy products.

The aim of the current study was to find out the prevalence of lactase deficiency in a Tajik population defined by genotype C/C-13910 and to assess for differences in the milk consumption and intolerance in individuals with different genotypes.

2. Materials and Methods

The study was carried out in the population group attached to the 'Shovgon' Rural General Practice (RGP) in the Peshky District of the Bukhara province of Uzbekistan. A simple random sample (n=120) of the population aged 16 to 70 years was drawn from the list of people permanently registered in the area (1181 people aged 16 to 70). All study subjects were Tajik (at least three maternal and paternal grandparents reported to be Tajik).

The study was approved by the National Ethics Committee at the Ministry of Health of Uzbekistan. Informed consent was obtained from the subjects who participated in the study.

The questionnaire developed by the authors consisted of 45 questions concerning the individual's health, personal data (ethnicity, including ethnicities going back three ancestral generations), symptoms of milk and dairy product intolerance, and attitude to milk consumption. The researchers filled out the questionnaire during face-to-face interviews with the participants. A blood sample was taken from each participant for lactase-deficiency genotyping. Blood sample collection and transportation to the laboratory were performed according to a protocol approved by the Laboratory of Genomics of the Institute of Bioorganic Chemistry Academy of Sciences Republic of Uzbekistan on labeling, storing and delivering blood. DNA was isolated using standard procedures.

2.1. Genotyping

One millilitre blood samples were obtained from the cubital vein of each participant. DNA was isolated from this venous blood using the Diatom ™ DNA Prep 200 DNA purification kit (Isogene Lab. Ltd. Invitrogen, USA) according to the manufacturer's protocol. The presence of genomic DNA was verified by 0.9% agarose gel electrophoresis with ethidium bromide, followed by visualisation under UV light on a WiseDOC gel documentation device. SNP identification was achieved by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP). To identify alleles of the SNP C/T-13910, a fragment of 201 bp was first amplified using the primers: 5'-TGGCAATACAGATAAGATAATGGA-3' and 5'-GCTTTGGTTGAAGCGAAGAT- 3'. Polymerase chain reaction (PCR) amplifications were carried out in 0.2-mL PCR tubes, using a GenePack PCR Core kit (Isogene Lab. Ltd., Invitrogen) with lyophilised components. Each tube contained 1.5 units of Taq DNA polymerase, 10 mM of Tris-HCl (pH 9), 50 mM of KCl, 1.5 mM of MgCl2 and 200 mM of each dNTP. 2.5 μL of each primer (10 μM solution), 10 μL of the PCR diluent and 5 μL of DNA were added to each tube and PCR was performed using an Applied Biosystems GeneAmp 9700 PCR thermal cycler system. The PCR cycle conditions were as follows: an initial round of denaturation at 95°C, then 35 cycles at 95°C for 60 seconds, 59 ° C for 60 seconds, 72°C for 60 seconds, and a final extension of 72°C for 1 minute. PCR products were digested with 5u Hinf I (Fermentas). Amplification and digestion products were run on an 8% polyacrylamide gel and visualised by ethidium bromide staining. Samples that only presented a 201 -bp (C) fragment or a 177-bp (T) fragment were interpreted as CC and TT genotype, respectively, while those that presented two fragments of 201 bp and 177 bp were interpreted as the CT genotype.

The association between milk consumption /intolerance symptoms and genotype variants was tested using Fisher's Exact Test.

3. Results

From the random sample of 120 Tajik adults, 100 respondents (58 males and 42 females) answered the questionnaire on tolerance of milk and dairy products. The age of the subjects was as follows: 45 in the 16-29 years age group, 36 in the 30-49 years age group, and 19 in the 50-70 years age group.

Genotyping revealed the presence of the hypolactasia allele (C/C-13910) in 83 % of the study population. The subjects had only two variants of nucleotide polymorphism (C/T-13910 and C/C-13910), the T/T-13910 genotype was not found in any of the cases (Table 1).

Table 1. *Frequency of C/T-13910 variants in an adult Tajik population*

Genotype of C/T-13910 variant	Frequency (%)
C/ C	83
C/T	17
T/T	0

Milk consumption among the Tajik population was low. It did not differ between the subjects with C/C and C/T genotypes ($p>0.05$). The reasons for avoiding fresh milk in their diet were as follows: 9 people reported abdominal discomfort after drinking milk and 2 participants did not like the taste of milk. The results of the questionnaire on the frequency of intolerance to milk, comparing the intake quantities with the different variants of the genotype C/T-13910, are shown in Table 2.

Table 2. *Quantity of milk consumption (dl/day) and number (%) with reported milk intolerance in an adult Tajik population according to different genotypes of the C/T-13910 variant.*

Genotype	C/C-13910 N=83	C/T-13910 N =17	T/T-13910	Total N =100
Drinking milk (dl/day)				
0	10 (12)	1 (5,9)	0	11(11)
1-2	67 (80,7)	16 (94,1)	0	83 (83)
3-7	6 (7,2)	0	0	6 (6)
8 and more	0	0	0	0
Symptoms from milk intolerance	21 (25,3)	0	0	21 (21)

21 respondents who reported gastro-intestinal (GI) complaints had the C/C genotype (C/C and C/T $p<0.05$), characteristic of hypolactasia. They all noted abdominal bloating and diarrhea, and 3 people indicated pain in the abdomen. 9 out of the 21 participants with GI complaints avoided fresh milk consumption while 12 did not stop milk consumption despite having lactase non-persistence and milk intolerance symptoms.

4. Discussion

This work is the first study of the prevalence of hypolactasia genotypes and frequency of consumption of milk among ethnic Tajiks. An earlier study of lactase persistence in a mixed Tajik-Uzbek population indicates the presence of lactase persistence in 18% of the population. [15]

Our study was conducted in a remote region, far from the major administrative centers - the area where this isolated population of the ethnic Tajiks is located.

The strong correlation of genotype variant C/T-13910 with lactase persistence or non-persistence justifies its use as a reliable test to detect hypolactasia. Importantly, genotyping is a minimally invasive method and the results of the tests are available quickly [19, 20].

The questionnaire to determine the tolerance to milk and dairy products has been used as a tool to identify the frequency and volume of drinking fresh milk and dairy products [21-23]. The assessment of milk intolerance symptoms combined with the testing for C/T-13910 SNP variants are considered reliable methods to determine a diagnosis of hypolactasia [19, 24].

83% of the surveyed Tajiks were found to have hypolactasia (C/C-13910 genotype allele). However, despite this, the majority of them (89%) consume milk in small amounts (100-200 ml per day). The population with lactase non-persistence (genotype C/C-13910) had more GI complaints after drinking fresh milk. Some respondents with milk intolerance notified that they include fresh milk in their diet as a mild laxative for the regulation of stool frequency.

Most people in the world are lactase non-persistent and have varying degrees of lactose intolerance in the adult period of life. However, not all individuals with a lactase non-persistence genotype have expressed lactose intolerance and not all the people who are intolerant to lactose are carriers of lactase non-persistence alleles [1, 4, 25].

Previously reported results indicate that consumption of milk is lower in lactase non-persistent subjects than in lactase persistent subjects, suggesting that lactase non-persistence does not prevent the intake of milk. Not all the subjects with lactase non-persistence reported symptoms after consuming milk and most of them were able to consume a small amount of milk per day (12g of lactose), especially during mealtimes [26-28]. This may explain the fairly high rate (78%) of milk consumers among the genotype C/C-13910 [29, 30]. It has elsewhere been shown that drinking 150ml to 250 ml of milk a day may not cause symptoms of milk intolerance in people with hypolactasia [31, 32]. Our results demonstrate that presence or absence of hypolactasia does not affect fresh milk consumption in small amounts. As milk has not been a very important component of the Tajik diet, there has not been a strong obvious preference in natural selection for genotypes of C/T-13910 or T/T-13910.

5. Conclusion

The Tajik population studied is characterized by a high prevalence of hypolactasia, which does not prevent them ingesting milk in small amounts.

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References

[1] J.T. Troelsen "Adult-type hypolactasia and regulation of lactase expression". *Biochim. Biophys. Acta* Vol.1723(1–3): 2005, pp.19–32.

[2] N. Kretchmer "Expression of lactase during development". *Am. J. Hum. Genet.* Vol. 45 (4): 1989, pp.487–8.

[3] Y. Itan, B.L.Jones, CJE Ingram, D.M.Swallow and M.G. Thomas. A worldwide correlation of lactase persistence phenotype and genotypes. BMC Evol Biol. Vol. 9; 2010, pp.10-36.

[4] D.M. Swallow "Genetics of lactase persistence and lactose intolerance". *Annual Review of Genetics* Vol.37:2003, pp.197–219.

[5] T. Sahi "Genetics and epidemiology of adult-type hypolactasia". *Scand. J. Gastroenterol. Vol.* 202: 1994, pp.7–20.

[6] C.E. Woteki, E. Weser and E.A. Young "Lactose malabsorption in Mexican-American children". *Am. J. Clin. Nutr.* Vol. 29 (1): 1976, pp.19–24.

[7] Y. Itan, A. Powell, M.A Beaumont, J. Burger and M.G. Thomas. "The origins of lactase persistence in Europe". In Tanaka, Mark M. *PLoS Comput. Biol. Vol.* 5 (8): 2009

[8] J.Hussin, P. Nadeau, J.F. Lefebvre and D. Labuda. "Haplotype allelic classes for detecting ongoing positive selection". *BMC Bioinformatics* 11: 65, 2010.

[9] S. Myles, N. Bouzekri, E. Haverfield, M. Cherkaoui, J.M.Dugoujon and R.Ward "Genetic evidence in support of a shared Eurasian-North African dairying origin". *Biomedical and Life Sciences* Vol. 117 (1): 2005, pp. 34–42.

[10] N.S. Enattah, T. G. K. Jensen, M.Nielsen, R. Lewinski, M.Kuokkanen, H.Rasinpera, El- H.Shanti, J.Kee Seo, M.Alifrangis, F.K.Insaf, N. Abdrazak, A. Ahmed, N. Sirajedin , D.Comas, S. Q.m Mehdi, L. Groop, E. M. Vestergaard, F. Imtiaz, M.S. Rashed, B. Meyer, J. Troelsen and L. Peltonen. "Independent Introduction of Two Lactase-Persistence Alleles into Human Populations Reflects Different History of Adaptation to Milk Culture". *American Journal of Human Genetics* Vol. 82(1):2008, pp.57–72.

[11] C.Ghudini, A. Sottini, C. Zanotti, F. Serana, M. Marini, L.Caimi and L.Imberti, "Adult-type hypolactasia genotyping in Northern Italy: prevalence of C/T-13910 polymorphism and questions after comparison with existing date". Minevra Gastroenterol Dietol. Vol. 56 (1), 2010, pp.19-23.

[12] T. Bersaglieri, P.C. Sabeti, N.Patterson, T.Vanderploeg, S.F. Schaffner, J. A. Drake, M.Rhodes, D.E. Reich and J.N. Hirschhorn "Genetic Signatures of Strong Recent Positive Selection at the Lactase Gene". *Am J Hum Genet* Vol.74 (6): 2004, pp.1111.

[13] G.Flatz, H.J. Henze, E. Palabiyikoglu, K.Dagalp and T. Turkkan. Distribution of the adult lactase phenotypes in Turkey. Tropical and geographical medicine. Vol.38: 1986. pp. 255-258

[14] Sh.Kasimov, M.Lember. Adult-Type Hypolactasia in an ethnic Uzbek population. Int.J.Health&Nutrition. Vol. 4(1): 2013, pp. 46-49.

[15] E. Heyer, L.Brazier, L.Ségurel, T. Hegay, F. Austerlitz, L.Quintana-Murci, M.Georges, P. Pasquet and M Veuille. Lactase persistence in Central Asia: Phenotype, genotype, and evolution. Human Biology. Vol. 83(3): 2011, pp. 379-92.

[16] Y.G. Wang, Y.S. Yan, J.J. Xu, R.F. Du, S.D. Flatz, W. Kühnau and G. Flatz. Prevalence of primary adult lactose malabsorption in three populations of northern China. Human Genetics; Vol. 67(1): 1984. pp. 103-6.

[17] NS. Enattah, T. Sahi, E. Savilahti, JD. Terwilliger, L. Peltonen and I. Järvelä. "Identification of a variant associated with adult-type hypolactasia". *Nat. Genet.* Vol.30 (2): 2002, pp.233–7.

[18] M Sadre, K. Karbazi. Lactose intolerance in Iran. American Journal of Clinical Nutrition; Vol. 32 (9): 1979, pp.1948-54

[19] M. Lember, S.Torniainen, M. Kull, R. Kallikorm, P. Saadla, T. Rajasalu, H. Komu and I. Järvelä.. Lactase non-persistence and milk consumption in Estonia. World Journal of Gastroenterology; Vol. 12(45): 2006, pp.7329-7331

[20] O. Hovde, P. Farup. "A comparison of diagnostic test for lactose malabsorbtion – which one is the best?", BMC Gastroenterol, Vol. 9, 2009, pp.82-88.

[21] M.Lember, A.Tamm, A.Piirsoo, K.Suurmaa, R.Kermes, R.Kermesand, T.Sahi and M.Isokoski "Lactose malabsorbtion in Khants in western Siberia". Scand. Journal of Gastroenterology: Vol.30, 1995, pp.225-227.

[22] M.Saberi-Firoozi, F.Khademolhosseini, D.Mehrabani, M.Yousefi, M.Salehi and ST Heidary "Subjective lactose intolerance in apparently healthy adults in southern Iran: Is it related to irritable bowel syndrome?". Indian Journal Medical Sciences, Vol.61 (11), 2007, pp.591-597.

[23] Adams, Anne and Cox, Anna L. "Questionnaires, in-depth interviews and focus group". Research Methods for Human Computer Interaction. Cambridge, UK:Cambridge University Press, 2008, pp.17-34.

[24] R.Almon, P.Engfeld, C.Tysk, M.Sjöström and T.K.Nilson, "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.., Vol. 42 (2), 2007, pp.165-70.

[25] JT Trolsen, J.Olsen, J.Møller and H. Sjöström "An upstream polymorphism associated with lactase persistence has increased enhancer activity". Gastroenterology Vol.125, (6): 2003, pp.1686-94.

[26] M.Lember, A.Tamm, K.Villako. Lactose malabsorption in Estonians and Russian. European Journal of Gastroenterology & Hepatology; Vol. 3: 1991, pp. 479-481.

[27] R.Mattar, D F. de Campos Mazo and FJ Carrilho. Lactose intolerance: diagnosis, genetic, and clinical factors. Clin Exp Gastroenterol. Vol.5:2012, pp.113–121.

[28] F. Suarez, DA Savaiano and VD Levitt. "A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe lactose intolerance". The New England Journal of Medicine; Vol. 333(1): 1995, pp.1-4.

[29] Raja A.H.Kuchay, Mumtaz Anvar, Baby R.Thapa, Akhtar Mahmood and Safrun Mahmood. "Correlation of G/A-22018 single-nucleotide polymorphism with lactase activity and its usefulness in improving the diagnosis among North Indian children". Genes Nutr. Vol.8(1): 2013, pp.145-151.

[30] S.Torniainen, M.Hedelin, V.Autio, H.Rasinperä, KA. Bälter, A.Klint, R.Bellocco, F.Viklund, P.Stattin, T.Ikonen, T.L.J. Tammela, J.Schleutker, H.Grönberg and I. Järvelä. "Lactase persistence, dietary intake of milk, and the risk for prostate cancer in Sweden and Finland". Cancer Epidemiol Biomarkers Prev. Vol. 16(5): 2007, pp. 956-61.

[31] Y.Yoshida, G. Sasaki, S. Goto, S. Yanagiya and K. Takashina. "Studies on the etiology of milk intolerance in Japanese adults". Gastroenterologia Japonica. Vol. 10 (1): 1975, pp 29-34

[32] R. Qiao, C.Huang, H.Du, G. Zeng, L. Li and S.Ye. "Milk consumption and lactose intolerance in adults". Biomed Environ Sci. Vol. 24(5): 2011, pp. 512-7.