Original Article

The lactase gene -13910T allele can not predict the lactase-persistence phenotype in north China

Hai-ming Sun MD[1], Yuan-dong Qiao MD[1], Feng Chen MD[1], Li-dan Xu MD[1], Jing Bai MD[1] and Song-bin Fu MD PhD[1,2]

[1]*Laboratory of Medical Genetics, Harbin Medical University, P.R.China*
[2]*Bio-pharmaceutical Key Laboratory of Heilongjiang Province, P.R.China*

The frequency of lactase persistence varies widely in human populations. Study showed that the T allele of a C/T transition 13910bp upstream from exon 1 of lactase gene (*LCT*) was completely associated with lactase persistence in a Finnish population. To evaluate if the frequency of -13910T allele was in concordance with the lactase persistence in northern Chinese populations, in this study, we used Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) to detect the lactase -13910T allelic frequency in 5 northern Chinese populations for the first time. Results showed that the T allele frequency was low in these populations and that it did not match the lactase persistence phenotype in these populations. Therefore the -13910T allelic frequency can not serve as a predictor of the lactase persistence in these populations and this suggests the existence of other possible mechanisms of lactose tolerance in Chinese populations.

Key Words: lactase, lactose intolerance, China, population, polymorphism, single nucleotide

INTRODUCTION

Lactose, which is a disaccharide composed of linked molecules of the simple sugars glucose and galactose by a beta 1-4 glycosidic bond, is a major constituent of the milk of all mammals except sea lions.[1] Dietary lactose is obtained almost exclusively from milk. Infants and young children digest lactose with an enzyme, lactase, which splits the molecule into the two readily absorbable simple sugars. The glycosidic bond makes lactose a very strange sugar in animal biochemistry. This strange bond is presumably a reason for the evolution of the lactase enzyme. In most mammals, the level of the lactase enzyme is severely reduced some time after weaning, so the majority of adults lost this ability and are lactose malabsorbers. Those malabsorbers who display clinical symptoms after milk consumption are described as lactose intolerant.[2]

The ability to digest lactose in adults is an autosomal dominant hereditary condition caused by the persistence of lactase activity in the small intestine after weaning. The frequency of lactase persistence varies widely in human populations. It is generally found at high frequencies in populations of European descent while at low frequencies in the native populations of Australia and America, and in the Asia and Africa.[3]

The mechanisms controlling lactase production were disputed for many years. Recently, Enattah et al, using linkage disequilibrium and haplotype analysis, have identified a C/T transition which located -13910 bp upstream from exon 1 of the human lactase gene (*LCT*) that are completely associated with lactase persistence/ nonpersistence in Finnish families.[4] The authors report complete correlation between the lactase persistence phenotype and the presence of the T variant allele. The research of Olds and colleagues shows that this SNP is located in an enhancer element and that the allele shows some differences in function. The T varient may create a binding site for a transactivating protein that is capable of enhancing lactase transcription in adults with lactase persistence.[5] The study of Lewinsky suggest that the binding of Oct-1 to the -13910T variant directs increased lactase promoter activity and this might provide an explanation for the lactase persistence phenotype in the human population.[6] Typing this SNP is therefore considered as a genetic test for lactase persistence in Finland and Austria.[7-9] However, in a recent paper, it has been reported that a lack of -13910T alleles has been found in some African populations where the lactase persistent phenotype is common, indicating that the C-13910T polymorphism may not be a predictor of lactase persistence in sub-Saharan Africans.[10] Altogether, the mechanisms of lactase persistence remain controversial.

China is a multinational country in which some ethnic populations are nomadic. The frequency of the lactose tolerant phenotype of these nomadic populations is obviously higher than that of non-nomadic populations. We have found that the frequency of the -13910T is extremely low in the Han population, it does not match the frequency of the lactose tolerant phenotype (data not show). To investigate the mechanisms of the lactose intolerant in the

Corresponding Author: Dr. Song-bin Fu, Baojian Road 157, Nangang District, Harbin 150081, P. R. China
Hai-ming Sun, Yuan-dong Qiao and Feng Chen contributed equally to this paper
Tel: +86 451 86674798; Fax: +86 451 86632768
Email: fusb@ems.hrbmu.edu.cn; fusongbin@yahoo.com
Manuscript received 9 January 2007. Initial review completed 2 February 2007. Revision accepted 14 March 2007.

Table 1. The location of samples involved in present study

Population	Sample size	Sampling place	Location	
			Longitude	Latitude
Kazak	94	Yining City of Xinjiang Province	81°3′E	43°9′N
Man	75	Xiuyan City of Liaoning Province	123°2′E	40°2′N
Mongol	82	Hailar City of Inner Mongolia Province	119°7′E	29°2′N
Oroqen	45	Arlihe of Inner Mongolia Province	131°0′E	53°5′N
Hezhen	77	Tongjiang of Heilongjiang Province	132°5′E	47°7′N

minorities of north China, in this study, we evaluated the -13910T allelic frequency of 5 northern Chinese populations for the first time to determine the relationship of the polymorphism and the lactase persistence in northern Chinese populations.

MATERIALS AND METHODS

Samples

A total of 373 healthy unrelated individuals were randomly selected from 5 northern Chinese populations. The sources of the samples are listed in Table 1 in detail. Each one was the offspring of a non-sanguineous marriage of members of the same nationality for within at least three generations. All samples were collected with informed consent from the participants. DNA samples were extracted from peripheral blood samples anticoagulated with ACD using the standard phenol-chloroform extraction method.

Typing the C-13910T polymorphism

The C-13910T was typed by PCR-restriction fragment length polymorphism (PCR-RFLP) methods. The polymorphism was amplified within a 201-bp fragment with primers 5'-GCTGGCAATACAGATAAGATAATGGA-3 (forward) and 5'-CTGCTTTGGTTGAAGCGAAGAT-3 (reverse). The underlined G was a introduced base change so that the PCR product will be digested by Hinf I into two fragments of 177 bp and 24 bp when the T allele is present, while it is not digested when the allele is C. PCR reactions contained 0.5 μM of each primer, 200 μM of each deoxynucleotide triphosphate (dNTP), 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$ and 1 U Taq polymerase in a total volume of 20 μL. Samples were denatured for 5 min at 94℃, followed by 35 cycles of 94℃ for 30 sec, 58℃ for 20 sec, and 72℃ for 20 sec, followed by a 10 min extension at 72℃. Then the PCR products were digested with Hinf I restriction endonuclease at 37℃ overnight. Each reaction contained the PCR product 10 μL, 5 U of Hinf I and 2 μL 10×New England Biolabs Buffer 2, as recommended by the manufacturer. The digestion products were run on 3% agarose gel and stained with ethidium bromide. The CC homozygote yielded the 201 bp fragment only, while the CT heterozygote yielded the 201, 177 and 24 bp fragments.

Statistical analysis

After the genotype of each individual was acquired, allele and genotype frequencies of each population were calculated by direct counting. Then the fit to Hardy–Weinberg equilibrium among the studied populations was evaluated by the chi-squared test. Genetic differentiation among population samples was estimated with the Fst statistic, which measures the fraction of total genetic variation that is distributed among rather than within populations, by the statistic software Arlequin 3.01 (http://cmpg.unibe.ch/software/arlequin3/). The significance level (α) of the analysis was 0.05.

Table 2. The distribution of *LCT* C-13910T in 5 Chinese populations

Population	Number	Genotype			$T(10^{-4})$
		CC	CT	TT	
Kazak	94	85	9	0	479
Man	75	75	0	0	0
Mongol	82	78	4	0	244
Oroqen	45	44	1	0	110
Hezhen	77	77	0	0	0
Total	373	359	14	0	188

RESULTS

The frequency of the *LCT* -13910T allele was surveyed in 373 individuals from 5 populations of north China. These populations included Mongol, Kazak, Man, Oroqen and Hezhen. The frequency of this polymorphism was low or zero in these 5 populations. Among the 373 individuals, 14 individuals were found with the -13910T allele, they were all heterozygotes. Of these individuals, there were 9 Kazak, 4 Mongol and 1 Oroqen. We did not find any homozygote individuals of the -13910T. The mean frequency of the -13910T allele was 1.88%. The detail was listed in Table 2. There were no significant departures from Hardy–Weinberg equilibrium in any of the ascribed ethnic groups where T alleles were observed by the chi-squared test ($p>0.05$). The Fst results showed that there were significant difference between Kazak and Hezhen as well as between Kazak and Man ($p<0.05$), while the differences between the other populations were not significant.

We had successfully genotyped the *LCT* -13910T allele by PCR-RFLP in 5 northern Chinese populations. The results showed that the frequency of the -13910T was low even zero in these populations.

DISCUSSION

Lactose intolerant people who can not produce lactase in the cells of the epithelium of the small intestine have the deficiency of digesting lactose. If they consume significant quantities of milk or other dairy products, unmetabolized lactose can cause symptoms like diarrhoea, flatulence and abdominal pain. This occurs because the lactose is not efficiently hydrolysed in the small intestine and therefore reaches the distal ileum and the colon where the

Table 3. Comparisons with published lactose-digester frequencies in matching populations

Population	Genotyped sample size (No.)	Expected frequency of lactose digesters	Phenotyped sample size (No.)	Test method	Observed frequency of lactose digesters in phenotyped sample	Reference
Mongol	82	2.44%	198	Breath hydrogen	12.1%	Wang et al. 1984
Kazak	94	4.79%	195	Breath hydrogen	23.6%	Wang et al. 1984
Han	197	0.5%	248	Breath hydrogen	7.7%	Wang et al. 1984

lactose is fermented by bacteria. The fermentative products result in the symptoms of lactose intolerance. There is significant difference of the frequency of lactose-intolerance amongst the populations of the world. It is low in the European descent populations while it is high in the African, Asian and Australian populations.

The *LCT* -13910T has been shown to be a predictor of lactase persistence for European populations but not Africans.[10] Here we genotyped the polymorphism in 5 ethnic groups of north China. Among these populations, Kazak, Man and Mongol are traditional "milkers" who have lived as nomads in the grasslands of central Asia for many generations. Animal milk is a main kind of food for them, so the ability of absorbing lactose was especially important for these populations. The frequency of the lactose tolerant phenotype of these populations is obviously higher than that of non-nomadic populations.

Our results showed that the frequency of the -13910T was low even zero in these 5 populations, in which Kazak had the highest T allele frequency of 4.79%, then Mongol was 2.44%, Oroqen was 1.1% while Man and Hezhen were zero. These results did not accord with the former studies of Bersaglieri,[11] in which they showed that the -13910T allele frequency of the Mongols were 10%, which was strikingly higher than ours. Due to the fact that they only typed 10 individuals of Mongol, we believed there could be a sampling deviation in their study.

The Kazak and Mongol are milk-drinking populations. The previous study had shown that the phenotype frequencies of lactose tolerance were 12.1% and 23.6% for the Mongol and Kazak populations respectively.[12] There was substantial difference between the predicted frequencies which were deduced from the frequency of -13910T genotypes and the reported frequencies obtained from lactose tolerance testing. Our previous study showed that the -13910T allele frequency of the Han population was 0.5% (n=197, in publication), it was in discrepancy with the phenotype frequency too, the detail was listed in Table 3. Taken together, these data showed that the -13910T allele can not predict the lactase persistence in the populations of north China. The explanation of this phenomenon maybe that the -13910T allele was not the cause or not the only cause of lactase persistence. Some mechanisms other than lactase persistence may allow people to tolerate lactose, such as non-specific hydrolysis of the disaccharide. This conclusion was consistent with the previous study of Mulcare and Poulter.[10,13]

The Fst results showed no significant variance between these populations except between the Kazak and Hezhen as well as between the Kazak and Man. The Kazak in the north-west of China are of the Tujia language group of the Altaic language family, whose ancestors lived near Lake Baikal, and therefore some gene frequencies of it were similar to the Europeans. Together with the fact that the *LCT* -13910T allele frequency was ~10% in most Europeans,[11] we propose that this maybe the reason the allele frequency of the Kazak was higher than that of the other populations of north China. It is well known that Genghis Khan had established the largest land empire in history from the end of the 12th century to the beginning of the 13th century, which was a process of conquering and amalgamation resulting in the formation of modern Mongolia, and a large multioriginal nationality spread widely throughout China.[14] In addition, Hezhen, Oroqen and Man ethnic groups in the north-east China were all Manchu-Tungusic language group of the Altaic language family, and these several ethnic groups lived near each other for a long time. The wide ethnic mixture among these populations could be the reason that the distribution of *LCT* -13910T allele frequency did not vary significantly among these populations.

In summary, our results show that the frequency of *LCT* -13910T was very low in the 5 populations of north China studied, and that the allele is not a suitable predictor of lactose tolerance for these populations.

ACKNOWLEDGEMENTS

This work was funded by the Basic Condition Construction Program of Science and Technology Research of MOE, P.R.C. (505015) and the National Natural Science Foundation of China (No.30671146). We also gratefully acknowledge the numerous sample donors for making this work possible.

AUTHOR DISCLOSURES

Hai-ming Sun, Yuan-dong Qiao, Feng Chen, Li-dan Xu, Jing Bai and Song-bin Fu, no conflicts of interest.

REFERENCES

1. Kretchmer, Norman. Lactose intolerance and malabsorption. In: Kenneth F. Kiple, eds. The Cambridge world history of human disease. Cambridge and New York, 1993; 813-817.
2. Swallow DM, Hollox EJ. The genetic polymorphism of intestinal lactase activity in adult humans. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular basis of inherited disease. McGraw-Hill, New York, 2000.
3. Troelsen JT. Adult-type hypolactasia and regulation of lactase expression. Biochim Biophys Acta. 2005;1723:19-32.
4. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat Genet. 2002;30:233-237.

5. Olds LC, Sibley E. Lactase persistence DNA variant enhances lactase promoter activity in vitro: functional role as a cis regulatory element. Hum Mol Genet. 2003;12:2333-2340.
6. Lewinsky RH, Jensen TG, Moller 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.
7. Rasinpera H, Savilahti E, Enattah NS, Kuokkanen M, Totterman N, Lindahl H, Jarvela I, Kolho KL. A genetic test which can be used to diagnose adult-type hypolactasia in children. Gut. 2004;53:1571-1576.
8. Hogenauer C, Hammer HF, Mellitzer K, Renner W, Krejs GJ, Toplak H. Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. Eur J Gastroenterol Hepatol. 2005; 17:371-376.
9. Bodlaj G, Stocher M, Hufnagl P, Hubmann R, Biesenbach G, Stekel H, Berg J. Genotyping of the lactase-phlorizin hydrolase -13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. Clin Chem. 2006;52:148-151.
10. Mulcare CA, Weale ME, Jones AL, Connell B, Zeitlyn D, Tarekegn A, Swallow DM, Bradman N, Thomas MG. 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. Am J Hum Genet. 2004;74:1102-1110.
11. Bersaglieri T, Sabeti PC, Patterson N, Vanderploeg T, Schaffner SF, Drake JA, Rhodes M, Reich DE, Hirschhorn JN. Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet. 2004;74:1111-1120.
12. Wang YG, Yan YS, Xu JJ, Du RF, Flatz SD, Kuhnau W, Flatz G. Prevalence of primary adult lactose malabsorption in three populations of northern China. Hum Genet. 1984; 67:103-106.
13. Poulter M, Hollox E, Harvey CB, Mulcare C, Peuhkuri K, Kajander K, Sarner M, Korpela R, Swallow DM. The causal element for the lactase persistence/non-persistence polymorphism is located in a 1 Mb region of linkage disequilibrium in Europeans. Ann Hum Genet. 2003;67:298-311.
14. Du, RF, Ye FS. Chinese Nationalities. Beijing: Science Publishing House, 1994.

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[1]*Laboratory of Medical Genetics, Harbin Medical University, P.R.China*
[2]*Bio-pharmaceutical Key Laboratory of Heilongjiang Province, P.R.China*

乳糖酶基因-13910T 等位基因無法預測中国北方人的乳糖耐受

乳糖耐受在不同人群中频率差异很大，有研究表明乳糖酶基因（*LCT*）第一外显子上游13910bp处的C/T转换与芬兰人群的乳糖耐受表型完全相关。为了分析-13910T等位基因是否与中国北方人群的乳糖耐受表型相关，我们应用聚合酶链反应－限制性片段长度多态性的方法对中国北方5个少数民族的-13910T等位基因频率进行了检测。结果显示在这些民族中，T等位基因频率很低，而且与乳糖耐受表型频率并不一致。因此，-13910T等位基因频率并不能反映这些民族的乳糖耐受表型，进而暗示在中国人群中存在着其他可能的乳糖耐受机制。

关键字：乳糖酶、乳糖耐受、中国、民族、单核苷酸多态性。