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Genetic Association of *LCT*-13910C/T and *LCT*-22018G/A with Adult Type Hypolactesia in Pakistan

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ABSTRACT

Adult type hypolactesia commonly known as lactose intolerance usually occur in adulthood when expression of the lactase gene diminishes. Two SNP's LCT-13910C/T and LCT-22018G/A are considered to be associated with the development of lactose intolerance in humans. The aim of the study was to evaluate the role of LCT-13910C/T and LCT-22018G/A with the development of lactose intolerance in Pakistani population. A total of 160 samples were collected and tetra primer ARMS-PCR was used for genotyping. 79.48% lactose intolerant individuals showed homozygous LCT-13910CC genotype and 16.66% showed heterozygous CT genotype. For LCT-22018G/A, 43.75% lactose intolerant individuals showed homozygous GG genotype, while 43.58% patients showed heterozygous GA genotype. Thus, different variants of genotype -13910T/C and -22018G/A of the individuals were found to be associated with the development of lactose intolerance in patients of the studied population. Milk intake status and gastrointestinal disease are among the other factors that may influence the onset of lactose intolerance in patients and controls.

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SM presented the concept, designed the study and revised the article. HSH collected, analysed and interpreted the data and drafed the manuscript.

Key words
Hypolactesia, LCT, Bloating, Milk, Intolerance.

INTRODUCTION

Lactose intolerance is a clinical condition that occurs commonly among people throughout the world. Milk, being a primary nutritional source especially during infancy, is rich in lactose sugar. Lactase enzyme in the intestine breaks lactose into glucose and galactose (Biller and Grand, 1990). There is ample secretion of lactase in case of high intake of milk, but its production gradually decreases as the milk consumption is mitigated resulting in a condition known as hypolactesia or lactase non-persistence (Sebastio *et al*., 1989). The individuals with lactose intolerance either show a decreased expression of the lactase gene or absent expression (Obermayer-Pietsch *et al*., 2004).

When individuals with lactose intolerance consume diet containing lactose the intestinal bacteria decompose undigested lactose and produce various gases thus causing bloating, abdominal pain, and diarrhea (Bayless and Rosensweig, 1966; Di Stefano *et al*., 2007). In order to avoid such excruciating condition, lactose intolerant individuals avoid milk products and in turn develop calcium and vitamin D deficiency, which make them vulnerable to develop osteoporosis etc. (Heyman, 2006).

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The frequency of lactose intolerance has been reported as 5% in Northern Europe and about 90% in Asian and African countries (Bulhões *et al*., 2007). As milk intake controls lactase production, it is reported that about 75% of any population becomes lactose intolerant at some stage (Savaiano and Levitt, 1987; Hertzler and Savaiano, 1996; Enattah *et al*., 2008; Mattar *et al*., 2009).

Decreased expression of lactase gene in adulthood is usually considered genetic such as because of certain single nucleotide polymorphisms (SNP's) (Swallow, 2003) while, secondary hypolactesia also known as lactose maldigestion or lactose malabsorption is often caused by intestinal injury, Crohn's disease, celiac disease etc. or infections like giardiasis which is quite prevalent in individuals belonging to a specific occupation (Vesa *et al*., 2000; Swagerty *et al*., 2002; Krawczyk *et al*., 2008; Wali *et al*., 2018).

Lactase is encoded by the *LCT* gene which is located at 2q21 and is regulated by the *MCM6* gene (minichromosome maintenance complex component 6). Different genetic studies stated that hypolactesia is an inherited autosomal recessive trait, while lactase persistence is a dominant trait (Ferguson and Maxwell, 1967; Ferguson and Anne, 1967). Two major SNPs -13910C/T and -22018G/A which are located upstream of *LCT* gene are known in association with *LCT* expression (Bulhões *et al*., 2007; Tishkoff *et al*., 2007). The "T" allele of -13910 and "G" allele of -22018 is reported to cause

lactase persistence as in Brazilian population (Bulhões *et al*., 2007; Ponte *et al*., 2016), while -13910C and -22018A is reported in association with lactase non-persistence as documented in the Finnish individuals among others (Enattah *et al*., 2002; Kuokkanen *et al*., 2003). Additional SNPs including *LCT*-13915 T/G in Arabian, Middle East, and East African groups and *LCT*-14010G/T in Northern Africans have been reported in association with lactose intolerance (Imtiaz *et al*., 2007; Gerbault *et al*., 2009).

There are different methods to diagnosis lactose intolerance including hydrogen breath test and the stool acidity test, in addition to intestinal biopsy (Metz *et al*., 1976; Heyman, 2006). Genetic diagnostic tests deploying SNPs such as -13910C/T and -22018G/A are also considered promising towards diagnosis of primary lactose intolerance (Bodlaj *et al*., 2006).

The current study was aimed to find out lactose intolerance associated genotypes for the SNPs -13910C/T and -22018G/A and the allele frequency in Pakistani population. Additionally, the study also targeted to analyze different stratification factors that can act with the genetic variants towards lactose intolerance. Based on the allele frequency, an important application of the current study could involve the screening of the above alleles in Pakistani population by tetra-primer ARMS PCR as a rapid and inexpensive strategy before doing more costly tests.

MATERIALS AND METHODS

The study was approved by the Institutional Review Board (IRB) of University of the Punjab Lahore, Pakistan. After gaining informed written and verbal consent, a total of 160 peripheral blood samples were collected. Out of 160 samples, 80 samples were from affected individuals who were positive for the symptoms of lactose intolerance/lactase non-persistence, while 80 samples were lactase persistent. The samples were collected from different areas of the country making sure that both patients and controls belong to same locality. The enrolled patients were interviewed in detail and the data was recorded in the questionnaire form. All important social and demographic factors were included in the study i.e. age, age of disease onset, sex, height, weight, cast, sensitivity towards milk, living background (rural or urban), family history and present milk intake status. The important associated factors are shown in Table I.

The blood samples were used for DNA extraction by using the standard method (Grimberg *et al*., 1989; Miller *et al*., 1988). Primers were designed to genotype -13910C/T and -22018G/A alleles for tetra-primer ARMS PCR (http://primer1.soton.ac.uk/primer1.html). The primer sequences were as follows:
For the SNP -13910C/T outer forward primer 5′-TTGCATGTTTTTAATCTTTGGTATGGGACA-3′, outer reverse primer 5′-TGCCCTTTCGTACTACTCCCCTTTTACC-3′, “C” allele primer 5′-CGCTGGCAATACAGATAAGATAATGTCGC-3′ and allele “T” primer 5′-GAGGAGAGTTCCTTTGAGGCCAGTGAA-3′. For the SNP -22018G/A outer forward primer 5′-GTTTTCCTGGGAAGGGTGCTGTATAATCA-3′, outer reverse primer 5′-GATCCTCCCACCTCAGCCTCTTGAGTAG-3′, allele specific primer for the “G” allele 5′-CCTTAAAAACAGCATTCTCAGCTGGTCG-3′, and allele specific primer for the “A” allele 5′-TACTGGGACAAAGGTGTGAGCCACAGT-3′. For 10 µl PCR, 1:2 of outer primers to allele specific primers were used in addition to 50ng of template DNA, 1X KCl buffer (Thermo Scientific, #EP0402), 2mM of $MgCl_2$, 0.2 mM of dNTPs, and 0.5 units of *Taq* polymerase (Thermo Scientific, #EP0402). Touchdown PCR protocol was used for amplification using the annealing temperature dropping from 74ºC to 64ºC for 45 seconds followed by final amplification at 64ºC for 33 cycles.

Table I.- Important social and demographic factors recorded in patients.

Variables		No. of patients
Gender	Male	58
	Female	22
Background	Rural	63
	Urban	17
Sensitivity to lactose (grams)	Low (up to 24g)	29
	Moderate (up to 12g)	37
	High (≤6g)	14
Family History	Yes	23
	No	57
Age of disease onset	Childhood	33
	Adulthood	41
	After 25 years	6
Milk intake status	Daily	4
	Once in a week	10
	Several times a week	2
	Occasionally	18
	Hardly ever	46

RESULTS

The presence of different symptoms among the lactose intolerant individuals was assessed. The most common symptoms recorded were diarrhea (30%), bloating (16.25%), flatulence (15%), and Borborygmus (2.5%). Many of the individuals showed combination of symptoms i.e. flatulence + abdominal pain (13.75%),

flatulence + bloating (6.25%), diarrhea + abdominal pain (3.75%) and 12.5% patients showed multiple (more than two of the above-mentioned) symptoms (Table II). Most of the controls showed no apparent symptoms of lactose intolerance upon milk ingestion.

Table II.- Distribution of patients according to symptoms.

Symptom	Number of patients (%)
Diarrhea	24 (30)
Bloating	13 (16.25)
Flatulence	12 (15)
Borborgymus	2 (2.5)
Flatulence + abdominal pain	11 (13.75)
Flatulence + bloating	5 (6.25)
Diarrhea + abdominal pain	3 (3.75)
Multiple (more than 2) symptoms	10 (12.5)

For the SNP -13910C/T, homozygous CC genotype was more common among patients (80%) than controls (28.75%). While the homozygous TT genotype was more common in controls (15%) than patients (3.75%). The heterozygous CT genotype in patients was 16.25% and in controls 65.25% (Fig. 1A).

For the SNP -22018G/A, homozygous GG genotype was found more common among patients (46.25%) than controls (8.75%). While the homozygous AA genotype was more common in controls (38.75%) than patients (11.25%). The heterozygous GA genotype in patients was 42.50% and in controls 52.50% (Fig. 1B).

DISCUSSION

Most of the patients showed -13910CC and -22018AA genotype indicating the relationship with the onset of lactose intolerance (Enattah *et al*., 2002), while most of the controls showed the -13910TT and -22018GG genotype which indicates their association with lactase persistence. Heterozygous condition for both of the SNPs also showed significant relationship with the development of the phenotype. The single nucleotide polymorphism at position -13910T/C is already reported to be strongly associated with lactose persistence/non-persistence (Tishkoff *et al*., 2007).

Some patients also showed -13910TT and -22018GG genotype, while some controls possessed -13910CC and -22108AA genotype. The likely reason for this could be the role of nutritional factors like milk intake status which is known to influence the onset of the disease (Pribila *et al*., 2000). According to the literature, if a person does not cutoff milk consumption during the entire life time, it may not develop lactose intolerance despite of possessing the right genotype for it. Similarly, a person can show the symptoms of lactose intolerance if a disease such as celiac disease, Crohn's disease, or ulcerative colitis is accompanied. (Lomer *et al*., 2008; Alpers, 2006). So, it is not unusual for a person bearing normal genotype of lactase persistence to appear as lactose intolerant (Mishkin *et al*., 1997).

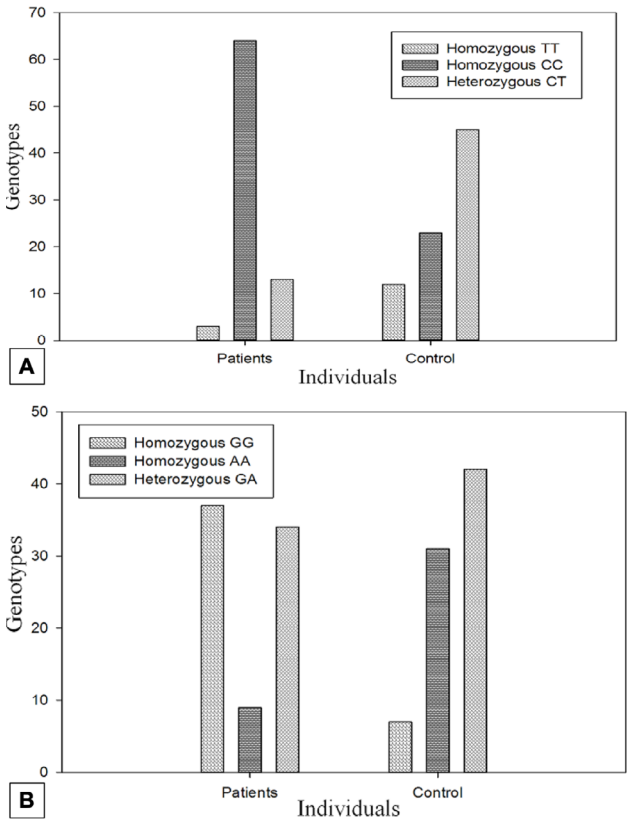

Fig. 1. Genotyping of SNP -13910C/T (A) SNP -22018G/A (B) among patients and controls.

The findings of the current study were prospective. The study finds out the association of SNP's -13910T/C and -22018G/A with the onset of lactose intolerance. As the genotype -13910CC and -22018AA show an association with the occurrence of lactose intolerance in the Pakistani individuals, the current study holds the potential for devising low-cost genetic tests based on the commonly found lactose intolerance associated alleles for rapid and effective population screening. Furthermore,

such genetic test could be proved helpful in the screening of lactose intolerance, if the environmental factors like nutrition and disease history be considered. If these factors are present hydrogen breath test (HBT) is recommended along with the genetic assessment for complete and successful diagnosis of lactose intolerance (Mulcare *et al*., 2004). Additionally, for future considerations, a more comprehensive study with a larger sample size from all over the country is needed to draw a detailed conclusion about the role of these SNPs in lactose intolerance and to add further SNP alleles in the genetic screening of the population.

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Statement of conflict of interest

Authors have declared that there is no conflict.

REFERENCES

Alpers, D.H., 2006. Diet and irritable bowel syndrome. *Curr. Opin. Gastroenterol.*, **22**: 136-139 https://doi.org/10.1097/01.mog.0000208462.92136.02

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. *J. Am. med. Assoc.,* **197**: 968-972. https://doi.org/10.1001/jama.197.12.968

Biller, H.A. and Grand, R.J., 1990. Lactose intolerance. *Annu. Rev. Med.*, **41**: 141-148. https://doi.org/10.1146/annurev.me.41.020190.001041

Bodlaj, G., Stöcher, M., Hufnagl, P., Hubmann, R., Biesenbach, G., Stekel, H. and Berg, J., 2006. Genotyping of the lactase-phlorizin hydrolase−13910 polymorphism by LightCycler PCR and implications for the diagnosis of lactose intolerance. *Clin. Chem.,* **52**: 148-151. https://doi.org/10.1373/clinchem.2005.057240

Bulhões, A.C., Goldani, H.A.S., Oliveira, F.S., Matte, U.D.S., Mazzuca, R.B. and Silveira, T.R.D., 2007. Correlation between lactose absorption and the C/T-13910 and G/A-22018 mutations of the lactase-phlorizin hydrolase (*LCT*) gene in adult-type hypolactasia. *Brazil. J. Med. biol. Res.*, **40**: 1441-1446. https://doi.org/10.1590/S0100-879X2007001100004

Di Stefano, M., Miceli, E., Mazzocchi, S., Tana, P., Moroni, F. and Corazza, G.R., 2007. Visceral hypersensitivity and intolerance symptoms in lactose malabsorption. *Neurogastroenterol. Motil.*, **19**: 887-895. https://doi.org/10.1111/j.1365-2982.2007.00973.x

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. and Natah, A., 2008. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *Am. J. Hum. Genet.*, **82**: 57-72. https://doi.org/10.1016/j.ajhg.2007.09.012

Enattah, N.S., Sahi, T., Savilahti, E., Terwilliger, J.D., Peltonen, L. and Järvelä, I., 2002. Identification of a variant associated with adult-type hypolactasia. *Nature Genet.*, **30**: 233. https://doi.org/10.1038/ng826

Ferguson, A. and Maxwell, J.D., 1967. Genetic aetiology of lactose intolerance. *The Lancet*, **290**: 188-191. https://doi.org/10.1016/S0140-6736(67)90009-8

Gerbault, P., Moret, C., Currat, M. and Sanchez-Mazas, A., 2009. Impact of selection and demography on the diffusion of lactase persistence. *PLoS One*, **4**: e6369. https://doi.org/10.1371/journal.pone.0006369

Grimberg, J., Nawoschik, S., Belluscio, L., McKee, R., Turck, A. and Eisenberg, A., 1989. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucl. Acids Res.*, **17**: 8390-8390. https://doi.org/10.1093/nar/17.20.8390

He, T., Venema, K., Priebe, M.G., Welling, G.W., Brummer, R.J. and Vonk, R.J., 2008. The role of colonic metabolism in lactose intolerance. *Eur. J. clin. Investig.*, **38**: 541-547. https://doi.org/10.1111/j.1365-2362.2008.01966.x

Hertzler, S.R. and Savaiano, D.A., 1996. Colonic adaptation to daily lactose feeding in lactose maldigesters reduces lactose intolerance. *Am. J. clin. Nutr.*, **64**: 232-236. https://doi.org/10.1093/ajcn/64.2.232

Heyman, M.B., 2006. Lactose intolerance in inf ants, children, and adolescents. *Pediatrics*, **118**: 1279-1286. https://doi.org/10.1542/peds.2006-1721

Imtiaz, F., Savilahti, E., Sarnesto, A., Trabzuni, D., Al-Kahtani, K., Kagevi, I., Rashed, M.S., Meyer, B.F. and Järvelä, 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. *J. med. Genet.*, **44**: e89-e89. https://doi.org/10.1136/jmg.2007.051631

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. *J. Gastrointest. Liver Dis.*, **17**: 135-9.

Kuokkanen, M., Enattah, N.S., Oksanen, A., Savilahti, E., Orpana, A. and Järvelä, I., 2003. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. *Gut*, **52**: 647-652. https://doi.org/10.1136/gut.52.5.647

Lomer, M.C.E., Parkes, G.C. and Sanderson, J.D., 2008. lactose intolerance in clinical practice–myths and realities. *Aliment. Pharmacol.Therap.*, **27**: 93-103. https://doi.org/10.1111/j.1365-2036.2007.03557.x

Mattar, R., Monteiro, M.S., Villares, C.A., Santos, A.F., Silva, J.M. and Carrilho, F.J., 2009. Frequency of *LCT*-13910C> T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups. *Nutr. J.,* **8**: 46. https://doi.org/10.1186/1475-2891-8-46

Metz, G., Drasar, B.S., Gassull, M.A., Jenkins, D.J.A. and Blendis, L.M., 1976. Breath-hydrogen test for small-intestinal bacterial colonisation. *The Lancet*, **307**: 668-669. https://doi.org/10.1016/S0140-6736(76)92779-3

Miller, S.A., Dykes, D.D. and Polesky, H.F.R.N., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucl. Acids Res.*, **16**: 1215. https://doi.org/10.1093/nar/16.3.1215

Mishkin, B., Yalovsky, M. and Mishkin, S., 1997. Increased prevalence of lactose malabsorption in Crohn's disease patients at low risk for lactose malabsorption based on ethnic origin. *Am. J. Gastroenterol.*, **92**: 1148-53.

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− 13.9 kbT) does not predict or cause the lactase-persistence phenotype in Africans. *Am. J. Hum. Genet.*, **74**: 1102-1110. https://doi.org/10.1086/421050

Obermayer-Pietsch, B.M., Bonelli, C.M., Walter, D.E., Kuhn, R.J., Fahrleitner-Pammer, A., Berghold, A., Goessler, W., Stepan, V., Dobnig, H., Leb, G. and Renner, W., 2004. Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures. *J. Bone Min. Res.*, **19**: 42-47. https://doi.org/10.1359/jbmr.0301207

Ponte, P.R.L., de Medeiros, P.H.Q.S., Havt, A., Caetano, J.A., Cid, D.A., de Moura G.P.M., Soares, A.M., Guerrant, R.L., Mychaleckyj, J. and Lima, A.Â.M., 2016. Clinical evaluation, biochemistry and genetic polymorphism analysis for the diagnosis of lactose intolerance in a population from northeastern Brazil. *Clinics*, **71**: 82-89. https://doi.org/10.6061/clinics/2016(02)06

Pribila, B.A., Hertzler, S.R., Martin, B.R., Weaver, C.M. and Savaiano, D.A., 2000. Improved lactose digestion and intolerance among African-American adolescent girls fed a dairy rich-diet. *J. Am. Dietet. Assoc.*, **100**: 524-528. https://doi.org/10.1016/S0002-8223(00)00162-0

Savaiano, D.A. and Levitt, M.D., 1987. Milk intolerance and microbe-containing dairy foods. *J. Dairy Sci.*, **70**: 397-406. https://doi.org/10.3168/jds.S0022-0302(87)80023-1

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. *Am. J. Hum. Genet.*, **45**: 489- 497.

Swagerty Jr, D.L., Walling, A.D. and Klein, R.M., 2002. Lactose intolerance. *Am. Fam. Physic.*, **65**: 1845-1850.

Swallow, D.M., 2003. Genetics of lactase persistence and lactose intolerance. *Annu. Rev. Genet.*, **37**: 197-219. https://doi.org/10.1146/annurev.genet.37.110801.143820

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. and Ibrahim, M., 2007. Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genet.*, **39**: 31-40. https://doi.org/10.1038/ng1946

Vesa, T.H., Marteau, P. and Korpela, R., 2000. Lactose intolerance. *J. Am. Coll. Nutr.*, **19**(sup2): 165S-175S. https://doi.org/10.1080/07315724.2000.10718086

Wali, K, Noor, U.N. and Aly, K., 2018. Prevalence of potentially important intestinal pathogenic protozoan parasitic infections in different occupational groups of Swat, Pakistan. *Pakistan J. Zool.*, **50**: 123-129.