TRANSACTIONS OF THE AMERICAN CLINICAL AND CLIMATOLOGICAL ASSOCIATION, VOL. 122, 2011

## THEODORE E. WOODWARD AWARD

# LACTASE PERSISTENCE SNPS IN AFRICAN POPULATIONS REGULATE PROMOTER ACTIVITY IN INTESTINAL CELL CULTURE

ERIC SIBLEY, MD, PhD, and (*by invitation*) JONG KUN AHN,

PALO ALTO, CALIFORNIA

## ABSTRACT

Lactase-phlorizin hydrolase, lactase, is the intestinal enzyme responsible for the digestion of the milk sugar lactose. The majority of the world's human population experiences a decline in expression of the lactase gene by late childhood (lactase non-persistence). Individuals with lactase persistence, however, continue to express high levels of the lactase gene throughout adulthood. Lactase persistence is a heritable autosomal dominant condition and has been strongly correlated with several single nucleotide polymorphisms (SNPs) located ~14 kb upstream of the lactase gene in different ethnic populations: -13910*T in Europeans and -13907*G, -13915*G, and -14010*C in several African populations. The coincidence of the four SNPs clustering within 100 bp strongly suggests that this region mediates the lactase non-persistence/persistence phenotype. Having previously characterized the European SNP, we aimed to determine whether the African SNPs similarly mediate a functional role in regulating the lactase promoter. Human intestinal Caco-2 cells were transfected with lactase SNP/promoter-reporter constructs and assayed for promoter activity. The -13907*G and -13915*G SNPs result in a significant enhancement of lactase promoter activity relative to the ancestral lactase non-persistence genotype. Such differential regulation by the SNPs is consistent with a causative role in the mechanism specifying the lactase persistence phenotype.

Lactase-phlorizin hydrolase (LPH, lactase) possesses two enzymatic activities: lactase ($\beta$-D-galactoside galactohydrolase, EC 3.2.1.23) and phlorizin hydrolase (phlorizin glucohydrolase, EC 3.2.1.62) (1). LPH enzyme present on the brush-border membrane of mammalian small intestinal absorptive epithelial cells is responsible for the hydrolysis of

Correspondence and reprint requests: Eric Sibley, M.D., Ph.D., Associate Professor of Pediatrics, Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition Stanford University School of Medicine, 300 Pasteur Drive, G310, Stanford, CA 94305, Tel: 650.723.5070, E-mail: eric.sibley@stanford.edu

Potential Conflicts of Interest: None disclosed.

the disaccharide lactose, the predominant carbohydrate in milk, to yield glucose and galactose. The resulting monosaccharides can be absorbed across the brush-border membrane for use as a source of energy or as components of glycolipids and glycoproteins. The development of assays for lactase activity and the application of molecular genetic methods has accelerated investigation of the ethnic distribution of lactose intolerance and expression of the LPH gene (*LCT*) (2–8).

The human *LCT* gene located on chromosome 2q21 comprises 17 exons spanning ~49 kb (7, 9, 10) and is transcribed to yield a mRNA transcript of ~6 kb encoding 1927 amino acids (11). The LPH mRNA translation product is heavily glycosylated, with a mass of ~220 kD, and is processed at the brush-border membrane by proteolytic trimming to a mature ~160-kD protein (12). Lactase activity at the mucosal surface of the human small intestine is highest at birth. Seventy percent of humans worldwide undergo a permanent maturational decline in lactase activity by late childhood (lactase non-persistence) resulting in lactose intolerance (5, 13). In certain populations, however, high-level lactase activity persists into and throughout adulthood (lactase persistence). Such lactase persistence occurs predominantly in people of northern European descent and in some populations in Africa that have a history of pastoralism (14). Although lactase non-persistence is the more common human phenotype (6, 7, 15), lactase persistence is believed to have occurred as a result of a selection process within the last ~10,000 years enabling sustained dairy consumption in certain populations (2, 5, 8, 16). Lactase persistence is inherited in an autosomal dominant manner (17, 18).

Recent interest in lactase non-persistence/persistence has focused on the molecular biological mechanisms regulating the maintenance or decrease of intestinal lactase gene expression during maturation. It is generally agreed that lactase persistence or non-persistence in humans is transcriptionally regulated at the *LCT* messenger RNA level (6, 19, 20). In 2002, Enattah et al. reported the identification of single nucleotide polymorphisms (SNPs), -13910*C/T and -22018*G/A, upstream of the *LCT* locus, that are associated with lactase non-persistence/persistence in Finnish families (21). The -13910*C/T and -22018*G/A SNPs are located within intron 13 and intron 9, respectively, of the adjacent *MCM6* gene on chromosome 2q21. Subsequently, the -13910*T allele was shown to enhance transcription of lactase gene promoter-luciferase reporter constructs in intestinal Caco-2 cells (22, 23). Additional reports have stated that the -13910*T allele correlates well with lactase persistence in European countries (24–26) and some Brazilian populations (27). In regions of Africa with a history of

milk consumption and a high prevalence of lactase persistence, however, the frequency of the -13910*T allele is low or zero (28, 29), as is also the case for lactase persistence in northern Chinese populations (30). New polymorphisms, -3712*T/C, -13907*C/G, -13913*T/C, -13915*T/G, and -14010*G/C, associated with lactase non-persistence/persistence, have recently been identified in African and Saudi Arabian populations (15, 28, 31–34). In the African populations, the -13907*G, -13915*G (15, 28, 33), and -14010*C (33) variants were widely found, whereas the -13913*C variant was found rarely (28). In Saudi Arabian populations, the -13915*G (28, 31, 32) and -3712*C (31) variants were identified.

Investigative efforts have focused on elucidating whether the various lactase SNPs function to regulate lactase non-persistence/persistence in adulthood. Such molecular mechanisms have not been fully defined. In the present study, we describe functional characterization of differential effects on lactase promoter activity in intestinal cell culture mediated by the lactase SNPs (-13907*G, -13915*G, and -14010*C) present in Africa.

## MATERIALS AND METHODS

***Cloning of African SNP Region DNA Fragments in Promoter-reporter Constructs.*** 215-bp DNA fragments spanning nucleotides -14014 to -13800 upstream of the human *LCT* gene and including one of the African lactase persistence SNPs (-13907*G, -13915*G, and -14010*C) or the ancestral allelic sequence were amplified by polymerase chain reaction (PCR) from chromosomal DNA constructs33 generously provided by G.A. Wray, of Duke University, as templates. Specifically, for amplification of DNA fragments corresponding to the ancestral allele or to the lactase persistent SNPs -13907*G and -13915*G, oligonucleotides 5′GGACGCGTAGACGTAAGTTACCATTTAATAC3′ (-14014 to -13922 bp upstream of *LCT*) and 5′GGACGCGTCGTTAATACCCACTGACCTATC3′ (-13800 to -13821 bp) were used as forward and reverse primers, respectively. For amplification of the DNA fragment corresponding to the lactase persistent SNP -14010*C, the oligonucleotide 5′GGACGCGTAGACCTAAGTTACCATTTAATAC3′ (-14014 to -13922 bp) was used as the forward primer. All primers were synthesized with a 5′ terminal *Mlu*I restriction enzyme site for subsequent cloning. The amplified DNA fragments corresponding to the ancestral allele or the African SNPs were cloned in both orientations (forward and reverse) at the *Mlu*I site upstream of the lactase

promoter in the p2kLac promoter-reporter construct to generate p2kLac-Ancestral, p2kLac-Ancestral' (reverse), p2kLac-13907*G, p2kLac-13907*G′ (reverse), p2kLac-13907*G, p2kLac-13907*G' (reverse), p2kLac-14010*C, and p2kLac-14010*C′ (reverse). The previously described p2kLac construct contains a 2.0-kb fragment of the rat lactase proximal promoter cloned upstream of firefly luciferase cDNA in the reporter plasmid pGL3Basic (Promega) (35). Incorporation of the ancestral allele or SNP fragment sequences was confirmed by sequencing.

***Cell Transfection and Assay of Luciferase Activity.*** Caco-2 cells were initially cultured in 100-mm dishes at 37°C in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Upon reaching 90% confluency, cells were seeded into 35-mm dishes with $2.5 \times 10^5$ cells per dish and cultured for 48 hours (50–80% confluent) and then transiently transfected with the experimental construct (0.4 pmol) and 10 ng of pRL-CMV (used as an internal control to normalize the transfection efficiency; Promega). Transfections were performed with Lipofectamine reagent (BRL) in triplicate according to the manufacturer's protocol. Transfected cells were cultured for 48 hours (70–90% confluence), harvested, and assayed for luciferase activity with the Dual-Luciferase™ Reporter Assay System (Promega). Firefly luciferase activities for each experimental construct were normalized to the activity of the internal control (pRL-CMV), and expressed as relative luciferase activity (mean ± SD). Statistical analysis was performed with Student's unpaired *t*-test.

## RESULTS

African SNP regions for lactase persistence were characterized with respect to their ability to regulate lactase promoter activity in Caco-2 cells, a human colonic adenocarcinoma cell line expressing a number of small-intestinal brush-border enzymes including LPH (36). Specifically, 215-bp DNA fragments corresponding to nucleotides -14010 to -13800 of the human *LCT* gene and including either a specific SNP or the ancestral sequence were cloned in both orientations into luciferase report constructs upstream of a 2-kb 5′ flanking region of the rat lactase gene previously shown to direct intestine-specific promoter activity in cell culture and in transgenic mice (37, 38). Caco-2 cells were transiently transfected with the lactase SNP-promoter-reporter constructs and assayed for relative luciferase activity.

As shown in Figure 1, the 2-kb rat lactase promoter fragment in p2kLac directed a 5.4-fold increase in luciferase activity relative to the construct pGL3-Basic lacking the promoter. Addition of the 215-bp fragment corresponding to the ancestral allelic sequence (-13907*C, -13915*T, and -14010*G) to generate the p2kLac-Ancestral promoter-reporter construct resulted in an additional 3-fold enhancement of luciferase activity.

To determine whether the different African SNP regions associated with lactase persistence are capable of functioning to regulate the lactase promoter, Caco-2 cells were similarly transfected with the individual SNP (13907*G, -13915*G, and -14010*C) promoter-reporter constructs and assayed for luciferase activity. Transfection with the -13907*G promoter-reporter constructs, p2kLac-13907*G (forward) and p2kLac-13907*G′ (reverse), resulted in a statistically significant enhancement of luciferase reporter gene expression over that of cells transfected with the p2kLac-Ancestral constructs (Figure 1). Specifically, the -13907*G allelic DNA region in the forward orientation resulted in a 1.2-fold enhancement ($P < 0.05$) of reporter activity as compared with the ancestral allele. Similarly, the -13907*G allelic DNA region in the reverse orientation resulted in a 1.5-fold enhance-

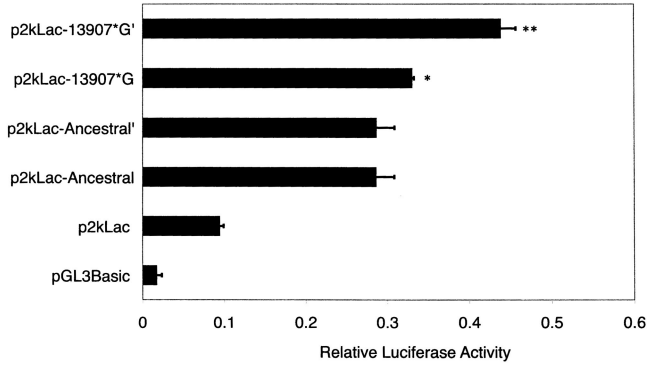


FIG. 1. Characterization of lactase promoter activity regulated by the -13907*G SNP associated with lactase persistence. Caco-2 cells were transiently transfected with the lactase promoter-luciferase reporter constructs and assayed for luciferase activity. pGL3-Basic: promoterless firefly luciferase reporter construct. p2kLac: firefly luciferase reporter construct with 2.0-kb fragment of the rat lactase promoter region. p2kLac-Ancestral and p2kLac-Ancestral': p2kLac with the 215-bp fragment of ancestral sequence (nucleotides -14014 to -13800) cloned in the forward or reverse orientation, respectively. p2kLac-13907*G and p2kLac-13907*G′: p2kLac with the 215-bp -13907*G SNP fragment cloned in the forward or reverse orientation, respectively. Transfection efficiencies were normalized to renilla luciferase expression from a co-transfected pRL-CMV vector and expressed as relative luciferase activity (mean ± SD, $n = 3$). *$P < 0.05$, **$P < 0.001$ versus the relative firefly luciferase activity of p2kLac-Ancestral.

ment ($P < 0.001$) of reporter activity as compared with the ancestral allele.

Transfection with the -13915*G promoter-reporter constructs, p2kLac-13915*G (forward) and p2kLac-13915*G′ (reverse), also resulted in a statistically significant enhancement of luciferase reporter gene expression over that of cells transfected with the p2kLac-Ancestral constructs (Figure 2). The -13915*G allelic DNA region in the forward orientation resulted in a 1.6-fold enhancement ($P < 0.001$) of reporter activity as compared with the ancestral allele. The -13915*G allelic DNA region in the reverse orientation resulted in a 1.8-fold enhancement ($P < 0.001$) of reporter activity as compared with the ancestral allele. In contrast to the enhanced promoter activation mediated by the -13907*G and -13915*G DNA regions, the -14010*C allelic region in both the forward and reverse orientations (p2kLac-14010*C and p2kLac-14010*C′, respectively) did not enhance luciferase activity as compared with that mediated by the ancestral allele (Figure 3). Thus, the -13907*G and -13915*G SNP variants were both capable of functioning to enhance activity of the 2-kb rat lactase promoter in Caco-2 cells. The -14010*C SNP variant did not function to enhance promoter activity.

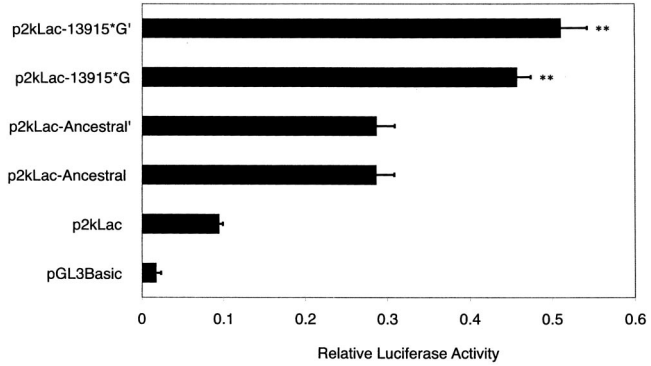


FIG. 2. Characterization of lactase promoter activity regulated by the -13915*G SNP associated with lactase persistence. Caco-2 cells were transiently transfected with the lactase promoter-luciferase reporter constructs listed below and assayed for luciferase activity. pGL3-Basic: promoterless firefly luciferase reporter construct. p2kLac: firefly luciferase reporter construct with 2.0-kb fragment of rat lactase promoter region. p2kLac-Ancestral and p2kLac-Ancestral': p2kLac with the 215-bp fragment of ancestral sequence (nucleotides -14014 to -13800) cloned in the forward or reverse orientation, respectively. p2kLac-13915*G and p2kLac-13915*G′: p2kLac with the 215-bp -13915*G SNP fragment cloned in the forward or reverse orientation, respectively. Transfection efficiencies were normalized to renilla luciferase expression from a co-transfected pRL-CMV vector and expressed as relative luciferase activity (mean ± SD, $n = 3$). **$P < 0.001$ versus the relative firefly luciferase activity of p2kLac-Ancestral.

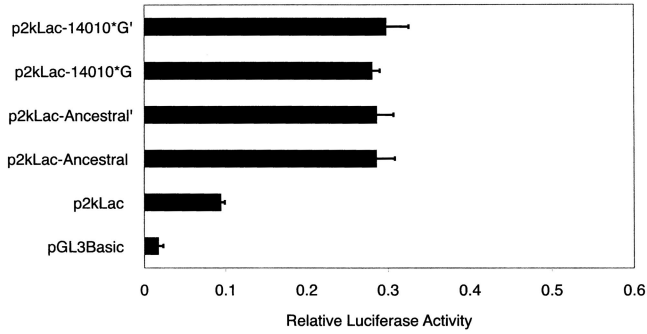


FIG. 3. Characterization of lactase promoter activity regulated by the -14010*C SNP associated with lactase persistence. Caco-2 cells were transiently transfected with the lactase promoter-luciferase reporter constructs listed below and assayed for luciferase activity. pGL3-Basic: promoterless firefly luciferase reporter construct. p2kLac: firefly luciferase reporter construct with 2.0-kb fragment of rat lactase promoter region. p2kLac-Ancestral and p2kLac-Ancestral': p2kLac with the 215-bp fragment of ancestral sequence (nucleotides -14014 to -13800) cloned in the forward or reverse orientation, respectively. p2kLac-14010*C and p2kLac-14010*C′: p2kLac with the 215-bp -14010*C SNP fragment cloned in the forward or reverse orientation, respectively. Transfection efficiencies were normalized to renilla luciferase expression from a co-transfected pRL-CMV vector and expressed as relative luciferase activity (mean ± SD, $n = 3$).

## DISCUSSION

In most mammals, the *LCT* gene is expressed maximally in the proximal and middle small intestine at birth, with its expression then declining after weaning. Regulation of the spatial and temporal expression patterns of the *LCT* gene is presumed to be primarily at the level of gene transcription. In previous studies, a 2-kb 5′ flanking fragment of the rat lactase promoter has been shown to mediate both spatial restriction and temporal downregulation of reporter gene expression in transgenic mice (37, 38). In the present study, in order to attribute function to recently identified SNPs associated with lactase persistence in African populations, DNA fragments in the SNP region were characterized with respect to their ability to regulate promoter activity when cloned upstream of the 2-kb fragment of the rat lactase promoter and assayed in intestinal Caco-2 cells.

Subsequent to the identification of the European SNP (-13910*C/T) associated with lactase non-persistence/persistence (21), additional SNPs (-13907*C/G, -13915*T/G, and -14010*G/C) have been identified in African populations (15, 28, 33, 34). The -13907*C/G SNP is widespread in Sudan (28, 33) and is present at a high frequency in Ethiopia (26). The -13915*T/G SNP is prevalent in Sudan and Ethiopia (15, 28), and the -14010*G/C was reported to be common in Tanzania and

Kenya (33, 34). In the present study, 215-bp fragments encompassing the -13907*G or -13915*G lactase persistence-associated SNPs significantly enhanced lactase promoter activity as compared with the 215-bp fragment corresponding to the ancestral sequence (Figures 1 and 2). In contrast to a previous report (33), however, the 215-bp fragment encompassing the -14010*C SNP did not enhance promoter activity relative to the ancestral fragment in our study (Figure 3). It is possible that the -14010*C SNP-region fragment did not enhance lactase promoter activity because of the cloned location of the SNP just five bases from the 5′ end of the DNA fragment insert. It therefore remains to be determined whether the -14010*C SNP is a functional mutation or merely a DNA polymorphism that is associated with the lactase persistence phenotype.

The mechanism by which the -13907*G, -13910*T, and -13915*G SNP region sequences can function to enhance lactase promoter activity in cell culture has yet to be fully defined. Lewinsky et al. found that the Oct-1 transcription factor can interact differentially with the European -13910*T region sequence as compared with the ancestral sequence in *in vitro* binding assays (39). Subsequent reports, summarized in Figure 4, have confirmed that the -13907*G and -13915*G SNP region sequences associated with lactase persistence in African and Middle Eastern populations can similarly interact with the Oct-1 transcription factor (31, 40). Future studies will focus on defining the role of Oct-1 and other transcriptions factors as candidate transcription factors involved in mediating temporal expression of the *LCT* gene.

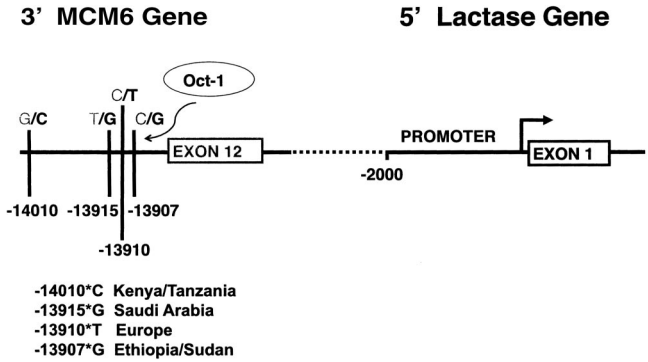


FIG 4. Schematic diagram of human lactase gene 5′ flanking region. The relative locations (within intron 13 of the *MCM6* gene) of SNPs associated with lactase persistence identified in different populations are indicated. The transcription factor Oct-1 interacts differentially with the -13903 to -13926 SNP region DNA oligonucleotides in *in vitro* DNA-binding assays.

## ACKNOWLEDGEMENTS

This work was supported by grants DK60074 and DK60715 from the National Institute of Diabetes and Digestive and Kidney Diseases.

## REFERENCES

1. Leese HJ, Semenza G. On the identity between the small intestinal enzymes phlorizin hydrolase and glycosylceramidase. J Biol Chem 1973;248:8170–3.
2. Grand RJ, Montgomery RK, Chitkara DK, Hirschhorn JN. Changing genes; losing lactase. Gut 2003;52:617–9.
3. Ingram CJ, Mulcare CA, Itan Y, Thomas MG, Swallow DM. Lactose digestion and the evolutionary genetics of lactase persistence. Hum Genet 2009;124:579–91.
4. Jarvela I, Torniainen S, Kolho KL. Molecular genetics of human lactase deficiencies. Ann Med 2009;41:568–75.
5. Lomer MC, Parkes GC, Sanderson JD. Review article: lactose intolerance in clinical practice-myths and realities. Aliment Pharmacol Ther 2008;27:93–103.
6. Montgomery RK, Buller HA, Rings EH, Grand RJ. Lactose intolerance and the genetic regulation of intestinal lactase- phlorizin hydrolase. Faseb J 1991;5:2824–32.
7. Sibley E. Genetic variation and lactose intolerance: detection methods and clinical implications. Am J Pharmacogenomics 2004;4:239–45.
8. Troelsen JT. Adult-type hypolactasia and regulation of lactase expression. Biochim Biophys Acta 2005;1723:19–32.
9. Boll W, Wagner P, Mantei N. Structure of the chromosomal gene and cDNAs coding for lactase-phlorizin hydrolase in humans with adult-type hypolactasia or persistence of lactase. Am J Hum Genet 1991;48:889–902.
10. Kruse TA, Bolund L, Grzeschik KH, et al. The human lactase-phlorizin hydrolase gene is located on chromosome 2. FEBS Lett 1988;240:123–6.
11. Mantei N, Villa M, Enzler T, et al. Complete primary structure of human and rabbit lactase-phlorizin hydrolase: implications for biosynthesis, membrane anchoring and evolution of the enzyme. EMBO J 1988;7:2705–13.
12. Jacob R, Radebach I, Wuthrich M, Grunberg J, Sterchi EE, Naim HY. Maturation of human intestinal lactase-phlorizin hydrolase: generation of the brush border form of the enzyme involves at least two proteolytic cleavage steps. Eur J Biochem 1996; 236:789–95.
13. Savaiano DA, Levitt MD. Milk intolerance and microbe-containing dairy foods. J Dairy Sci 1987;70:397–406.
14. Matthews SB, Waud JP, Roberts AG, Campbell AK. Systemic lactose intolerance: a new perspective on an old problem. Postgrad Med J 2005;81:167–73.
15. Ingram CJ, Raga TO, Tarekegn A, et al. Multiple rare variants as a cause of a common phenotype: several different lactase persistence associated alleles in a single ethnic group. J Mol Evol 2009;69:579–88.
16. Simoons FJ. The geographic hypothesis and lactose malabsorption. A weighing of the evidence. Am J Dig Dis 1978;23:963–80.
17. Sahi T, Isokoski M, Jussila J, Launiala K, Pyorala K. Recessive inheritance of adult-type lactose malabsorption. Lancet 1973;2:823–6.
18. Sahi T, Launiala K. More evidence for the recessive inheritance of selective adult type lactose malabsorption. Gastroenterology 1977;73:231–2.

19. Escher JC, de Koning ND, van Engen CG, et al. Molecular basis of lactase levels in adult humans. J Clin Invest 1992;89:480–3.
20. Fajardo O, Naim HY, Lacey SW. The polymorphic expression of lactase in adults is regulated at the messenger RNA level. Gastroenterology 1994;106:1233–41.
21. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat Genet 2002;30:233–7.
22. 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;12:2333–40.
23. Troelsen JT, Olsen J, Moller J, Sjostrom H. An upstream polymorphism associated with lactase persistence has increased enhancer activity. Gastroenterology 2003; 125:1686–94.
24. Bersaglieri T, Sabeti PC, Patterson N, et al. Genetic signatures of strong recent positive selection at the lactase gene. Am J Hum Genet 2004;74:1111–20.
25. Nagy D, Bogacsi-Szabo E, Varkonyi A, et al. Prevalence of adult-type hypolactasia as diagnosed with genetic and lactose hydrogen breath tests in Hungarians. Eur J Clin Nutr 2009;63:909–12.
26. Smith GD, Lawlor DA, Timpson NJ, et al. Lactase persistence-related genetic variant: population substructure and health outcomes. Eur J Hum Genet 2009;17:357–67.
27. Mattar R, Monteiro MS, Villares CA, Santos AF, Silva JM, Carrilho FJ. Frequency of LCT -13910C>T single nucleotide polymorphism associated with adult-type hypolactasia/lactase persistence among Brazilians of different ethnic groups. Nutr J 2009;8:46.
28. Ingram CJ, Elamin MF, Mulcare CA, et al. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? Hum Genet 2007;120:779–88.
29. Mulcare CA, Weale ME, Jones AL, et al. 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; 74:1102–10.
30. Sun HM, Qiao YD, Chen F, Xu LD, Bai J, Fu SB. The lactase gene -13910T allele cannot predict the lactase-persistence phenotype in north China. Asia Pac J Clin Nutr 2007;16:598–601.
31. Enattah NS, Jensen TG, Nielsen M, et al. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. Am J Hum Genet 2008;82:57–72.
32. Imtiaz F, Savilahti E, Sarnesto A, et al. 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 2007;44:e89.
33. Tishkoff SA, Reed FA, Ranciaro A, et al. Convergent adaptation of human lactase persistence in Africa and Europe. Nat Genet 2007;39:31–40.
34. Torniainen S, Parker MI, Holmberg V, Lahtela E, Dandara C, Jarvela I. Screening of variants for lactase persistence/non-persistence in populations from South Africa and Ghana. BMC Genet 2009;10:31.
35. Fang R, Santiago NA, Olds LC, Sibley E. The homeodomain protein Cdx2 regulates lactase gene promoter activity during enterocyte differentiation. Gastroenterology 2000;118:115–27.
36. Hauri HP, Sander B, Naim H. Induction of lactase biosynthesis in the human intestinal epithelial cell line Caco-2. Eur J Biochem 1994;219:539–46.

37. Lee SY, Wang Z, Lin CK, et al. Regulation of intestine-specific spatiotemporal expression by the rat lactase promoter. J Biol Chem 2002;277:13099–105.
38. Wang Z, Maravelias C, Sibley E. Lactase gene promoter fragments mediate differential spatial and temporal expression patterns in transgenic mice. DNA Cell Biol 2006;25:215–22.
39. 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;14:3945–53.
40. Olds LC, Ahn JK, Sibley E. -13915*G DNA polymorphism associated with lactase persistence in Africa interacts with Oct-1. Hum Genet 2010; Oct 20:[Epub ahead of print].

## DISCUSSION

**Howley, Boston:** Thank you for the talk, Eric. Given that the element you found, which may or may not bind Oct-1, functions as an enhancer, does it have an effect on the upstream *MCM6* gene?

**Sibley, Palo Alto:** The lactase persistence polymorphisms are located in intron 13 of the *MCM6* gene. We have not looked; but those who have characterized *MCM6* have not described any transcriptional regulation by Oct-1 or the lactase persistence SNP region.

**Clark, San Antonio:** I was wondering if you have considered whether those lactase persistence SNPs might also affect epigenetic factors that could be regulating expression.

**Sibley, Palo Alto:** We have looked for methylation differences or sequences that may be involved in methylation. Also, other colleagues in the field have looked for differences in histone modification but have not yet identified any epigenetic effects. I would be surprised if epigenetic factors are not involved, however, since epigenetic regulation would seem to be a more likely mechanism for mediating the maturational changes in lactase gene expression.

**Rosenwasser, Kansas City:** I enjoyed your presentation. I am curious about relative allele frequencies in the African populations versus the European populations at that particular site, and whether you've done any preferential affinity binding with Oct-1 and those nucleotides?

**Sibley, Palo Alto:** Regarding the second part of your question, we actually have purified Oct-1 and performed binding reactions the same way we've done with the EMSAs that I described, using intact nuclear protein extracts isolated from intestinal cells. The same type of interactions that we see with nuclear protein extracts are seen as well with purified Oct-1. As for the allele frequencies, although we are not a population genetics laboratory, I believe the region-specific lactase persistence allele frequencies are generally less in the various African populations studied than in the Northern European populations studied.

**Kenney, Madison:** As you probably know, there is a whole series of members in the Oct transcription factor family. Some of them are widely expressed, liked Oct-1, and others, like Oct-2, are very cell-type dependent. Are there any Oct family members that are specifically regulated in the small bowel by age?

**Sibley, Palo Alto:** I am not aware if any Oct family members are regulated in the small bowel by age.