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–13915*G DNA polymorphism associated with lactase persistence in Africa interacts with Oct-1

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Abstract

Lactase gene expression declines with aging (lactase non-persistence) in the majority of humans world-wide. Lactase persistence is a heritable autosomal dominant condition and has been strongly correlated with several single nucleotide polymorphisms (SNPs) located ~14-kb upstream (–13907, –13910 and –13915) of the lactase gene in different ethnic populations. In contrast to the –13907*G and –13910*T SNPs, the –13915*G SNP was previously believed not to interact with Oct-1. In the present study, however, Oct-1 is shown to interact with the –13915*G SNP region DNA sequence by EMSAs and gel supershift. In addition, Oct-1 is capable of enhancing promoter activity of a lactase promoter-reporter construct harboring the 13915*G SNP sequence in cell culture. Oct-1 binding to the –13907 to –13915 SNP region therefore remains a candidate interaction involved in lactase persistence.

Introduction

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 ethnic pastoral populations (Enattah et al. 2002,2008;Ingram et al. 2007;Tishkoff et al. 2007). Though strongly correlated with the lactase persistence phenotypes, a functional mechanism for the SNPs in mediating lactase persistence has not been clearly defined. Transfection experiments have shown that the lactase persistence SNP region DNA can function as a cis element capable of enhancing differential transcriptional activation of the lactase promoter in cell culture (Olds and Sibley 2003;Troelsen et al. 2003;Lewinsky et al. 2005). While previous reports have indicated that the –13907*G and –13910*T SNP region sequences can interact with the Oct-1 transcription factor (Lewinsky et al. 2005;Ingram et al. 2007), Oct-1 binding to the –13915*G SNP was minimal or not detected (Ingram et al. 2007;Enattah et al. 2008). In the

present study, however, we demonstrate that the –13915*G region can interact with the Oct-1 protein.

Results and discussion

The lactase persistence SNP region –13915*G interacts with the Oct-1 transcription factor

To determine the identity of intestinal nuclear proteins interacting with the African –13915*G SNP region DNA sequence, electrophoretic mobility shift assays (EMSA) were performed. Specifically, nuclear extracts were prepared from differentiated Caco-2 cells, a human adenocarcinoma derived intestinal cell line (Hauri et al. 1994), and incubated with a biotinylated oligonucleotide probe corresponding to the human DNA sequences flanking the –13915*G SNP AGATAAAGATAAGGTAGCCCCTGGC (Oct-1 consensus binding site underlined and –13915 in bold). EMSA reactions were performed in the presence or absence of excess unlabeled oligonucleotides corresponding to the –13915*G SNP region, to an Oct-1 consensus binding sequence, or to an unrelated non-specific control sequence. As shown in Fig. 1a, the –13915*G probe interacts with Caco-2 cell nuclear extract to result in a specific DNA/protein complex (lane 2, arrow) that is competed by 200-fold excess unlabeled probe (lane 3) but not by 200-fold excess oligonucleotide corresponding to the unrelated non-specific sequence (lane 5). As described above, the sequence surrounding the –13907*G and –13910*T SNPs associated with lactase persistence and the ancestral sequence have previously been shown to interact with the Oct-1 transcription factor protein. In the present report, EMSA performed with the –13915*G probe and 200-fold excess unlabeled oligonucleotide corresponding to a Oct-1 consensus DNA binding sequence results in competition for nuclear protein binding (lane 4). This result suggests that Oct-1 is capable of interacting with the –13915*G region sequence. In addition, EMSA performed with the –13915*G probe resulted in a more abundant and faster migrating DNA/protein complex than with the ancestral sequence probe (Fig. 1b, compare lanes 3 and 5). The –13915*G complex, however, was not as abundant as the –13910*T complex bands. It is likely that multiple nuclear proteins interact with this SNP region. The detection of Oct-1 interaction with the –13915*G SNP region is in contrast to previous reports and may be due to differences in binding reaction conditions (e.g. ionic strength, temperature) or sensitivity of band detection methods (radiographic vs. chemiluminescent).

To confirm that Oct-1 interacts with the –13915*G SNP region, gel supershifts were performed in the presence of Oct-1 specific antibody or non-specific antibody control. Incubation of the –13915*G probe with Caco-2 cell nuclear extract in the presence of control non-specific IgM antibody results in the specific DNA/protein complex band (lane 7, arrow). Incubation in the presence of Oct-1 specific antibody, however, results in decreased abundance of the specific DNA/protein complex band (lane 6). This result is consistent with Oct-1 interaction to form the –13915*G DNA/Protein complex. The presence of a faint –13915*G band after Oct-1 competition (Fig. 1a, lane 4) or supershift (Fig. 1b, lane 2) suggests that additional nuclear protein(s) may also interact with the –13915*G probe.

In order to determine whether the DNA sequence surrounding the –13915*G human lactase persistence variant is capable of regulating lactase promoter activity, Caco-2 cells were transfected with lactase promoter-reporter constructs harboring the –13915*G or ancestral –13915*T sequences. The constructs were generated by cloning the G/T-13915 SNP region of the human lactase promoter upstream of the 2.0-kb rat lactase promoter in the luciferase reporter plasmid pGL3Basic. Transfected Caco-2 cell extracts were assayed for relative luciferase activity 48 h after transfection as shown in Fig. 1c. Transfection with the lactase persistence associated –13915*G SNP construct, p2K.Lac-13915G, results in significantly greater promoter activity than the ancestral p2K.Lac-13915T lactase promoter construct.

Differential enhancement of lactase promoter activity mediated by the G/T-13915 SNP variant region suggests a functional role for the DNA variant in specifying the lactase persistence/non-persistence phenotypes. Co-transfection with an Oct-1 expression construct results in a ~2-fold increase in promoter activity driven by both promoter-reporter constructs, consistent with reports (Lewinsky et al. 2005; Ingram et al. 2007) that Oct-1 can function to regulate the human lactase promoter. Similar fold increase of both promoter-reporter constructs by the Oct-1 expression construct, however, suggests that excess Oct-1 does not result in the differential lactase promoter activity associated with the G/T-13915 SNP region but rather that Oct-1 general upregulation may be mediated by an invariant region of the promoter constructs.

In summary, we have demonstrated that the -13915*G SNP region (associated with lactase persistence) of the lactase gene interacts with the Oct-1 transcription factor in in vitro binding reactions. Such interaction is contrary to previous reports in which Oct-1 binding to the -13915*G SNP was not detected suggesting that the SNP mutation abolishes Oct-1 binding. With the current report, Oct-1 has now been shown to interact with the sequence surrounding each of the currently identified SNPs located between -13907 and -13915. Oct-1 binding to the SNP region therefore remains a candidate interaction involved in lactase persistence.

Acknowledgments

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Abbreviations

LPH	Lactase-phlorizin hydrolase
RLU	Relative light units
SNP	Single nucleotide polymorphism
EMSA	Electrophoretic mobility shift assay

References

1. 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(2):233–237. [PubMed: 11788828]
2. Enattah NS, Jensen TG, Nielsen M, Lewinski R, Kuokkanen M, Rasinpera H, El-Shanti H, Seo JK, Alifrangis M, Khalil IF, Natah A, Ali A, Natah S, Comas D, Mehdi SQ, Groop L, Vestergaard EM, Imtiaz F, Rashed MS, Meyer B, Troelsen J, Peltonen L. Independent introduction of two lactase-persistence alleles into human populations reflects different history of adaptation to milk culture. *Am J Hum Genet* 2008;82(1):57–72. [PubMed: 18179885]
3. Hauri HP, Sander B, Naim H. Induction of lactase biosynthesis in the human intestinal epithelial cell line caco-2. *Eur J Biochem* 1994;219(1–2):539–546. [PubMed: 7508390]
4. Ingram CJ, Elamin MF, Mulcare CA, Weale ME, Tarekegn A, Raga TO, Bekele E, Elamin FM, Thomas MG, Bradman N, Swallow DM. A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Hum Genet* 2007;120(6):779–788. [PubMed: 17120047]
5. 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(24):3945–3953. [PubMed: 16301215]
6. 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(18):2333–2340. [PubMed: 12915462]

7. Tishkoff SA, Reed FA, Ranciaro A, Voight BF, Babbitt CC, Silverman JS, Powell K, Mortensen HM, Hirbo JB, Osman M, Ibrahim M, Omar SA, Lema G, Nyambo TB, Ghorri J, Bumpstead S, Pritchard JK, Wray GA, Deloukas P. Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet* 2007;39(1):31–40. [PubMed: 17159977]
8. Troelsen JT, Olsen J, Moller J, Sjostrom H. An upstream polymorphism associated with lactase persistence has increased enhancer activity. *Gastroenterology* 2003;125(6):1686–1694. [PubMed: 14724821]

Fig. 1.

a Electrophoretic mobility shift and supershift assays with Caco-2 cell nuclear extract and –13915*G SNP region probe. Caco-2 cell nuclear extract protein (7 days post-confluent, 12.5 μ g) was incubated with biotin-labeled –13915*G oligonucleotide probe (AGATAAGATAAGGTAGCCCCTGGC) alone (*lane 2*) or in the presence of 200-fold excess unlabeled –13915*G probe (*lane 3*), Oct-1 consensus (ATGTCGAATGCAAATCACTAGAACT) (*lane 4*), or non-specific (GTCACCCATTAGCACAGGCC) (*lane 5*, NS) oligonucleotides. Gel supershifts were performed with incubation in the presence of 2 μ g Oct-1 specific antibody (Santa Cruz Biotech, *lane 6*) or control IgM (*lane 7*). Probe incubated in the absence of nuclear protein is shown in *lane 1*. EMSA reaction conditions and detection were performed as previously described (Olds and Sibley 2003). Arrow indicates specific DNA/protein complex. **b** Electrophoretic mobility shift assays with Caco-2 cell nuclear extract and –13915*G, –13910*T and ancestral SNP region probes. EMSAs with 10 μ g Caco-2 cell nuclear protein and biotin-labeled –13915*G, –13910*T (AGATAAGATAAGGTAGTCCCTGGC) (*lane 4*) or ancestral (AGATAAGATAATGTAGCCCCTGGC) (*lane 5*, AC) oligonucleotide probes. Gel supershifts were performed with incubation in the presence of Oct-1 specific antibody (*lane 2*) or control IgM (*lane 1*). Arrow indicates specific DNA/protein complex. **c** Luciferase activity of intestinal cells transfected with G/T-13915 SNP region lactase promoter-reporter constructs. Caco-2 cells were transfected with lactase promoter-luciferase reporter constructs in the presence of Oct-1 expression construct (Lewinsky et al. 2005; Ingram et al. 2007) (*black bars*) or empty vector (*white bars*) and assayed for luciferase expression as previously described (Olds and Sibley 2003). The p2kLac-13915 reporter constructs were generated by cloning a 218-bp fragment (–14017 to –13994) containing the –13915*G or –13915*T sequence upstream of the 2.0-kb rat lactase promoter cloned in pGL3 as previously described (Olds and Sibley 2003). Transfection efficiencies were normalized to *Renilla* luciferase expression from a co-transfected pRL-CMV vector and expressed as relative luciferase activity (mean $\pm$ SD, $n = 3$)