TECHNICAL NOTE

LCT-22018G>A single nucleotide polymorphism is a better predictor of adult-type hypolactasia/lactase persistence in Japanese-Brazilians than LCT-13910C>T

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INTRODUCTION

Adult type hypolactasia, the genetically programmed down-regulation of lactase enzyme activity in the intestinal wall after weaning, is a common condition worldwide, except for in northwestern Europe, where the prevalence is less than 10%. Lactose intolerant individuals complain of abdominal cramps, bloating, distention, flatulence and diarrhea after milk or lactose-containing food ingestion.[1]

The diagnosis of adult-type hypolactasia can be achieved by a hydrogen breath test that is cumbersome and provokes symptoms, or more recently, using a genetic approach.[2] Lactase persistence and adult-type hypolactasia have been associated with the *LCT*-13910C>T and *LCT* -22018G>A polymorphisms in introns 13 and 9, respectively, of the minichromosome maintenance type 6 gene (*MCM6*) upstream of the *LCT* locus in several populations.[3]

In Brazil, the lactase persistence allele, *LCT*-13910T, was found in approximately 43% of both white (European descent) and brown (European and African descent), and 20% of black (African descent) Brazilians, but was absent in all Japanese-Brazilians studied.[4] Recent epidemiological data regarding lactose intolerance/hypolactasia are lacking in Japan. This lack of information may be because of the relative rarity of symptoms; it has been shown that, although 92% of tested subjects were lactase deficient, only 2% were milk intolerant and 13% were lactose intolerant.[5]

Recent evidence in northern China suggests that *LCT*-22018G>A, rather than *LCT*-13910C>T, *LCT*-13907C>G, *LCT*-13915T>G, or *LCT*-14010G>C, matched the lactase persistence phenotype.[6] Therefore, the purpose of this study was to ascertain whether *LCT*-22018G>A would also be a predictor of lactase persistence in Japanese-Brazilians.

MATERIALS AND METHODS

This study was approved by the local Ethics Committee. The study population consisted of 56 Japanese-Brazilians with the *LCT*-13910CC genotype according to a previously described genotyping technique,[4] with a mean age of 47.1 ± 17.6 years. Seventeen (30.4%) men gave written informed consent to participate.

DNA was extracted from leukocytes. Primers 5′-AACAGGCACGTGGAGGAGTT-3′ (position 18261-18280) and 5′-CCCACCTCAGCCTCTTGAGT-3′ (position 18708-18689), Accession number *AY220757*, spanning the *LCT*-22018 region, were used in a polymerase chain reaction (PCR) with Premium *Taq* DNA polymerase (Invitrogen, São Paulo, Brazil) and 2.5 mM $MgCl_2$. Amplification was performed in 38 cycles at 95℃ for 1 min, 67℃ for 1 min, and 72℃ for 1 min. The PCR product was digested with *Hha*I, resulting in one fragment of 448 bp (the AA genotype), two fragments of 264 and 184 bp (the GG genotype) and three fragments of 448, 264, and 184 bp (the GA genotype), which were visualized on a 2% agarose gel stained with ethidium bromide, as has been previously described by Büning et al.[7]

RESULTS AND DISCUSSION

Among the 56 Japanese-Brazilians who were previously genotyped as *LCT*-13910CC (hypolactasia),[4] 3 (5.4%) had the *LCT*-22018GA genotype associated with lactase persistence (Fig. 1), and 53 (94.6%) had the *LCT*-22018GG genotype associated with hypolactasia (Table 1).

The incidence of lactase deficiency gradually increases with age from 3 years, and approximately 90% of all normal Japanese adults are lactase-deficient.[8] Among Japanese-Brazilians, 100% had lactose malabsorption;[9] therefore, these values are in accordance with lactose malabsorption in Japanese people who have been diagnosed by the hydrogen breath test.

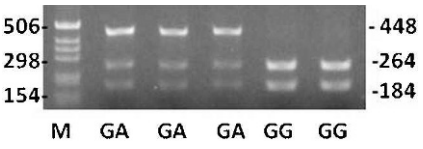

Figure 1- Agarose gel (2%) electrophoresis of *Hha*I-digested PCR products; M, 1kb DNA ladder (Invitrogen, USA) was used as marker of DNA fragment sizes; GA, bands of 448 bp, 264 bp, and 184 bp connote the GA genotype of three individuals; GG, two bands of 264 bp and 184bp connote the GG genotype of the other participants.

Table 1- Genotypic distribution of lactase persistence/ hypolactasia in Japanese-Brazilians.

Japanese-Brazilian	*LCT*-22018GA	*LCT*-22018GG	Total
LCT-13910CC	3 (5.4%)	53 (94.6%)	56 (100%)

In Brazilians, both the *LCT*-13910C>T allele[2] and the *LCT*-22018G>A allele[10] have been associated with lactase persistence phenotypes. As such, genetic analysis for Japanese-Brazilians should include an assessment for the *LCT*-22018G>A allele, as the *LCT*-13910C>T polymorphism is already routinely performed for hypolactasia/lactase persistence diagnosis.[2]

CONCLUSION

The *LCT*-22018G>A allele is a better predictor of lactase persistence in Japanese-Brazilians than the *LCT*-13910C>T allele.

REFERENCES

1. Mattar R, Mazo DFC. Intolerância à lactose: mudança de paradigmas com a biologia molecular. Rev Assoc Med Bras. 2010;56:230-6, doi: 10.1590/S0104-42302010000200025.
2. Mattar R, Monteiro MS, Villares CA, Santos AF, Carrilho FJ. Single nucleotide polymorphism C/T_{-13910}, located upstream of the lactase gene, associated with adult-type hypolactasia: validation for clinical practice. Clin Biochem. 2008;41:628-30, doi: 10.1016/j.clinbiochem.2008.01.006.
3. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jãverlã I. Identification of a variant associated with adult-type hypolactasia. Nat Genet. 2002;30:233-7, doi: 10.1038/ng826.
4. Mattar R, Monteiro MS, Villares CA, Santos AF, Silva JMK, 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, doi: 10.1186/1475-2891-8-46.
5. Kondo T, Liu F, Toda Y. Milk is a useful test meal for measurement of small bowel transit time. J Gastroenterol. 1994;29:715-20, doi: 10.1007/BF02349276.
6. Xu L, Sun H, Zhang X, Wang J, Sun D, Chen F, et al. The -22018A allele matches the lactase persistence phenotype in northern Chinese populations. Scand J Gastroenterol. 2010;45:168-174, doi: 10.3109/00365520903414176.
7. Büning C, Ockenga J, Krüger S, Jurga J, Baier P, Dignass A,et al. The C/C-13910 and G/G-22018 genotypes for adult-type hypolactasia are not associated with inflammatory bowel disease. Scand J Gastroenterol. 2003;38:538-42.
8. Nose O, Iida Y, Kai H, Harada T, Ogawa M, Yabuuchi H. Breath hydrogen test for detecting lactose malabsorption in infants and children. Arch Dis Child. 1979;54:436-40, doi: 10.1136/adc.54.6.436.
9. Sevá-Pereira A, Beiguelman B. Primary lactose malabsorption in healthy Brazilian adult caucasoid, negroid and mongoloid subjects. Arq Gastroenterol. 1982;19:133-8.
10. Bernardes-Silva CF, Pereira AC, da Mota GFA, Krieger JE, Laudanna AA. Lactase persistence/non-persistence variants, C/T_13910 and G/A_22018, as a diagnostic tool for lactose intolerance in IBS patients. Clin Chim Acta. 2007;386:7-11, doi: 10.1016/j.cca.2007.07.012.