

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 MgCl₂. Amplification was performed in 38 cycles at 95°C for 1 min, 67°C for 1 min, and 72°C 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 GA genotype) and three fragments of 448, 264, and 184 bp (the GG 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.

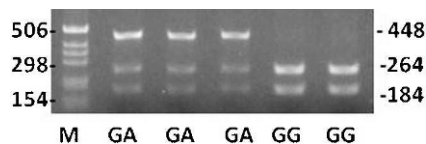


Figure 1- Agarose gel (2%) electrophoresis of *Hha*I-digested PCR products; M, 1kb DNA ladder (Invitrogen, USA) was used as marker of DNA fragment sizes; GA, bands of 448 bp, 264 bp, and 184 bp connote the GA genotype of three individuals; GG, two bands of 264 bp and 184bp connote the GG genotype of 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* allele² and the *LCT-22018G>A* allele¹⁰ 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.

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