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DIAGNOSIS OF ADULT-TYPE HYPOLACTASIA/LACTASE PERSISTENCE: genotyping of single nucleotide polymorphism (SNP C/T_{-13910}) is not consistent with breath test in Colombian Caribbean population

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ABSTRACT – ***Context*** **-** Genotyping of single nucleotide polymorphism (SNP C/T_{-13910}) located upstream of the lactase gene is used to determine adult-type hypolactasia/lactase persistence in North-European Caucasian subjects. The applicability of this polymorphism has been studied by comparing it with the standard diagnostic methods in different populations. ***Objective*** **-** To compare the lactose hydrogen breath test with the genetic test in a sample of the Colombian Caribbean population. ***Methods*** **-** Lactose hydrogen breath test and genotyping of SNP C/T_{-13910} were applied to 128 healthy individuals (mean age 35 ± 1). A positive lactose hydrogen breath test was indicative of hypolactasia. Genotyping was done using polymerase chain reaction/restriction fragment length polymorphism. The kappa index was used to establish agreement between the two methods. ***Results*** **-** Seventy-six subjects (59%) were lactose-maldigesters (hypolactasia) and 52 subjects (41%) were lactose-digesters (lactase persistence). The frequencies of the CC, CT and TT genotypes were 80%, 20% and 0%, respectively. Genotyping had 97% sensitivity and 46% specificity. The kappa index = 0.473 indicates moderate agreement between the genotyping of SNP C/T_{-13910} and the lactose hydrogen breath test. ***Conclusion*** **-** The moderate agreement indicates that the genotyping of the SNP C/T_{-13910} is not applicable to determine adult-type hypolactasia/lactase persistence in the population participating in this study.

HEADINGS - Lactose intolerance. Lactase. Polymorphism, single nucleotide. Breath tests. Colombia. Caribbean region.

INTRODUCTION

Lactase-florizine hydrolase (E.C. 3.2.1.23/62), commonly known as lactase, is the intestinal enzyme that allows lactose digestion to yield its absorbable constituent monosaccharides, glucose and galactose[25]. This ability persists throughout adulthood only in a minority of human subjects, resulting in the lactase persistence (LP) phenotype, while lactase enzyme is significantly reduced after weaning in most adults worldwide, resulting in lactase non-persistence or adult-type hypolactasia (ATH) phenotype[27]. Both conditions are genetically determined and their frequencies vary in human populations according to geographical and ethnic variations[7].

The direct method to diagnose ATH is to assay lactase activity in intestinal biopsies[4]. However, a non invasive method, the hydrogen breath test after lactose ingestion, allows to indirectly determine the phenotype with high sensitivity and specificity[1, 24]. With the discovery of phenotype-genotype association in the past few years, a direct genetic diagnostic method becomes relevant. In fact, genotyping of the single nucleotide polymorphism (SNP) C/T_{-13910}, located upstream of the lactase gene (LCT), has shown that CC-genotype is associated with ATH phenotype, whereas the TC and TT-genotypes are associated with LP phenotype in Europeans of Caucasian origin[6, 14, 23].

Taking into account the relevance of the genetic test as a predictor of ATH in some Europeans populations and the European contribution to the ethnic admixture of the Colombian Caribbean population, the purpose of this study was to compare the lactose hydrogen breath test (LHBT) with the genetic test in order to ascertain the applicability of genotyping to diagnose LP/ATH in a Colombian Caribbean population.

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METHODS

Subjects

A group of 128 healthy individuals, men and women (mean age 35 ± 1; range 17–69 years) without family ties among them, born in the Colombian Caribbean were selected. Their parents and grandparents were also born in this geographical zone. None of the individuals were smokers, showed digestive disease, had undergone abdominal surgery nor had undergone antibiotic treatment within the last 3 months preceding the study.

The subjects gave their informed consent. The study was approved by the Ethics Committee of Universidad Libre of Barranquilla, Colombia.

Lactose hydrogen breath test (LHBT)

The LHBT was performed after ingestion of 250 mL of 10% lactose. Ten-hours of fasting were required before testing. The breath hydrogen level was detected by Microlyzer Quintron Model 12i plus (Quintron Instrument Company Inc., Milwaukee, USA) at baseline and after the ingestion of lactose every hour over a period of 3 hours. The LHBT was considered positive if the hydrogen production was equal to or above 20 ppm compared to baseline in any measurement during the 3 hours after the ingestion of lactose. A positive LHBT showed that the subject was lactose maldigester (hypolactasia)[10, 20].

Genotyping of the SNP C/T_{-13910}

DNA was obtained from venous blood using the Wizard® Genomic DNA Purification Kit (Promega, USA). Polymorphism identification was done by Polymerase Chain Reaction/Restriction Fragment Length Polymorphism (PCR/RFLP). The fragment was amplified using the primers: 5'-GCTGGCAATACAGATAAGATAATGGA-3' and 5'-CTGCTTTGGTTGAAGCGAAGAT-3'[18]. Reaction was carried out in a total volume of 20 µL, with Tris HCl buffer 1X pH 9.0; $MgCl_2$ 1.5 mM; dNTPs 0.2 mM; Taq polymerase 1.5U and 1 µM for each primer (Invitrogen®, USA). PCR conditions were: 95°C for 10 min (denaturation cycle), followed by 35 cycles of 95°C for 1 min, 59°C for 1 min and 72°C for 1 min, and a final elongation cycle at 72°C for 8 min. PCR product was digested with Hinf I (New England Biolabs) generating fragments of 177 and 24 bp. Amplification and digestion products were run in an 8% polyacrylamide gel electrophoresis. Fragments were visualized with ethidium bromide stain. Samples that only presented a 201 bp (C) fragment or a 177 bp (T) fragment were interpreted as CC and TT genotype, respectively. While samples that presented two fragments of 201 bp and 177 bp, were interpreted as the CT genotype. The 24 bp fragment was not visualized.

Statistical analysis

Allelic and genotypic frequencies, as well as Hardy-Weinberg equilibrium and linkage disequilibrium were determined with Arlequin version 3.1 software[8]. The agreement between the genotyping of the SNP C/T_{-13910} and the LHBT was assessed by Kappa statistics. All of the analyses were done with a confidence interval of 95%.

RESULTS

LHBT analysis

Seventy-six of the 128 subjects (59%) had positive and 52 (41%) had negative LHBT. There was a significant difference ($P = 0.0005$) of hydrogen averages between lactose-digester and lactose-maldigester subjects. Figure 1 shows breath hydrogen average during testing time.

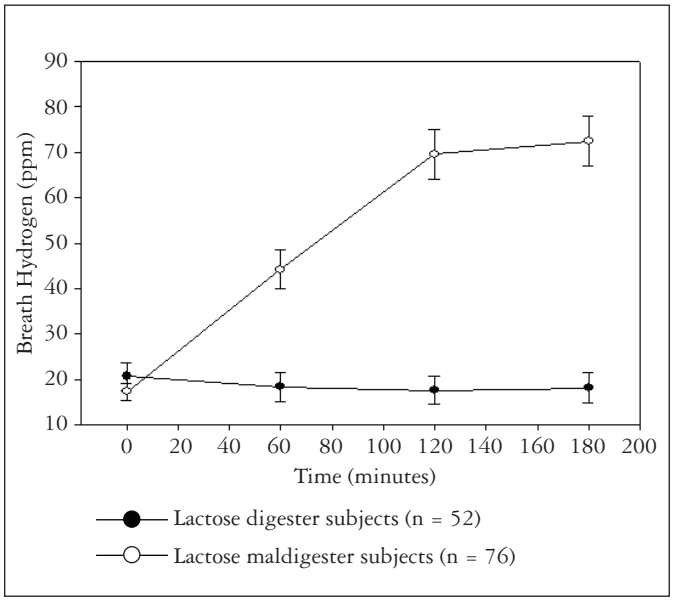

FIGURE 1. Hydrogen in breath test

Genotyping results

The frequencies of the CC, CT and TT genotypes were 80%, 20% and 0%, respectively. The frequency of the -13910*C allele was 90% and the frequency of the -13910*T allele was 10%.

The population in this study was in Hardy-Weinberg equilibrium for the SNP C/T_{-13910} ($P = 0.3667$).

Correlation between the LHBT and the genotyping of the SNP C/T_{-13910}

The results of genotyping compared with lactose digestion are presented in Table 1.

TABLE 1. Genotyping of SNP C/T_{-13910} compared with the lactose digestion evaluated by the LHBT

CT (lactase persistence)	Lactose digester (n = 24) 92%
n = 26	Lactose maldisgester (n = 2) 8%
CC (ATH)	Lactose digester (n = 28) 27%
n = 102	Lactose maldigester (n = 74) 73%

Seventy-four of 102 individuals (73%) with CC genotype were lactose maldigesters and 24 of 26 individuals (92%) with CT genotype were lactose digesters.

Using the LHBT as the standard method, genotyping of C/T_{-13910} had 97% sensitivity and 46% specificity. The positive predictive value of genotyping was 73% and the negative predictive value was 92%. The Kappa index for the agreement between the genotyping of C/T_{-13910} and the HBT was 0.473, suggesting a moderate agreement.

DISCUSSION

In this study, no close correlation between the CT genotype and the digesters (LP) or between CC genotype and the maldigesters (ATH) was found. The genetic test showed high sensitivity (97%) but low specificity (46%) and poor positive predictive value (71%). These last data suggest that the probability that a subject with a positive hypolactasia result actually has this genetic condition is not high, which means that the test is susceptible to false positives and is inconsistent compared to the LHBT for the ATH diagnosis in the studied population.

The results of this study differ from those of Di Stefano et al.[5], Hogenauer et al.[10], Krawczyk et al.[13], Nagy et al.[19], and Pohl et al.[22], carried out on European populations which showed strong agreement between LHBT and the genetic studies. It is possible that the discrepancy can be due to the genetic heterogeneity of the Colombian Caribbean population that arose from the crossing of different races as opposed to the studies with Europeans with Caucasian descent only[26]. Racial difference has implications on the genotypes that are reflected on the distribution of lactase phenotypes. In Europeans, the most common phenotype (lactase persistence) is strongly associated with the dominant and most prevalent -13910*T allele due to strong positive selection; but the ATH phenotype is rare and it is associated with the recessive and less prevalent -13910*C allele[2, 12]. In people of mixed ancestry, the genetic influence of their racial background is reflected. Thus, the low frequency of the -13910T allele found in the present study most likely represents the European contribution to the mixture of the three main ethnicities of the Colombian Caribbean people, whereas the high frequency of -13910C allele would represent the contribution from the other two ethnicities (Amerindians and Africans)[17].

The above hypothesis agrees with the Torniainen et al.[29] and Mattar et al.[16] studies. The former found in South Africa and Ghana that both -13910*T and -14010*C alleles reflected the contribution from Europeans and Africans in mixed ancestry subjects, respectively; the latter study reported that the lactase persistence allele (-13910*T) in mestizos subjects, product of white and African-Brazilian interbreeding, is present not only as a consequence of the European contribution but also of the ancestral contribution of Africans who have that allele[9, 16, 28]. This is not the case for African Caribbean Colombians, because the blacks brought as slaves from Africa during the colonial period came mainly from Western Africa where the -13910*T allele is scarce[18, 29].

The importance of the ethnic origin, when doing genotype-phenotype correlation studies is evident with the results recently published in Brazil by Bulhões et al.[3] and Mattar et al.[15] who reported perfect correlation between the SNP C/T_{-13910} and lactose digestion. They got this result because the subjects in their study were Brazilians of Caucasian descendence.

On the other hand, the low prevalence of -13910*T allele found in Colombian Caribbean population would indicate that some different lactase persistence associated allele is present in the population studied. Indeed, the C/G_{-13907}, T/C_{-13913}, G/C_{-14010} and T/G_{-13915} polymorphisms, all of them located upstream of the LCT gene, have been identified in some African and Middle Eastern populations[7, 11, 21, 28]. This finding shows that the genotyping of C/T-13910, valid as a diagnostic resource for Northern Europeans, does not apply worldwide[28].

It would be interesting to verify if those polymorphisms found in African populations are associated with LP/ATH in the Colombian Caribbean population, because of its African ancestry. Likewise, since the heterogeneity has implications on the lactase genotypes, new studies about the true contribution of Blacks, Spanish and Amerindians to the genetic structure of the population being studied are necessary.

In our study, no subjects had lactose intolerance symptoms such as diarrhea or abdominal distention at the time of the LBHT test. It would be interesting to study a group of symptomatic Colombian Caribbean subjects and correlate the lactose test with the -13910 polymorphism.

CONCLUSION

In a sample of the Colombian Caribbean population, no close correlation between the genotyping of the SNP C/T_{-13910} polymorphism and the LHBT was found. From these findings we conclude that the genetic test is not reliable to diagnose ATH/lactase persistence in Colombian Caribbean subjects.

Mendoza Torres E, Varela Prieto LL, Villarreal Camacho JL, Villanueva Torregroza DA. Diagnóstico de hipolactasia tipo adulto/persistência da lactase: a genotipagem do polimorfismo de um único nucleotídeo (SNP) C/T_{-13910} não é consistente com o teste de hidrogênio na população do Caribe Colombiano. Arq Gastroenterol. 2011;49(1):5-8.

RESUMO – ***Contexto*** **-** A genotipagem do SNP C/T_{-13910} localizado corrente acima do gene da lactase é usada para determinar hipolactasia e persistência da lactase tipo adulto em indivíduos caucasianos do Norte da Europa. A aplicabilidade deste polimorfismo tem sido estudada em comparação com métodos padronizados de diagnóstico em diferentes populações. ***Objetivo*** **-** Comparar o teste de hidrogênio expirado após a ingestão de lactose com o teste genético em uma mostra da população do Caribe Colombiano. ***Métodos*** **-** O teste de hidrogênio expirado após a ingestão de lactose e a genotipagem do SNP C/T_{-13910} foram aplicados em 128 sujeitos sadios (idade media 35 ± 1). O teste de hidrogênio positivo foi indicativo de hipolactasia. A genotipagem foi feita pelo método "polymerase chain reaction/restriction fragment length polymorphism". O índice Kappa foi utilizado para estabelecer a concordância entre os dois métodos. ***Resultados*** **-** Setenta e seis indivíduos (59%) foram mau digestores da lactose (hipolactasia) e 52 outros (41%) foram digestores da lactose (persistência da lactase). As frequências dos genotipos CC, CT e TT foram 80%, 20% e 0%, respectivamente. A genotipagem mostrou 97% da sensibilidade e 46% da especificidade. O índice kappa: 0,473 indicou moderada concordância entre os dois métodos. ***Conclusão*** **-** A moderada concordância indica que a genotipagem do SNP C/T_{-13910} nao é aplicável para determinar hipolactasia tipo adulto/persistência da lactase na população estudada.

DESCRITORES – Intolerância à lactose. Lactase. Polimorfismo de um único nucleotídeo. Testes respiratórios. Colômbia. Região do Caribe.

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