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Evaluation of agreement between C/T-13910 polymorphism genotyping results and lactose tolerance test results: a retrospective population-based study in Brazil

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HIGHLIGHTS

- Lactose intolerance is highly prevalent and may be implicated as a cofactor, or as a differential diagnosis, in many gastrointestinal conditions.
- The C/T-13910 polymorphism in lactase persistence is well characterized in Caucasian populations for lactase persistence.
- Concordance between genotyping and functional tests does not occur in all patients.
- Brazil has a highly mixed population and knowledge regarding presence of other polymorphisms is of importance in clarifying difficult cases.

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ABSTRACT – Background – Lactose tolerant test (LTT) is the most broadly used diagnostic test for lactose intolerance in Brazil, is an indirect, minimally invasive and a low-cost test that is widely available in primary care and useful in clinical practice. The C/T-13910 polymorphism in lactase persistence has been well characterized in Caucasian populations, but there are no studies evaluating the concordance between C/T-13910 polymorphism genotyping results and LTT results in Brazil, where the population is highly mixed. **Objective** – We aimed to evaluate agreement between presence of C/T-13910 polymorphism genotyping and malabsorption in LTT results. **Methods –** This is a retrospective analysis of a Brazilian population whose data were collected from a single laboratory database present in several Brazilian states. Results of individuals who underwent both genetic testing for lactose intolerance (C/T-13910 polymorphism genotyping) and an LTT from April 2016 until February 2019 were analysed to evaluate agreement between tests. Groups were classified according to age (<10-year-old (yo), 10–17 yo, ≥18 yo groups) and state of residence (São Paulo or Rio Grande do Sul). **Results** – Among the 404 patients evaluated, there was agreement between the genotyping and LTT results in 325 (80.4%) patients and discordance in 79 (19.6%) patients (k=0.42 -moderate agreement). Regarding the genotype, 47 patients with genotype C/C (lactase nonpersistence) had normal LTT results, and 32 with genotype C/T or T/T (indicating lactase persistence) had abnormal LTT results. Neither age nor state of residence (Rio Grande do Sul or São Paulo) affected the agreement between test results. **Conclusion** – Considering the moderate agreement between C/T-13910 polymorphism genotyping and LTT results ($\varkappa$=0.42) in the Brazilian population, we hypothesize that an analysis of other polymorphisms could be a strategy to improve the agreement between genotyping and established tests and suggest that additional studies should focus on exploring this approach.

Keywords – Food intolerance; lactose; genetic testing; lactose tolerance test.

INTRODUCTION

Lactose is the main carbohydrate in milk obtained from mammals. Its digestion requires lactase enzyme-mediated hydrolysis into glucose and galactose, monosaccharides that are absorbed[1].

Lactase persistence in adults is a late evolutionary trait that emerged frequently in populations working with domestic cattle, such as populations in Northern Europe and some African herding populations[2]. Mutations in regulatory genes encoding lactase (*LCT*), located on chromosome 2, allow for the persistence of lactase production. This persistence depends on a single-nucleotide polymorphism in the DNA sequence in a regulatory region of the *LCT* gene that controls the expression of the protein product (the lactase enzyme)[3]. At this time, there are 23 known genetic variants for lactase persistence[4]. One of the variants related to lactase persistence in the Caucasian population is the polymorphism C/T-13910. At position -13.910 of the *LCT* gene, genotype C/C is associated with a predisposition to lactose intolerance, while genotypes T/T and C/T are associated with lactose tolerance[4-6].

Diagnostic methods for lactose intolerance include investigations of lactase enzyme activity in jejunal biopsies, genetic testing, the lactose tolerance test (LTT), the hydrogen breath test[7] and the rapid test for lactase dosage in postbulbar duodenum samples[8]. LTT that is the most broadly used diagnostic test for lactose intolerance in Brazil. It is an indirect, minimally invasive test that determines serum glycemia levels in previously determined intervals after an oral overload administration of lactose. The LTT is a low-cost test that is widely available in primary care[9] and useful in clinical practice. Regarding to genetic testing, studies from Brazil have shown correlations between the C/T-13910 polymorphism, malabsorption[10] and lactose intolerance[11] as assessed using the hydrogen breath test[10,11]. A Swedish study analysed the concordance between this polymorphism genotyping results and lactose tolerance test (LTT) results in adults and found a 94% concordance[12]. There are no studies evaluating the concordance between C/T-13910 polymorphism genotyping results and LTT results in Brazil, where the population is highly mixed.

Therefore, our aim was to evaluate the diagnostic agreement between C/T-13910 polymorphism genotyping results and LTT results in the Brazilian population. In order to achieve this aim, we studied the tests results of patients from different Brazilian states attending a private laboratory facility.

METHODS

Study design

This is a retrospective, data mining study of a database from Group Fleury, a private laboratory facility located in 8 Brazilian states. The results of LTTs and genotyping for the C/T-13910 polymorphism from patients who were submitted to both tests between April 2018 and February 2019, were evaluated. All patients were anonymous to the researchers. The variables evaluated included patient sex, age, state of residence, qualitative genotyping results (alleles C/C, C/T or T/T) and quantitative LTT interval data.

Patients

A total of 452 patients submitted to both LTT and genetic test were identified. Forty-eight patients were excluded as they presented a fasting glucose ≥100 mg/dL in LTTs, as alterations in glucose metabolism are considered a limitation for the interpretation of LTT results[11]. In the analysis of agreement between the C/T-13910 polymorphism genotyping results and the LTT results, 404 patients were classified according to age <10-year-old (yo), between 10 and 17 yo and ≥18 yo groups and state of origin.

Molecular analysis of the blood sample was performed by polymerase chain reaction (PCR), followed by gene sequencing for the analysis of the C/T-13910 variant (rs4988235:NM_005915.5:c.1917+326C >T) in the MCM6 gene in chromosome 2.

For LTT, glycemia was evaluated in the fasting state and after ingestion of a solution of lactose (2 g lactose/kg of body weight up to 50 g of lactose in total). Blood glucose levels were determined at 30, 60 and 90 minutes after lactose ingestion. Individuals with an increase in glucose levels of at least 20 mg/dL at any time after the ingestion of lactose were considered good absorbers. Conversely, individuals who did not show such an increase in glucose levels were considered to have lactose malabsorption[13].

Statistical analysis

Cohen's Kappa coefficient was used to evaluate the degree of agreement between the results of genotypes (C/T-13910 polymorphism) and lactose tolerance tests[14].

The Kappa coefficient for multiple samples (analogous to Cohen's Kappa) was used to verify the degree of agreement between the methods (genotyping and LTT), according to the influence of the analysed variables (age and state of origin)[15]. Statistical significance was considered when the *P*-value was less than 0.05.

Ethical considerations

The project was approved by the National Research Ethics Commission (Plataforma Brazil) and by the Research Ethics Committee of Fleury Group (3.610.202, date: 09/30/2019).

RESULTS

Patients: the 404 patients included in the study were geographically distributed as follows: São Paulo state (n=361; 89.4%), Rio de Janeiro state (n=1; 0.24%), Paraná state (n=10; 2.5%), Rio Grande do Sul state (n=27; 6.7%), Bahia state (n=4; 1.0%) and Pernambuco state (n=1; 0.24%). Due to the lack of an adequate number of tests performed in other states, we limited this analysis to individuals from the states of Rio Grande do Sul and São Paulo. The number (N) of individuals in each age group was <10 yo (N: 27; media: 6,57yo); between 10 and 17 yo (N: 29; media: 14yo); >18 yo (N: 348; media: 44,97yo).

The results of the association between C/T13910 polymorphism and LTT in the whole population is described in TABLE 1. Level of agreement between the C/T-13910 polymorphism test and LTT among all included patients was evaluated and the results were in agreement in 325 patients (80.4%). Among the seventy-nine patients with discordant results, 47 had the C/C genotype (lactase nonpersistence), and their LTT results indicated good absorption; 32 had the C/T or T/T genotype (lactase persistence), and their LTT results indicated malabsorption. Cohen's kappa coefficient indicated moderate agreement ($\varkappa$=0.42) between the two diagnostic methods.

TABLE 1. Association between genotyping results (C/T -13.910 polymorphism) and lactose tolerance test (LTT) results among 404 Brazilian individuals.

	LTT results indicating malabsorption	LTT results indicating good absorption	Total
CC genotype (lactase nonpersistence)	278 (85.5%)	47 (14.5%)	325
CT or TT genotype (lactase persistence)	32 (40.5%)	47 (59.5%)	79
Total	310	94	404

$\varkappa$=0.42 (Cohen's kappa coefficient).

TABLE 2 shows the association between C/T-13910 polymorphism and LTT according to age group. The agreement between the genotyping and LTT results was not statistically diferent among the three age groups (*P*>0.05). This suggests that age does not statistically significantly affect the degree of agreement between results. Brazilian population ancestry is very diverse and this diversity varies among different regions due to patterns of immigration. The distribution of patients according to the state of origin, namely, São Paulo and Rio Grande do Sul, is presented in TABLE 3. Because the number of tests from other states was too low (n ≤10), we could not include those data in the analysis; thus, we analysed patient data only from São Paulo and Rio Grande do Sul. We verified the homogeneity of the kappa values using the analysis with 1 degree of freedom (χ2HE=0.197) and found that the state-of-origin variable did not influence the degree of agreement between the results of the two methods.

DISCUSSION

Diagnostic methods for lactose intolerance include investigations of lactase enzyme activity in jejunal biopsies, genetic testing, the LTT, the hydrogen breath test[7] and the rapid test for lactase dosage in post bulbar duodenum samples[8]. The hydrogen breath test is considered the gold-standard test for the diagnosis of lactose malabsorption[16], and is therefore

TABLE 2. Association between genotyping (C/T -13.910 polymorphism) results and LTT results among 404 Brazilian individuals according to age group.

	<10 years old N=27		11-17 years old N=29		18 years or older N=348		Total
	LTTMA*	LTTGA**	LTTMA*	LTTGA**	LTTMA*	LTTGA**	
Genotype							
CC	6 (22.2%)	06 (22.2%)	08 (27.6%)	08 (27.6%)	132 (37.9%)	33 (9.5%)	193
CT or TT	0	15 (55.5%)	12 (41.4%)	01 (3.4%)	31 (8.9%)	152 (43.7%)	211
Total	6	21	20	9	163	185	404
Agreement	21 (77.8%)		20 (69.0%)		284 (81.6%)		

*LTTMA: lactose tolerance test with malabsorption; **LTTGA: lactose tolerance test with good absorption.

TABLE 3. Comparison of the association between genotyping (C/T -13.910 polymorphism) results and lactose tolerance test results among Brazilians from the states of Rio Grande do Sul and São Paulo.

State	Rio Grande do Sul (N=27)		São Paulo (N=361)	
	LTTMA*	LTTGA**	LTTMA*	LTTGA**
Genotype				
CC	8 (29.6%)	0	133 (36.8%)	43 (11.9%)
CT/TT	4 (14.8%)	15 (55,5%)	27 (7.5%)	158 (43.8%)
Total	**12**	**15**	**160**	**201**
Agreement	**23 (85.1%)**		**281 (80.6%)**	

*LTTMA: lactose tolerance test with malabsorption; **LTTGA: lactose tolerance test with good absorption.

the most studied in comparison with other tests in literature[17,18]. However LTT is simpler as it does not require a complex infrastructure[9]. Hence we chose LTT, rather than the hydrogen breath test, to assess the agreement between an established and broadly available method and the genetic test genotyping results. In fact, a recent study carried out in Spain with 430 adults found good agreement between hydrogen breath test results and LTT results and concluded that the LTT is a good alternative when the hydrogen breath test cannot be performed[19]. Furthermore, a meta-analysis comparing the C/T-13910 polymorphism genotyping results of Northern Europeans with the hydrogen breath test and LTT results, to determine the ability of these tests to predict the genotype-phenotype relationship, showed that the LTT had higher sensitivity and specificity (94% and 90%, respectively) than the hydrogen breath test (88% and 85%, respectively)[20].

In the present study, we found moderate agreement (κ=0.42) between results of genotyping and results of LTT. Concordant results between genotyping and LTT results was 80.4% (325/404) in our study in contrast to a study carried out in Sweden that found 94% (48/51)[12], in which the authors suggest that genotyping should be the test of choice test for adults of European descent[12]. The lower agreement found in our study may be associated with the presence of other polymorphisms in the Brazilian population. In 2002, Enattah et al. reported that the C/T -13910 polymorphism is involved in lactase persistence and that this polymorphism is largely found in Northern Europeans and their descendants[6]; however, another study showed that the frequency of this polymorphism is lower among East African and Arab populations[21].

Given the ancestry in the Brazilian population is diverse, we aimed to test the possible influence of ethnic diversity according to state of origin. Population genetic studies in Brazil have shown more individuals of European ancestry in the South, Southeast and Midwest regions; African ancestry is more frequent in the Northeast region comparing to Southeast region; and fewer individuals of indigenous ancestry in the South, Southeast and Northeast regions than in the Midwest and North regions[22]. This

diverse ancestry may be at least partially responsible for the 47/325 (14.5%) individuals with the CC genotype (lactase nonpersistence) and good lactose absorption, as they may have other polymorphisms, such as G/A-22018, which was not evaluated in this study. For example, in the Brazilian Japanese population, G/A-22018 allele is a better predictor of lactase persistence than the C/T-13910 allele[23]. Other Brazilian studies have also demonstrated an association between the G/A-22018 polymorphism and lactose absorption[24-26].

Although the state of origin did not influence the degree of agreement between test results, it is noteworthy that in Rio de Grande do Sul, a state with more individuals of European ancestry, all individuals with CC genotype showed malabsorption in the lactose tolerance test, suggesting that the C/T-13910 polymorphism is predominant in this state.

Another factor to be considered in individuals with the CC genotype (hypolactasia) and good lactose absorption is age, considering that the decrease in enzyme activity is progressive and the rate of this process is variable, with a mean age of onset between 5 and 7 years and a maximum impact between 30 and 40 years[27]. In this study, the rate of disagreement between the CC genotype and lactose malabsorption was higher in the <10 yo and 11–17 yo groups than in the ≥18 yo group; however, the difference was not significant.

A false-negative result in the LTT is another possible explanation for disagreement between CC genotyping results and LTT results. In individuals with false-negative LTT results, the increase in blood glucose may be due to changes in glucose metabolism and not due to lactose absorption[7]. Patients with fasting glucose >100 mg/dL were excluded to minimize the possibility of false-negative results, but such results can also occur in patients with altered glucose metabolism and normal fasting glucose[28].

Regarding disagreement found in individuals with the C/T or T/T genotypes (lactase persistence) who presented with lactose malabsorption (32/70; 40.5%), some factors, such as small intestinal bacterial overgrowth (SIBO), may be involved. In this situation, bacteria degrade lactose, preventing its absorption and consequent elevation of blood glucose levels. A recent study carried out in Hungary showed that individuals with SIBO had lower post lactose ingestion glycemia (P<0.001), and a positive LTT result was more frequent in this group[29].

Diseases that cause secondary lactose intolerance, such as celiac disease, can also lead to disagreement between test results. One study carried out with Italian adults using the hydrogen breath test showed that the frequency of celiac disease was significantly higher in patients with lactose malabsorption than in those without malabsorption[30]; celiac disease occurs due to a loss of intestinal mucosa integrity and secondary lactase deficiency resulting in lactose malabsorption[31].

In situations of accelerated intestinal transit, lactose quickly reaches the colon, which compromises its digestion and absorption in the small intestine, with a consequent false-positive result in the LTT[7].

CONCLUSION

Considering the moderate agreement between C/T-13910 polymorphism genotyping and LTT results ($\varkappa$=0.42) in the Brazilian population, we hypothesize that an analysis of other polymorphisms could be a strategy to improve the agreement between genotyping and established tests and think that additional studies should focus on exploring this approach.

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Authors' contribution

Cavichio MWE: data collection and results organization. Quaio CRD'AC: results organization and statistical analysis. Baratela WAR: analysis of the genetic part of the study. Oliveira PMC: critical review of manuscript. Tahan S: text writing.

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RESUMO – Contexto – O teste de tolerancia à lactose (TTL) é ampliamente utilizado por ser minimamente invasivo e de baixo custo, disponível na atenção primária e muito útil na prática clínica. Está bem estabelecido o polimorfismo C/T-13910 na persistência da lactase em populações caucasianas, mas não há estudos avaliando a concordância entre os resultados da genotipagem do polimorfismo C/T-13910 e do TTL no Brasil, onde a população é altamente miscigenada. **Objetivo** – Avaliar a concordância entre a presença do polimorfismo C/T-13910 e a má absorção nos resultados do TTL. **Métodos –** Análise retrospectiva de dados coletados de um laboratorio presente em vários estados brasileiros. Os resultados dos pacientes que realizaram um teste genético para intolerância à lactose (genotipagem do polimorfismo C/T-13910) e um TTL de abril de 2016 a fevereiro de 2019 foram analisados para avaliar a concordância entre os testes. Os grupos foram classificados de acordo com a idade (<10 anos; 10–17 anos, ≥18 anos) e estado de residência (São Paulo ou Rio Grande do Sul). **Resultados** – Entre os 404 pacientes avaliados, houve concordância entre os resultados de genotipagem e TTL em 325 (80,4%) pacientes e discordância em 79 (19,6%) pacientes (K=0,42 - concordância moderada). Em relação ao genótipo, 47 pacientes com genótipo C/C (não persistência de lactase) apresentaram TTL normal e 32 com genótipo C/T ou T/T (indicando persistência da lactase) apresentaram TTL anormal. A idade e o estado de residência (Rio Grande do Sul ou São Paulo) não afetaram a concordância entre os resultados dos exames. **Conclusão** – Considerando a concordância moderada entre a genotipagem do polimorfismo C/T-13910 e os resultados de TTL ($\varkappa$=0,42) na população brasileira, sugerimos que a análise de outros polimorfismos poderia ser uma estratégia para melhorar a concordância entre os testes.

Palavras-chave – Intolerância alimentar; lactose; teste genético; teste de tolerância à lactose.

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