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DNA polymorphisms associated with lactase persistence, self-perceived symptoms of lactose intolerance, milk and dairy consumption, and ancestry, in the Uruguayan population

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Abstract

Uruguay has one of the highest per capita milk intakes worldwide, even with a limited supply of lactose-free products; furthermore, the admixed nature of its population is well known, and various frequencies of lactase persistence (LP) are observed in the source populations. We aimed to contribute to the understanding of the relation between allelic variants associated with LP, milk consumption, digestive symptoms, and genetic ancestry in the Uruguayan population. Samples of saliva or peripheral blood were collected from 190 unrelated individuals from two regions of Uruguay, genotypes for polymorphic sites in a fragment within the LCT enhancer were determined and allelic frequencies calculated in all of them. Data were collected on frequency of milk and dairy consumption and self-reported symptoms in a subsample of 153 individuals. Biparental and maternal ancestry was determined by analyzing individual ancestry markers and mitochondrial DNA. Twenty-nine percentage of individuals reported symptoms attributed to the ingestion of fresh milk, with abdominal pain, bloating and flatulence being the most frequent. European LP-associated allele T-13910 showed a frequency of 33%, while other LP-associated alleles like G-13915 and T-14011 were observed in very low frequencies. Associations between self-reported symptoms, fresh milk intake, and C/T-13910 genotype were statistically significant. No evidence of association between genetic ancestry and C/T-13910 was found, although individuals carrying one T-13910 allele appeared to have more European ancestry. In conclusion, the main polymorphism capable of predicting lactose intolerance in Uruguayans is C/T-13910, although more studies are required to unravel the relation between genotype and lactase activity, especially in heterozygotes.

1 | INTRODUCTION

Lactase-phlorizin-hydrolase (LPH), or simply lactase, is the enteric enzyme responsible for the hydrolysis of lactose, the main carbohydrate of milk, being codified in humans by the lactase gene (LCT) located on chromosome 2 (2q21.3) (Amiri et al., 2015; Naim, 2001; Naim et al., 1991; Zecca et al., 1998). Approximately 70% of human beings experience a down regulation in lactase activity after weaning (Lomer et al., 2008). Lactase non persistence (LNP) in adults, the physiological decline in lactase activity, constitutes the most common trait worldwide and is often, but not always, related to lactose intolerance (LI). In the other hand, lactase persistence (LP), the ability to digest large amounts of lactose during adulthood owing to the maintenance of lactase activity, is prevalent in Europeans, Arabs and pastoralist populations of Africa (Campbell & Ranciaro, 2021; Swallow, 2003). Symptoms of LI usually include abdominal pain, bloating, flatulence acid stools, perianal erythema and diarrhea, and less commonly nausea, vomiting, constipation, anorexia and weight loss, being most of them driven by lactose colonic fermentation byproducts (Lomer et al., 2008; Toca et al., 2022; Ugidos-Rodríguez et al., 2018). Diverse factors remark the complex interrelations between symptomatology, physiology, microbiome and metabolome (Szilagyi, 2015; Usai-Satta et al., 2022).

The first single nucleotide polymorphism (SNP) associated with lactase phenotype was the transition C/T-13910 (rs4988235), located in intron 13 of the minichromosome maintenance complex component 6 gene (*MCM6*), upstream of the *LCT* starting codon. This SNP showed a complete co-segregation with the lactase phenotype, being T-13910, dominant (Enattah et al., 2002). The T-13910 allele exhibits its highest frequency in northwestern Europe, decreasing to the south and east of the continent, being the C/T-13910 genotype strongly associated with the distribution of AH phenotype (Ingram et al., 2009), and was introduced in two or more independent events in European populations in the last 12 000 years, reflecting a still ongoing effect of selection associated with dairy farming (Enattah et al., 2007, 2008).

The DNA sequence within intron 13 of *MCM6* is known to act as a specific enhancer of *LCT* expression (Lewinsky et al., 2005). In this intron, other SNPs like T/G-13915 (rs41380347) in Saudi Arabia or G/C-14010 (rs145946881), T/G-14009 (rs869051967), and C/G-13907 (rs41525747) in pastoralist populations of East Africa, have been associated with LP, showing evidence of ongoing positive selection and having been related with past events of domestication and spread of pastoralism (Campbell & Ranciaro, 2021; Enattah et al., 2008; Hassan et al., 2016; Ranciaro et al., 2014; Schlebusch et al., 2013; Tishkoff et al., 2007).

Latin American populations have different continental origins, mainly sub-Saharan Africans, Europeans, and Native Americans in different proportions, due to a continuous admixture process started 500 years ago (Galanter et al., 2012; Homburger et al., 2015; Salzano & Sans, 2014; Wang et al., 2008). In this context, diverse studies carried out in Brazil, Ecuador, Colombia, Peru, and Chile found the C/T-13910 SNP to be the most frequent polymorphism associated with LP. However, T-13910 allele frequency varies greatly depending on ethnic affiliation or genetic ancestry background of the population or individuals studied, being higher when European contribution increases (Fernández & Flores, 2014; Friedrich, Santos, et al., 2012; Guimarães Alves et al., 2021; Mattar et al., 2009; Mendoza Torres et al., 2012; Paz y Miño et al., 2016). Association between expected lactose digestion phenotype considering C/T-13910 genotype, and the effectively observed phenotype, was assessed in some studies in South America. Authors reported statistical association of phenotype, determined with lactose tolerance test (LTT) or hydrogen breath test (H_2BT), with expected phenotype in Colombian Caribbean and Ecuadorian populations, although with a modest agreement between them (Mendoza Torres et al., 2012; Paz y Miño et al., 2016). On the other hand, Montalvá et al. (2019) found a strong association of genotype C/T-13910 with lactose digestion phenotype, determined with LTT, in goat herders of central Chile. In spite of the strong genotype–phenotype association observed, in some Chilean populations there was no statistical difference in the milk intake frequency of genotypes carrying at least one T-13910 allele, inferred as LP phenotypes, and C-13910 homozygotes, inferred as LNP phenotype (Fernández et al., 2016; Montalvá et al., 2019).

Uruguayan population exhibits a predominantly European genetic background, with African and Native American contributions of 6%–9% and 10%–14% respectively (Bonilla et al., 2015; Hidalgo et al., 2005). Nevertheless, parental contributions are significantly different depending on the geographic region considered (Sans et al., 1997), socioeconomic status (Bonilla et al., 2015; Sans et al., 2021), and maternal or paternal genetic ancestry (Bertoni et al., 2005; Bonilla et al., 2004; Bravi et al., 1997; Gascue et al., 2005; Sans et al., 2002, 2006). The only study carried out about LI phenotype in Uruguay was performed using H_2BT (Maggi et al., 1987). They reported LNP (lactose malabsorption in the text) in children under 5 years old, increasing gradually with growth and reaching levels of 65% of LNP and 40% of LI in adult Uruguayans. Furthermore, they found significant differences in percentages of LNP and LI when "white"

and "black" participants were compared. Recently, Guimarães Alves et al. (2021) reported in a sample of 191 Uruguayans a frequency of the derived T-13910 allele of 35%, and a proportion of LP phenotype, considering T-13910 carriers, of 61%.

This article aims to contribute to the understanding of the relation of some genetic factors involved in lactose tolerance with milk and dairy products consumption in Uruguay, a Latin American country with one of the highest per capita milk consumption worldwide according to the MInisterio de Ganaderia y Agricultura (2016). We will be looking to find that C/T-13910 features a higher frequency when compared to other Latin American populations and lower than European populations, related with differences the proportions of European, African, and Native American ancestry among Uruguayan regions. Moreover, due to the fact of the population admixture, we will analyze alleles with other continental origins that have also been related to LP. We hope to find that individuals with these alleles will consume more fresh milk than individuals without them.

2 | MATERIALS AND METHODS

The sampling design was initially part of an ongoing project, the Human Genetic Diversity project of the CENUR Northeast (Tacuarembó), Universidad de la República, Uruguay (Udelar). In this context, samples of saliva or peripheral blood were obtained from 119 unrelated adult individuals living in the department of Tacuarembó. As that region presents one of the smallest contributions of European ancestry in the country, for analyzing the frequency of alleles related to LP as well as its relation with ancestry, we also included a random sample from the south, where European contribution is maximum (Bonilla et al., 2004, 2015; Sans et al., 1997, 2021). This sample was composed of 38 women, older than 45 years old, living in Montevideo and Maldonado, healthy controls part of a breast cancer case–control study (Bonilla et al., 2015). This last sample was considered regardless of their milk intake frequency or digestive symptoms background, since this information was not available. Sampling locations can be seen in Figure 1.

In order to better assess the relation between phenotype and genotype we increased the number of individuals who self-declared symptoms attributed to LI. For that purpose, a targeted sample of unrelated adults informing at least one digestive symptom associated with milk or dairy intake was considered. This sample included 10 individuals from Tacuarembó, enrolled in a hypertension study (Flores-Gutiérrez, 2019), and 23 from Montevideo.

All participants who agreed to participate in this study signed an informed consent based on a project approved by the Research Ethics Committee of the Facultad de Humanidades (FHCE), Udelar (number 121900-000087-14). All participants filled a questionnaire with information about sex, age, and place of birth; all except those of the breast cancer study also answered about the frequency of fresh milk and dairy products (cheese, cream cheese, pudding, yoghurt, and fermented milk) consumption in the last year, using the following predefined frequencies: daily (everyday), weekly (at least 1 day per week), monthly (at least once per month), rarely, and never.

In Montevideo, we extracted DNA from saliva samples using the saliva-adapted protocol of the Laboratorio de Antropología Biológica of the FHCE. Briefly, it consists of a cellular lysis step with Tris–HCL, EDTA, SDS and proteinase K, protein precipitation with ammonium acetate, DNA precipitation with isopropanol, and DNA elution with distilled water. A detailed description of DNA extraction methods used in Tacuarembó, and hypertension and breast cancer projects can be seen in Vega-Requena et al. (2020), Flores-Gutiérrez (2019), and Bonilla et al. (2015) respectively.

For identification of polymorphisms associated with LP, a fragment within the *LCT* enhancer, where most of the previously identified LP-associated variants are located, was amplified by polymerase chain reaction (PCR). A set of 28 samples were processed using primers and cycling conditions previously described (Coelho et al., 2009) which yield a PCR product of 359 bp. In order to improve PCR performance, we designed a new pair of primers to obtain a PCR product of 186 bp. Primer sequences are presented below and cycling conditions were the same described by Coelho et al. (2009) except for annealing temperature, which was set at 58°C:

Forward: (F): 5′-GGAGAGTTCCTTTGAGGCCA-3′
Reverse: (R): 5′-GCATTTGAGTGTAGTTGTTA-GACGG-3′

We also amplified a mitochondrial DNA (mtDNA) fragment comprising the hypervariable region I (HVR-I) (positions 16024–16400) using primers and cycling conditions as detailed by Sans and coworkers (Sans et al., 2011, 2012). All PCR amplifications were verified by 2% agarose electrophoresis, prepared for sequencing by incubation with exonuclease I and alkaline phosphatase at 37°C followed by enzyme inactivation at 85°C, and sequenced at Institut Pasteur of Montevideo.

All DNA sequences obtained were edited using Chromas 2.6.6 (http://www.technelysium.com.au/chromas.html) and BioEdit v.7.2.5 (Hall, 1999), and aligned

FIGURE 1 Map with the sampled localities: Tacuarembó, Maldonado, Montevideo

with CLUSTALW in MEGA v.6 (Tamura et al., 2013) using NC_000002.12 (NCBI) and the revised Cambridge reference sequence (rCRS; Andrews et al., 1999) as reference sequences for nuclear and mitochondrial DNA (nDNA and mtDNA) respectively. Allelic frequencies of polymorphic loci were obtained by direct counting and Hardy–Weinberg equilibrium (HWE) was evaluated with Arlequin v.3.5.2.2 (Excoffier & Lischer, 2010). To compare Tacuarembó with other European, African, American and Asian populations, we performed multidimensional scaling (MDS) with Python v3.9.0, from a Nei's D_a distance matrix calculated with POPTREE2 (Takezaki et al., 2010), using published allelic frequencies of six polymorphic loci associated with LP. For haplogroup assigning of mtDNA sequences we used the FosWIKI MITOMASTER platform (Center for Mitochondrial and Epigenomic Medicine, Children's Hospital, Philadelphia) and Haplogrep2 (V2.1.13) (Weissensteiner et al., 2016), in accordance with Phylotree 17 criteria (van Oven, 2015).

Purified DNA from a subset of samples was sent to the Centro Nacional de Genotipado of the Instituto de Salud Carlos III (CeGen-ISCIII), Universidad de Santiago de Compostela, Spain, to genotype a panel of 79 Ancestry Informative Markers (AIMs) selected from those previously used to estimate admixture in Latin American populations (Galanter et al., 2012). Genotypic frequencies of Europeans (Spain, Italy, CEPH-Utah) Africans (Luhya, Yoruba) and Native Americans (Quechua, Tepehuano, Zapoteca, Maya, Naymara, Nahua) were retrieved from 1000 Genomes Project (http://www.internationalgenome.org/data) or kindly ceded by Dr Raquel Cruz (CeGen-ISCIII). Finally, we estimated individual ancestry using STRUCTURE 2.3 (Pritchard et al., 2000).

To assess statistical association of inferred lactase phenotype (assuming dominance of the T-13910 allele) with observed phenotype, milk intake, dairy intake and maternal ancestry, we performed χ^2, Cramer's V, unweighted Cohen's Kappa and odds ratio tests.

Comparisons of bi-parental ancestry of both inferred phenotypes were done using a Wilcoxon's rank sum test. We performed all statistical tests with Stata®v.4 (Stata Corp. LLC). For instrumental purposes, we defined LI phenotype as the self-report of at least one clinical feature associated with milk or dairy intake, while participants reporting none of these features were classified as lactose tolerant (LT) phenotype.

3 | RESULTS

3.1 | LP-associated variants

We sequenced the previously described DNA genomic fragment in 116 residents of Tacuarembó, identifying three SNPs previously associated with LP: C/T-13910 (rs4988235), T/G-13915 (rs41380347) and C/T-14011 (rs4988233). The main polymorphism identified was the European C/T-13910 variant, with the derived allele T-13910 showing a frequency of 33% (Table 1). Both the G-13915 and T-14011 alleles appeared in single heterozygous individuals (allelic frequency of 0.4% each), who were homozygous for C-13910 ancestral allele. All these polymorphic loci were in HWE.

Thirty-three individuals, 23 living in Montevideo and 10 from the Tacuarembó hypertension study, selected as a targeted sample because they declared experiencing at least one symptom associated with milk or dairy intake, were also sequenced. The main LP polymorphism identified was C/T-13910, with a frequency of the T-13910 allele of 21%. One individual was found to be

TABLE 1 Summary of the C/T-13910 allele and genotype frequencies evaluated for three Uruguayan samples, associations of lactase genotype with phenotype, (combined Tacuarembó and targeted sample), frequencies of fresh milk and dairy consumption, and genetic ancestry (the last three only for Tacuarembó and considering CC-13910 homozygotes and T-13910 carriers genotypes)

Item	C/T-13910 genotype (%)			T-13910%	*p*
Population	*CC*	*CT*	*TT*		
Tacuarembó	52 (44.8)	51 (43.9)	13 (11.2)	33.2	.802
Targeted sample[a]	21 (63.6)	10 (30.3)	2 (6.1)	21.2	
Southern Uruguay[b]	20 (52.6)	15 (39.5)	3 (7.9)	27.6	
Phenotype					
Lactose intolerant	37 (64.9)	16 (28.1)	4 (7.0)	21.0	**.004**
Lactose tolerant	29 (36.3)	42 (52.5)	9 (11.3)	37.5	
Fresh milk consumption frequency					
Daily	16 (32.0)	34 (68.0)			**.005**
Weekly-monthly	8 (50.0)	8 (50.0)			
Rarely	8 (38.1)	13 (61.9)			
Never	15 (78.9)	4 (21.1)			
Dairy consumption frequency					
Daily	9 (30.0)	21 (70)			.09
Weekly-monthly	23 (56.1)	18 (43.9)			
Rarely-never	13 (43.3)	17 (56.7)			
AIMs Ancestry (%)					
European	77.7	83.6			.539
Native American	12.9	11.8			.779
African	9.4	4.6			.121
mtDNA Ancestry					
European	14 (43.8)	18 (56.2)			0.90
Native American	24 (48.0)	26 (52.0)			
African	7 (50.0)	7 (50.0)			

Note: Bold values denote statistical significance at the $p < 0.05$ level.

[a]Sample of individuals from Montevideo ($N = 23$) and Tacuarembó ($N = 10$) informing at least one digestive symptom associated with fresh milk consumption.

[b]Individuals from the South of the country, Montevideo ($N = 28$) and Maldonado ($N = 10$), who were part of a Breast Cancer case–control study (Bonilla et al., 2015). Probabilities calculated with Chi Square test, except genotype-population (Fisher's exact test) and AIMs ancestry percentage per genotypic group (Wilcoxon's rank sum test).

heterozygous for C/T-14011, being homozygous for the C-13910 allele. Finally, we analyzed the 38 sequences from individuals living in Montevideo or Maldonado (southern region) with unknown background both for milk intake and LI symptoms. The only LP polymorphism detected was C/T-13910, with a frequency of the T-13910 allele of 28%, slightly lower than that observed in Tacuarembó. Differences in genotypic frequencies of C/T-13910 among the three samples analyzed were not significant (Table 1).

The MDS performed to compare allelic frequencies of LP-related loci C/T-13910, G/C-14010, C/T-14011, T/G-13915, C/G-13907, and T/C-13913 (rs41456145) of other South American, European, African, Middle Eastern and Asian populations, with those obtained in Tacuarembó-Uruguay can be seen in Figure 2. As expected, Tacuarembó clustered with populations from Europe and South America, where C/T-13910 is the only or most frequent polymorphism observed, being more closely related with those who shared similar frequencies

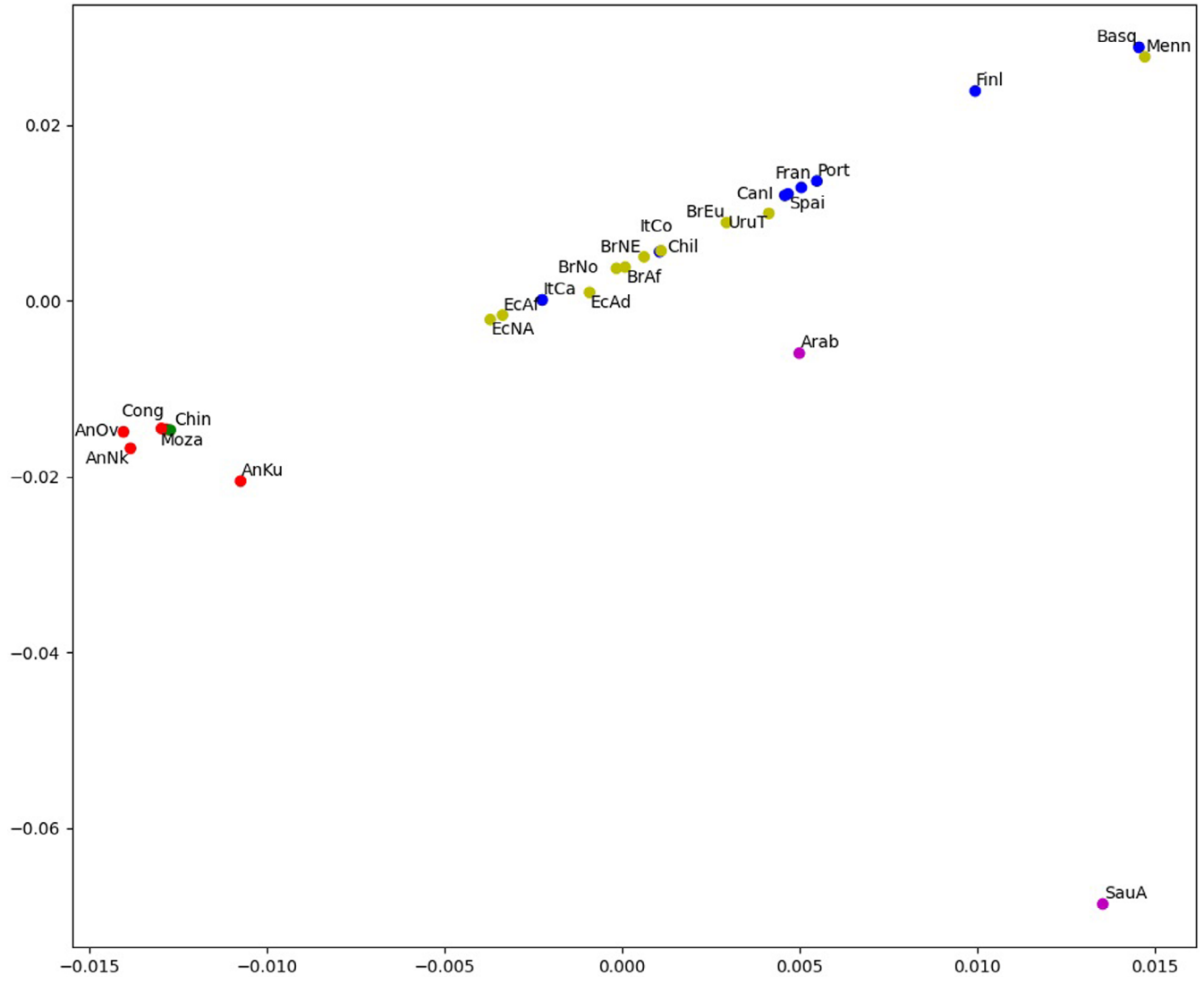

FIGURE 2 Multi-dimensional scaling (MDS) plot performed using a Nei's D_a distance matrix of allelic frequencies of six polymorphic loci associated with LP, from some populations of Europe, Asia, Africa and South America (see Table A1) and Tacuarembó-Uruguay. Red dots: African populations (AnOv, Angola-Ovimbundu; AnKu, Angola-Kuvale; AnNk, Angola-Nkhumbi; Cong, Congo-Bantu; Moza, Mozambique-Bantu). Green dot: (Chin, China-Han). Yellow dots: South American populations (EcNA, Ecuador-Native American; EcAf, Ecuador-Afroecuadorian; EcAd, Ecuador-Admixed; Chil, Chile-IV Region-Admixed; BrNo, Brazil-North; BrNE, Brazil-Northeast; BrAf, Brazil-South-Afrobrazilian; BrEu, Brazil-South-Eurobrazilian; Menn, Brazil-Mennonites; UruT, Uruguay-Tacuarembó). Blue dots: European populations (ItCa, Italy-Sardinia-Cases; ItCo, Italy-Sardinia-Controls; CanI, Canary Islands; Spai, Spain; Fran, France; Port, Portugal; Basq, Basques; Finl, Finland). Magenta dots: Middle East populations (Arab, Arabs-Middle East; SauA, Saudi Arabia)

of the T-13910 allele. On the other hand, populations where C/T-13910 polymorphism is rare or absent formed a second cluster, including African populations that feature low frequencies of the C-14010 allele, and populations where LP polymorphisms are absent. Arab populations appeared separate, owing to a high frequency of the G-13915 allele, especially in Saudi Arabia.

3.2 | Phenotype, genotype, and milk intake

Based on the data collected in the questionnaire, Tacuarembó residents were classified as LI observed phenotype ($N = 35$) or LT observed phenotype ($N = 84$) using the criteria described above. As it could be expected, fresh milk intake frequency differed markedly in these two groups ($\chi^2 = 33.3$, df $= 3$, $p < .0001$), with more than 60% of LT declaring a daily intake, while only 6% of LI reported a daily intake. In contrast with these findings, dairy product intake showed similar daily frequencies in both phenotypic groups ($\chi^2 = 2.38$, df $= 2$, $p = .30$), being this frequency slightly higher in the LT group (28.6%) compared with the LI group (25.7%).

The most frequent symptoms associated with milk or dairy consumption were abdominal pain ($N = 16$), bloating ($N = 13$) and flatulence ($N = 10$), in a total of 35 individuals of 69 analyzed. Other digestive symptoms not listed in the questionnaire were reported by seven participants, including heartburn, reflux, and unspecified enteric symptoms. Only one individual declared a systemic symptom associated with milk intake (hypotension). Finally, more than a half of individuals declaring symptoms ($N = 18$) reported only one symptom associated with milk or dairy consumption. Detailed data about the frequency of milk and dairy consumption and symptoms attributed to these can be seen in Table A2, for individuals for Tacuarembó as well as the ones with at list one symptom related to lactase (targeted sample).

In order to assess the relation of observed phenotype and C/T-13910 genotype, we included all the individuals from Tacuarembó and the targeted sample, whose lactose phenotype and CT-13910 genotype were determined. Then, we formed a LT group including 80 individuals, all from Tacuarembó, and a LI group, including 34 individuals from Tacuarembó and 23 from Montevideo. In each group we counted the three genotypes and compared its frequencies. While roughly two thirds of LI individuals presented a CC-13910 genotype, 63% of the LT group were heterozygous or presented homozygous TT-13910 genotypes ($\chi^2 = 11$, df $= 2$, $p = .0041$). Considering the proposed dominance of the T-13910 allele, we assessed significance, concordance, and odds ratio between genotype (CC or *T) and observed phenotype (LI or LT). Even when statistical significance of genotype/phenotype association was seen ($p = .0017$), concordance between inferred and observed phenotype was poor (unweighted Cohen's Kappa $= 0.28$). This is not surprising if we consider that observed phenotype was defined by self-perceived symptoms and not by determining lactase activity. Despite the bias inherent to this approach, we observed that individuals homozygous for the C-13910 allele had a threefold increase in probability of perceiving themselves as LI (OR $= 3.25$, 95% confidence interval 1.6–6.62).

Finally, assuming dominance of the T-13910 allele, we assessed if inferred phenotype was associated with the frequency of fresh milk intake in Tacuarembó. The group with inferred LNP phenotype presented a daily frequency of fresh milk intake of 32%, significantly lower than observed in the LP inferred phenotype group, which showed a daily intake frequency of 58% ($\chi^2 = 12.85$, df $= 3$, $p = .005$). Therefore, the pattern of fresh milk intake in Tacuarembó was notably impacted by C/T-13910 genotype, even though discrepancies between observed phenotype (LI or LT) and inferred phenotype (LNP or LP) were evidenced in this study.

3.3 | Genotype and genetic ancestry

From a random subset of 69 individuals of Tacuarembó, we estimated biparental genetic ancestry using the panel of AIMs previously described and setting in three ($k = 3$) the putative parental populations in STRUCTURE, in accordance to the ancestry of Uruguayan population (mostly of European, sub-Saharan African and Native American continental origins). European contribution was found to be predominant, with a mean of 80.4%, while mean Native American and African contributions were 12.7% and 6.9% respectively. We also observed a marked inter-individual variation in these parental contributions, with ranges 0.7%–99.2%, 0.3%–44.6%, and 0.1%–51.7% for European, Native American and African origin respectively.

To assess whether parental contribution was related to inferred lactase phenotype, we formed two groups, one including individuals carrying at least one T-13910 allele (inferred LP phenotype) and another with individuals with inferred LNP phenotype (CC-13910 genotype). The mean European contribution was slightly higher in the inferred LP phenotype group (83.6%) compared with the inferred LNP phenotype group (77.7%). The mean African contribution was higher in the inferred LNP phenotype group (9.4%) than in the group with inferred LP phenotype (4.6%), while Native American contribution

was very similar in both groups (12.9% and 11.8% respectively). However, there was no statistical support to suggest that the biparental ancestry background, inferred by nuclear DNA (nDNA-AIMs) analysis, of individuals carrying a T-13910 allele differs from the background of those who are homozygous for the ancestral allele (European: $p = .539$, Native American: $p = .779$, African: $p = .121$ respectively).

We also determined maternal genetic ancestry through mtDNA markers in 102 individuals from Tacuarembó, classifying their mtDNA haplogroups by continental origin. Native American haplogroups accounted for nearly half of the total, while European and African haplogroups were found in frequencies of 33% and 19% respectively. When comparing mtDNA haplogroup frequencies in relation to the inferred phenotypes, Native American, European and African haplogroups showed very similar frequencies in both groups; with LP inferred phenotype presenting a slightly higher European frequency than LNP inferred phenotype. In summary, we did not find evidence suggesting association of C/T-13910 genotype and maternal ancestry within the population of Tacuarembó ($\chi^2 = 0.21$, df = 2, $p = .90$).

All the information at the individual level can be seen in the Supporting Information.

4 | DISCUSSION

Here, we studied the association phenotype/genotype with LP, milk and dairy consumption and ancestry in a Uruguayan population for the first time. Several studies describe the Uruguayan population as having predominantly European origin, with modest Native American and African contributions (Bonilla et al., 2015; Hidalgo et al., 2005; Sans et al., 1997). Particularly, we have estimated in the population of Tacuarembó a mean of 80.4% of European contribution based on nDNA, and previously Bonilla et al. (2015) had estimated a mean of 77.1% for the whole country. Recently, Guimarães Alves et al. (2021) estimated 83.4% of European ancestry proportion for a sample of Uruguay, and reported a frequency of the LP associated allele (T-13910) of 35.3% in Uruguay, the highest among the American populations they have analyzed. Similar frequencies of T-13910 were reported in Euro-Brazilians from southern Brazil, while in other South American cosmopolitan populations, with smaller European contributions, T-13910 allele frequency ranged from 10% to 22%. The frequencies estimated for us were 33.2% in Tacuarembó and 27.6% in Montevideo, in agreement with the proposed introduction in the Americas of T-13910 by European immigrants since beginning of the colonization process.

The differences between Tacuarembó and Montevideo can be related with the parental populations. Tacuarembó was populated mostly by descendants of Portuguese, Brazilians, and Spaniards (Bonilla et al., 2004), and the frequency of the allele (33.2%) is slightly lower than the reported in the Iberian populations of Portugal, Spain and the Canary Islands where vary from 37% to 40%, and to 66% if Basques are considered (detailed data of populations, LP-associated allele frequencies and sources used can be seen in Table A1). Consequently, our findings are in agreement with the demographic background of the population of Tacuarembó. Interestingly, we obtained a T-13910 allele frequency of 27.8% in residents of the departments of Montevideo and Maldonado, in southern Uruguay, who showed a slightly higher European contribution than Tacuarembó (83.5%; Bonilla et al., 2015). Although lacking statistical support, this finding could be reflecting the higher contribution of Italian immigrants in the peopling of this region (Bresciano, 2010), considering that T-13910 allele frequency ranges from 6% to 18% in that country (see Table A1). A greater contribution of Italians could explain the lower frequency of this variant in southern Uruguay, even when more studies are required to ascertain this assumption.

The Arab variant G-13915 and the rare allele T-14011, both found in Tacuarembó with an allelic frequency of 0.4%, were previously reported at very low frequencies in Brazilians (Friedrich, Santos, et al., 2012; Guimarães Alves et al., 2021), while the first one was also reported in Ecuadorians (Paz y Miño et al., 2016). Variant G-13915 exhibits it highest frequency in Saudi Arabia (57%), with minor frequencies in other Middle East Arabs, North Africa, and East Africa (Table A1). Variant T-14011, found in many populations of Europe, India, and Iran, presents frequencies ranging from 1.5% in Swedish to 0.5% in Russians and Italians (Liebert et al., 2016). In our work, both LP-associated alleles were found in homozygous CC-13910 individuals, in accordance with the hypothesis of an independent origin of these variants (Enattah et al., 2008; Liebert et al., 2016).

Previously, Maggi et al. (1987) informed a prevalence of phenotype LI of 40% in a sample of Uruguayan adults who participated in a clinical trial were H_2BT was used to determine lactose digester status. Strikingly, only 29.6% of the Tacuarembó residents were classified as LI phenotype in our study, even when 44.8% of them showed an inferred LNP phenotype (CC-13910 genotype) under the assumption of dominance of the T-13910 allele. It is possible that the low frequency of LI found in our study could be due to the bias inherent to our approach, based on self-perceived symptoms associated with the frequency of fresh milk intake. Indeed, we observed that

phenotype (LI or LT) and inferred phenotype (genotype CC or *T) showed a poor concordance. This phenomenon had been reported in blind clinical trials that evidenced a low correlation between self-declared LI and clinical symptoms or H_2BT results (Deng et al., 2015; Yang et al., 2013). Some conditions, such as intolerance to dietary fermentable saccharides and polyols, giardiasis, intestinal bacterial overgrowth, inflammatory bowel disease, casein intolerance, and milk allergy, could lead to the manifestation of LI-like symptoms in non-hypolactasic individuals (Benhamou et al., 2009; Jianqin et al., 2016; Pal et al., 2015; Sicherer & Sampson, 2010; Szilagyi, 2015). On the other hand, adaptations in the intestinal microbiome, associated with a frequent lactose intake pattern, reduce the onset of LI symptoms and seem to have beneficial effects in LNP subjects (Deng et al., 2015; Misselwitz et al., 2019; Szilagyi, 2015), although the effects of dietary interventions on the intestinal microbiota are controversial (Smith et al., 2022). In our study, 15 out of 29 individuals with LP phenotype, but inferred LNP phenotype (CC-13910 genotype), declared a daily intake of fresh milk without noticing symptoms, which is probably explained by some extent of microbiome adaptation. Even when lactase activity was determined in intestinal biopsies, Leseva et al. (2018) found that the predictive value of genotype C/T-13910 was notably reduced in heterozygous individuals. These authors identified two differentially methylated positions in promoter and enhancer regions of *LCT* that showed a higher predictive value of phenotype than the genotype. In fact, most of individuals with an LP associated genotype in our study where heterozygous, so the findings reported by Leseva could be involved in the poor phenotype/genotype concordance found in our work.

Despite the bias and restrictions of our approach, we observed that phenotype, inferred through C/T-13910 genotype, was statistically associated with the frequency of fresh milk consumption in the population of Tacuarembó. Individuals inferred as LNP phenotype (CC-13910 genotype) declared a frequency of fresh milk consumption significantly lower than that declared by those inferred as LP phenotype (T*-13 910 genotype). These findings have been widely reported in populations of northwestern Europe (Bergholdt, Larsen, et al., 2018; Bergholdt, Nordestgaard, et al., 2018; Juhl et al., 2018; Pires-Hartwig & Davey Smith, 2018). Notwithstanding, a recent study using genotypic and phenotypic data from ~337 000 "white" British suggests that LP genotype has a small effect on milk consumption, even when it is statistically significant (Evershed et al., 2022). In South American populations, reports have been less clear. Studying Chilean populations, Fernández et al. (2016) found that milk intake was significantly higher in individuals carrying at least one copy of the T-13910 allele in populations of Easter Island and Santiago, while in the Fourth Region this association was not observed. In the same way; Montalvá et al. (2019) found non-significant association of both C/T-13910 genotype and H_2BT-determined phenotype with fresh milk intake in agro-pastoralist populations of central Chile.

Several studies carried out in admixed, African and Native American populations of south America have shown that the frequency of the T-13910 allele is markedly associated with the proportion of European ancestry in the population (Fernández et al., 2016; Fernández & Flores, 2014; Friedrich, Callegari-Jacques, et al., 2012; Friedrich, Santos, et al., 2012; Guimarães Alves et al., 2021; Mattar et al., 2009; Paz y Miño et al., 2016). In our study, we wanted to explore if C/T-13910 genotype and genetic biparental and maternal ancestry were statistically associated within the population of Tacuarembó. Considering the European origin of T-13910 allele, we expected that those individuals classified as LP inferred phenotype would exhibit a mean European contribution bigger than those with LNP inferred phenotype. As pointed out previously, our results do not show association between the proportions of genetic ancestry from European, African and Native American continental origins, and C/T-13910 genotype or inferred phenotype within the population of Tacuarembó. Montalvá et al. (2019) also found that the proportion of European ancestry estimated with AIMs did not differ significantly in groups inferred as LNP or LP in agro-pastoralist populations of central Chile. The most plausible explanation for this finding is the independent segregation of the different regions of genomic DNA where AIMs are located, and the *LCT* enhancer in Chromosome 2, running through again in every generation of admixture. Ten AIMs used in our study are located in Chromosome 2, being the region encompassing the *LCT* enhancer flanked by rs1567803 (2:100.726.556) and rs1196705 (2:149.362.708). Given the distance between those loci and C/T-13910 (2:135.851.076), we can safely assume that interactions in the segregation of these polymorphisms are not expected.

Given that we did not find association of genetic biparental ancestry with C/T-13910 genotype within the population of Tacuarembó, we expected that the same would happen with maternal ancestry through mtDNA. Indeed, biparental ancestry was not significantly different when we compared individuals with European and non-European maternal ancestry (data not shown). Biparental and maternal genetic ancestry tend to diverge in Uruguayan populations due to directional mating, leading to a higher proportion of Native American ancestry in the maternal line (Bonilla et al., 2004; Gascue

et al., 2005; Sans et al., 2006). On the other hand, paternal contribution, using specific polymorphisms located on the Y chromosome, showed a predominantly European male origin of Uruguayan populations (Bertoni et al., 2005; Bravi et al., 1997; Mut, 2019; Sans et al., 2002). In this context, the high frequency of Native American haplogroups found in Tacuarembó have been associated with an initial event of directional mating of European men and non-European, mostly Native American, women, followed by a fast demographic expansion via extra-regional, mainly European, migration of men (Bertoni et al., 2005; Bonilla et al., 2004).

5 | CONCLUSIONS

The main polymorphism associated with LP identified in Uruguayans was transition C/T-13910 (rs4988235), showing a frequency of the T-13910 derived allele of 33% in residents of Tacuarembó and 28% in the south. Very similar frequencies of this allele were reported in other predominantly European cosmopolitan populations of South America and slightly higher in Iberian populations, where most of Tacuarembó residents have their European ancestors.

Despite the bias related with phenotype classification based on self-reported symptoms, which lead to a poor phenotype–genotype concordance, fresh milk intake was significantly impacted by C/T-13910 genotype. Therefore, individuals with at least one T-13910 allele showed a higher intake of fresh milk, in accordance with a model of dominance of the LP-associated allele. On the other hand, we did not find association of the pattern of dairy product intake with genotype, as expected considering the lower contents of lactose in these products.

In this study, we assessed the association of C/T-13910 genotype with genetic ancestry at within-population level. Neither biparental ancestry nor maternal ancestry was significantly different in individuals carrying at least one copy of the T-13910 allele and those homozygous for the ancestral allele.

We also found two LP-associated polymorphisms, C/T-14011 (rs4988233) and T/G-13915 (rs41380347), both appearing in heterozygous genotypes and with a derived allele frequency of 0.4%. Individuals carrying this SNPs presented a homozygous CC-13910 genotype and were classified as LI phenotype.

Finally, we consider that this work may contribute to future research in the field of Mendelian randomized studies using the C/T-13910 polymorphism as a proxy of milk intake, evaluating its association with several pathologies or conditions prevalent in Uruguay.

AUTHOR CONTRIBUTIONS

Conceptualization; *methodology*, *writing original draft*; *writing-review and editing*: Raul German Negro and Mónica Sans. *Sampling*, *laboratory analyses*, *data curation*: Raúl Germán Negro Gaudin, Gonzalo Figueiro, Sara Flores-Gutiérrez, Patricia Mut, Yasser Vega-Requena, Lorena Luna-Andrada, Elizabeth Ackermann, Pedro C. Hidalgo. *Laboratory analyses*, *data curation*: Angel Carracedo and María Torres. *Funding acquisition*: Angel Carracedo and Mónica Sans. *Supervision*: Mónica Sans.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in Supporting Information.

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SUPPORTING INFORMATION

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TABLE A1 Populations, LP-associated allele frequencies and papers where data were obtained

Population	*N*	LP-associated allele frequency						References
		T-13910	G-13907	C-13913	G-13915	C-14010	T-14011	
Saudi Arabia[a]	248	0.004	0.008	0	0.570	0	0	Enattah et al., 2008
Arabs-Middle East[a]	40	0.130	0	0	0.105	0	0	Enattah et al., 2008
Morocco	24	0.210	0	0	0.083	0	0	Enattah et al., 2008
Saharawi	22	0.230	0	0	0.182	0	0	Enattah et al., 2008
Sudan-Beja	17	0	0.206	0	0.176	0	0	Ranciaro et al., 2014
Kenya-Afroasiatic	217	0	0.041	0	0.104	0	0	Ranciaro et al., 2014
Congo-Bantu[a]	11	0	0	0	0	0	0	Breton et al., 2014
Mozambique-Bantu[a]	111	0	0	0	0	0	0	Coelho et al., 2009
Angola-Ovimbundu[a]	96	0	0	0	0	0.010	0	Coelho et al., 2009
Angola-Nkhumbi[a]	153	0	0	0	0	0.030	0	Coelho et al., 2009
Angola-Kuvale[a]	54	0	0	0	0	0.060	0	Coelho et al., 2009
Italy-Central-South-Controls	1000	0.110	-	-	-	-	-	Santonocito et al., 2015
Italy/Central-South-Cases	1426	0.117	-	-	-	-	-	Santonocito et al., 2015
Italy South	100	0.055	-	-	-	-	-	Enattah et al., 2007
Italy	605	0.151	-	-	-	-	-	Sacerdote et al., 2007
Italy-Sardinia-Cases[a]	120	0.108	0	0	0	0	0	Obinu et al., 2009
Italy-Sardinia-Controls[a]	120	0.217	0	0	0	0	0	Obinu et al., 2009
Canary Islands[a]	551	0.365	0	0	0	0	0	Almon et al., 2012
Spain[a]	221	0.370	0	0	0	0	0	Rasinpera et al., 2005
Portugal[a]	447	0.402	0	0	0	0	0	Manco et al., 2017
Basques[a]	85	0.66	0	0	0	0	0	Enattah et al., 2007
China-Han[a]	100	0	0	0	0	0	0	Enattah et al., 2007
Finland[a]	1876	0.575	0	0	0	0	0	Enattah et al., 2008
France[a]	17	0.340	0	0	0	0	0	Enattah et al., 2007
Brazil-South-Afrobrazilian[a]	182	0.184	0	0	0	0.005	0	Friedrich, Santos, et al., 2012
Brazil-Afrobrazilian-Curitiba	241	0.187	-	-	0.002	-	0.002	Guimarães Alves et al., 2021
Brazil-South-Eurobrazilian[a]	337	0.295	0	0	0	0	0	Friedrich, Santos, et al., 2012
Brazil-Southeast-Bambuí	950	0.320	-	-	-	-	-	Guimarães Alves et al., 2021
Brazil-Salvador	1244	0.178	-	-	-	-	-	Guimarães Alves et al., 2021
Brazil-Northeast[a]	262	0.204	0	0	0	0	0.011	Friedrich, Santos, et al., 2012
Brazil-North[a]	200	0.175	0	0	0	0	0.005	Friedrich, Santos, et al., 2012
Brazil-Mennonites[a]	151	0.650	0	0	0	0	0	Boschmann et al., 2016
Chile-Araucanía-Mapuche	29	0.050	-	-	-	-	-	Fernández et al., 2014
Chile-Araucanía-Admixed	115	0.125	-	-	-	-	-	Fernández et al., 2014
Chile-IV Region-Admixed[a]	437	0.220	0	0	0	0	0	Fernández et al., 2016
Ecuador-Admixed[a]	488	0.137	0	0.002	0.005	0	0	Paz y Miño et al., 2016
Ecuador-Native American[a]	128	0.074	0	0	0	0	0	Paz y Miño et al., 2016
Ecuador-Afroecuadorians[a]	125	0.080	0	0	0	0	0	
Colombia-Caribbean	128	0.100	-	-	-	-	-	Mendoza Torres et al., 2012
Peru-Iquitos	30	0.058	-	-	-	-	-	Guimarães Alves et al., 2021
Colombia-Bogotá	23	0.283	-	-	-	-	-	Guimarães Alves et al., 2021

[a]Population included in the multidimensional scaling plot.

TABLE A2 Data about frequency of milk and dairy consumption, self-declared digestive symptoms, biparental (AIMs) and mitochondrial (mtDNA) genetic ancestry, in the samples used in this study

Frequency milk consumption	Tacuarembó *N* (%)	Targeted sample *N* (%)[a]
Daily	54 (45.4)	7 (20.6)
Weekly	16 (13.4)	3 (8.8)
Monthly	3 (2.5)	2 (5.9)
Rare	23 (19.3)	4 (11.8)
Never	23 (19.3)	18 (52.9)
Total	119 (100)	34 (100)
Frequency dairy consumption		
Daily	33 (28.2)	12 (35.3)
Weekly	40 (34.2)	8 (23.5)
Monthly	3 (2.6)	1 (2.9)
Rare	38 (32.5)	10 (29.4)
Never	3 (2.6)	3 (8.8)
Total	117 (100)	34 (100)
Digestive symptoms[b]		
Abdominal pain	16 (45.7)	20 (58.8)
Bloating	13 (37.1)	22 (64.7)
Flatulence	10 (28.6)	10 (29.4)
Diarrhea	7 (20.0)	14 (41.2)
Nausea/Vomiting	4 (11.4)	4 (11.8)
Constipation	8 (22.9)	1 (2.9)
Other/non specified	7 (20.0)	7 (20.6)
Total individuals	35	34
Genetic ancestry (AIMs)[c]		
European	80.4	93.3
African	6.9	2.5
Native American	12.7	4.2
Total individuals	69	14[d]
Mitochondrial ancestry		
European	34 (33.3)	17 (73.9)
African	19 (18.7)	0
Native American	49 (48.0)	6 (26.1)
Total	102 (100)	23 (100)[4]

[a]Sample of individuals from Montevideo and Tacuarembó informing at least one symptom associated with fresh milk consumption.
[b]Frequency of self-reported symptoms from individuals who relate those with milk consumption. Individuals can report more than one symptom.
[c]Mean proportion (%) of individual continental ancestry.
[d]Only include individuals from Montevideo.