Research paper

Association of *LCT* -13910C>T polymorphism and hip fracture in a cohort of older adult population from Northern Spain

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The corrections made in this section will be reviewed and approved by a journal production editor.

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Abstract

Hip fracture is a common health problem very frequent in the older adult population and is associated with significant morbidity, mortality, and societal costs. There are several factors that increase the risk of suffering a hip fracture, however, the effect of genetic lactase non-persistence is not clear-cut yet. For this reason, we investigated if the *LCT* -13910C>T polymorphism is a potential risk factor for osteoporotic hip fractures in older adult people from the Northern Spain population. A total of 740 individuals were included in this study. Of them, 364 belonged to the group of patients whit osteoporotic hip fracture while the control group consisted of 376 individuals without hip fracture. The genotypes for the *LCT* -13910C>T polymorphism were analyzed by using polymerase chain reaction and high resolution melting. The prevalence of the CC genotype, which is related to lactase non-persistence, did not differ significantly in both groups. Likewise, no differences were observed between groups when they were compared with regard to the C or the T allele, or when they were analyzed considering gender. Additionally, our results were compared with those obtained in a control group of 207 nonagenarian individuals originally from Northern Spain and no differences were observed. In conclusion, no significant association was observed between the *LCT* -13910C>T polymorphism and the risk for suffering hip fracture in the older adult population of Northern Spain.

Abbreviations: BMD, bone mineral density; Ca, calcium; K, potassium; *LCT*, *lactase* gene; LP, Lactase persistence; LNP, Lactase non-persistence; Mg, magnesium; OHF, osteoporotic hip fracture; ORs, odds ratios; P, phosphorus; Zn, zinc

Keywords: Hip fracture; Lactase persistence; Lactase non-persistence; Genotyping; PCR-HRM; Elderly people

1 Introduction

[Instruction: When I view the final pdf version, the abstract and introduction sections are not separated.]Hip fracture is a common health problem that increases over the years due to population aging (Hernández et al., 2006). In Spain, >90% of hip fractures occur in over 65 years old population, with an average age of 79 years and happening 74% of them in women, in which hip fracture has an incidence 2.6 times higher than in men (Alvarez-Nebreda et al., 2008). There are several factors that increase the risk of suffering a hip fracture, most of them influencing the bone mineral density (BMD). Age over 65 years, family history of fractures or osteoporosis, female sex, low socioeconomic status, and prior hip fractures are nonmodifiable factors influencing the risk of hip fractures. On the other hand, there are also modifiable factors, such as low BMD, falls, reduced physical activity, vitamin D deficiency, low calcium intake, excessive alcohol intake, eating disorders, low body mass index, as well as the use of some medications that decrease BMD (e.g.

cortisone, diuretics) (LeBlanc et al., 2014). Therefore, hip fractures are common injuries, especially in older adult women who often have osteoporosis and multiple comorbidities.

Dairy products provide bone-beneficial nutrients, such as protein, calcium (Ca), magnesium (Mg), potassium (K), zinc (Zn), and phosphorus (P) in the adult diet (van den Heuvel and Steijns, 2018). However, the relevance of dairy products for the prevention of osteoporotic fractures is still a matter of scientific debate as some large prospective studies have suggested that increased milk consumption during adolescence or adult life may be associated with a higher (future) hip fracture risk (Weaver et al., 2016), but there are others indicating that at very high age osteoporotic fracture risk decreases with a higher milk intake (van den Heuvel and Steijns, 2018).

Dairy intake in adult life is related to lactase persistence which is associated with noncoding variations in the *MCM6* gene on chromosome 2, located upstream of the *lactase* gene (*LCT*) in a region that appears to act as a *cis*-element capable of enhancing differential transcriptional activation of the *lactase* promoter (OMIM 223,100 entry) (Olds and Sibley, 2003). Primary adult lactase deficiency is a common autosomal recessive condition that produces a physiological decline in lactase phlorizin hydrolase activity (named in the present study as LCT) after weaning. Without this enzyme, the lactose cannot be hydrolyzed in glucose and galactose in the small intestine. In consequence, the lactose is fermented in the colon by bacteria, potentially causing discomfort, bloating, and flatulence (Montgomery et al., 1991). This condition is commonly described as lactose intolerance.

In 2002, Enattah *et al.* using linkage disequilibrium and haplotype analysis reported the identification of genetic polymorphisms located in the *MCM6* gene that are associated with *LCT* expression (Enattah et al., 2002). One of these polymorphisms is the *LCT* -13910C>T (rs4988235) that is located in the intron 13 of the *MCM6* gene and was associated with lactase expression in European populations (Enattah et al., 2002). The -13910T variant creates a new binding site for transcription factor Oct-1, increasing transcriptional activation of the *LCT* promoter (Olds and Sibley, 2003). Therefore, individuals with genotypes TT or TC show LCT persistence (LP) in adulthood, while individuals with the CC genotype lack this enzyme.

In recent years, several polymorphisms have been evaluated since they could present a significant relationship with different diseases. Some examples are described in (Zhuo et al., 2020a, 2020b, 2018c). In this sense, numerous studies have reported the significant role of the *LCT* -13910C>T polymorphism in osteoporosis, where the CC genotype has been associated with the decrease of BMD at different sites and the predisposition to bone fractures (Obermayer-Pietsch et al., 2004; Enattah et al., 2005; Laaksonen et al., 2009; Bácsi et al., 2009; Marozik et al., 2013). However, this is still a matter of debate since, in other studies, non-significant relationship was found (Enattah et al., 2004, 2005; Obermayer-Pietsch et al., 2007; Gugatschka et al., 2007; Esterle et al., 2009; Kull et al., 2009; Smith et al., 2009; Agueda et al., 2010; Koek et al., 2010; Tolonen et al., 2011; Bergholdt et al., 2018). Table 1 summarizes the studies that assessed *LCT* -13910C>T polymorphism with BMD and/or fracture risk in Europe. Although sixteen studies have been performed, only one of them focuses on people older than 80 years (Enattah et al., 2005).

Table 1

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Overview of studies where *LCT* -13910C>T polymorphism was assessed in relation to osteoporosis, BMD, and/or fracture risk in Europe. LP: LAC persistence; LNP: LAC non-persistence.

Study	Population	Cohort (n)	LP (TT + TC) frequency	LNP (CC) frequency	Association with osteoporosis, BMD, and/or fracture risk
(Obermayer-Pietsch et al., 2004)	Austria	Postmenopausal women (n = 258)	76.36	23.64	The LNP genotype is associated with reduced bone density, and they predispose to bone fractures in postmenopausal women
(Enattah et al., 2004)	Finland	Young men (n = 234)	82.91	17.09	No
(Enattah et al., 2005)	Finland	Postmenopausal women (n = 453)	82.12	17.88	No
(Enattah et al., 2005)	Finland	Very old age peple (>85) (n = 483)	84.89	15.11	Genetic LNP seems to be an independent risk factor for hip and wrist fracture in people aged 85 and older
(Obermayer-Pietsch et al., 2007)	Austria	Postmenopausal women (n = 73)	56.16	43.84	No
(Gugatschka	Austria	Men with a mean age	73	27	No

et al., 2007)		of 61 ± 9 yr (n = 239)			
(Laaksonen et al., 2009)	Finland	Baseline men (n = 126)	88	12	Genetic LNP may be associated with increased risk for greater bone loss in males in young adulthood
		Baseline women (n = 167)	85	15	
		Men follow-up after 12 years (n = 62)	90	10	
		Women follow-up after 12 years (n = 83)	84	16	
(Esterle et al., 2009)	France	Adolescent girls and young women (n = 173)	67	33	No
(Bácsi et al., 2009)	Hungary	Postmenopausal women (n = 595)	59.33	40.67	They report an association between LNP genotype and BMD
(Kull et al., 2009)	Estonia	Men and women from Northern Estonia (n = 356)	75.56	24.44	No
(Smith et al., 2009)	United Kingdom	Women ages 60 to 79 (n = 3344)	93.2	6.8	No
(Agueda et al., 2010)	Spain	Postmenopausal women (n = 964)	60.42	39.58	No
(Koek et al., 2010)	Netherlands	Men of Rotterdam study aged ≥ 55 yr (n = 2590)	89	11	No
		Women of Rotterdam study aged ≥ 55 yr (n = 3777)	90	10	
		Men of LASA population aged 55 to 85 yr (n = 415)	91	9	
		Women of LASA population aged 55 to 85 yr (n = 429)	89	11	
(Tolonen et al., 2011)	Finland	Men ages 31 to 46 (n = 669)	84.45	15.55	No
		Women ages 31 to 46 (n = 882)	83.67	16.33	
(Marozik et al., 2013)	Belarusian	Postmenopausal osteoporosis women (n = 54)	59.30	40.70	The LNP genotype showed a statistically significant association with postmenopausal osteoporosis
		Postmenopausal control group (n = 70)	81.80	18.20	
(Bergholdt et al., 2018)	Denmark	Individuals of Danish descent aged ≥ 20 years (n = 97811)	Data not shown	Data not shown	No

In view of this, the aim of the present study was to investigate the role of *LCT* -13910C>T polymorphism as a potential risk factor for osteoporotic hip fracture (OHF) in a cohort of elderly population (older than 80) from Northern Spain.

2 Materials and methods

2.1 Samples

In the present study, DNA samples of 740 individuals from Cantabria (North of Spain) were analyzed after approval by the Ethics Committee in Clinical Research of Cantabria (FIS PI060034) and by the Ethics Committee in Research with Drugs of Euskadi (PI2018022). Written informed consent was obtained from the patients, or legal representatives, in accordance with the declaration of Helsinki. The samples were classified into two groups: cases and controls. The case group consisted of 364 patients with OHF. The mean age of this group was 82 ± 9 yr. The inclusion criteria were OHF in the absence of other diseases (cancer, connective diseases, etc.) or treatments (corticosteroids, antiepileptic drugs, etc.) causing secondary osteoporosis. Furthermore, individuals with hip fractures caused by high-energy trauma were

excluded. On the other hand, the control group was composed of 376 healthy individuals without osteoporotic fractures in their medical history. The mean age of this group was 69 ± 7 yr. In addition, *LTC* -13910C>T polymorphism was analyzed in 207 nonagenarian individuals (95 ± 3 yr) living in senior nursing homes and originally from Northern Spain (Cantabria, Basque Country, and Asturias). Individuals with a history of clinical fragility fractures or joint replacement surgery due to osteoarthritis were excluded.

Genomic DNA was extracted by the Service of Internal Medicine of the Marqués de Valdecilla University Hospital (Cantabria, Spain) from 200 μL of peripheral blood by using commercially available methods (Qiagen or GE Q4 Healthcare) and resuspended in 50 μL of TE buffer. The DNA obtained was quantified using the NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA; TFS) and diluted to 1 ng/μL concentration. Subsequent analyses were carried out at the BIOMICs Research Group (Alava, Spain).

2.2 Real-time PCR amplification and HRM analysis

The *LCT* -13910C>T (rs4988235) polymorphism was genotyped by PCR-HRM. A fragment of 159 bp was amplified using 5′-TCACGTCATAGTTTATAGAGTGC-3′ as forward primer and 5′-AGGAGGAGAGTTCCTTTGAG-3′ as reverse primer. Primers were designed with PerlPrimer software v.1.1.21 (Marshall, 2004). The PCR-HRM analysis was performed on a C1000 Thermal Cycler coupled to CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, California, USA). Reactions were carried out in a final volume of 5 μL and contained: 0.5 μL of each forward and reverse primers (1 μM), 2.5 μL of SsoFast EvaGreen Supermix (Bio-Rad), and 1.5 μL of DNA template at 1 ng/μL. Real-time PCR conditions were: an initial denaturalization at 98 °C for 2 min, followed by 40 cycles at 98 °C for 10 s and 62 °C for 30 s. The melting curve was from 65 °C to 95 °C with an increment of 0.2 °C/sec. For each PCR-HRM reaction, three PCR reference controls (TT, TC, and CC genotypes) were included in duplicate. Melting profiles were analyzed with the Bio-Rad Precision Melt Analysis Software v1.0 (Bio-Rad).

2.3 Statistical analysis

The sample size required to achieve statistically significant associations was calculated using the Power for Genetic Associations (PGA) package in Matlab (available in https://dceg.cancer.gov/tools/design/pga). The parameters used were as follows: a power of 90%, an α level of 5%, LCT non-persistence (LNP) prevalence of 29% (Storhaug et al., 2017), the LNP frequency obtained in the present study, a case/control ratio of 1, and relative risk of 1.38 obtained in the present study.

Allele and genotype frequencies, as well as Hardy-Weinberg equilibrium, were calculated with Arlequin software v.3.5 (Excoffier and Lischer, 2010). The differences between controls and cases were analyzed by Pearson's chi-square (χ^2) test. The odds ratios (ORs) with 95% confidence intervals were also calculated. For both estimations, IBM SPSS Statistics 25.0 (SPSS Inc., IBM Corporation, NY, USA) software was used. P-values < 0.05 were considered statistically significant.

3 Results

A total of 364 patients with OHF were included in this study. The control group consisted of 376 individuals without OHF. DNA samples from all of them were analyzed by PCR-HRM to determine the *LCT* -13910C>T genotype (Fig. 1). This technique allows the identification of variations in the DNA sequence based on the analysis of the melting curve of the previously amplified DNA fragments. In this sense, the melting curve of each sample is compared with the obtained by the reference controls, i.e. samples with known genotype, enabling genotyping.

Fig. 1

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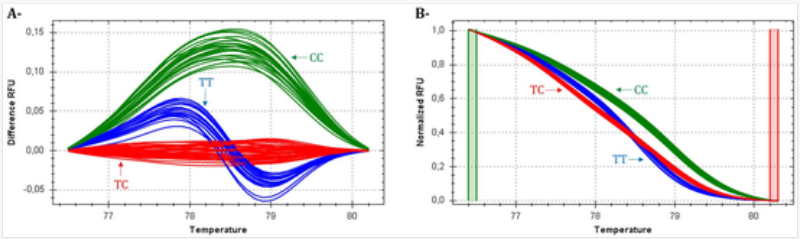

Example of melting curves obtained by PCR-HRM for the *LCT* -13910C>T polymorphism. In this run, 83 individuals from the control group and six reference controls (two for each genotype) were analyzed. Each color curve represents a different genotype: blue the TT genotype, red the TC genotype, and green the CC genotype. A- Difference curve. B- Normalized melt curve. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Individuals with the TT and TC genotypes maintain the LP in adulthood, unlike individuals with the CC genotype who lack this capacity. In the group of patients with OHF, the percentage of individuals with LP was 78.57% (TT = 27.20% and TC = 51.37%), while 21.43% showed an LNP genotype (Table 2). On the other hand, in the control group, 72.61% (TT = 25.80% and TC = 46.81%) of them showed LP while 27.39% of individuals lacked lactase in adulthood. The genotypic distribution of both groups followed the Hardy-Weinberg equilibrium ($P > 0.05$ in both cases) (Table 2).

Table 2

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Allele and genotype frequencies, and Hardy-Weinberg equilibrium and χ^2 test and the ORs for the patients with OHF and controls.

	Frequency of genotypes and alleles and SD				
Genotypes/Alleles	**Patients with OHF (n = 364)**	**Controls (n = 376)**	χ^2	***P*-value**	**OR (95% CI)**
TT + TC	0.786 ± 0.022	0.726 ± 0.023	3.562	0.059	1.383 (0.987–1.939)
CC	0.214 ± 0.022	0.274 ± 0.023			
T	0.529 ± 0.019	0.492 ± 0.018	2.007	0.157	1.159 (0.945–1.421)
C	0.471 ± 0.019	0.508 ± 0.018			
Hardy-Weinberg equilibrium (*P*-value)	0.601	0.218			

A PGA sample size analysis was performed to ensure that this study reaches sufficient statistical power to identify significant associations. The minimum required number of balanced case-control samples to obtain 90% of statistical power was between 330 and 335 for a risk of 1.38. Therefore, the number of case-control samples in this study was enough.

No statistically significant differences in the frequencies of LP/LNP were detected between the patients who had an OHF and those of the control group, i.e. individuals without OHF ($P = 0.059$). In the same way, no differences were found between the groups when they were compared regarding the C or the T allele ($P = 0.157$). The ORs were not significant in all cases (Table 2).

Finally, we divided our cohort sample considering sex (males and females) in order to determine if there were differences between the *LCT* -13910C>T polymorphism and case/control groups for the same sex. However, no differences were found in both sexes ($P > 0.05$ in all cases) (Table 3).

Table 3

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Allele and genotype frequencies, and the χ^2 test in males and females from the case and control groups.

	Frequency of genotypes and alleles and SD on males ~~and SD~~				Frequency of genotypes and alleles and SD on females ~~and SD~~			
Genotypes/Alleles	**Patients with OHF (n = 65)**	**Controls (n = 112)**	χ^2	***P*-value**	**Patients with OHF (n = 299)**	**Controls (n = 264)**	χ^2	***P*-value**
TT + TC	0.815 ± 0.048	0.750 ± 0.041	1.005	0.316	0.779 ± 0.024	0.716 ± 0.028	2.998	0.083
CC	0.185 ± 0.048	0.250 ± 0.041			0.221 ± 0.024	0.284 ± 0.028		
T	0.554 ± 0.044	0.540 ± 0.033	0.062	0.803	0.523 ± 0.020	0.472 ± 0.022	3.012	0.083
C	0.446 ± 0.044	0.460 ± 0.033			0.477 ± 0.020	0.528 ± 0.022		

On the other hand, data about the *LCT* -13910C>T polymorphism in nonagenarian individuals showed that 77.29% (TT = 25.12% and TC = 52.17%) of them presented LP while 22.71% had LNP genotype (Table 4). These results were compared with the obtained by the older adult group of patients with OHF analyzed in the present study. No statistically significant differences were found between both groups (Table 4). Additionally, the case group was divided into three according to the patient's age (≥80 years old, ≥85 years old, and ≥90 years old) and the comparisons with the nonagenarian group were performed again. However, no differences were found in all cases (Supplemental Table S1).

Table 4

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Data about the allele and genotype frequencies and χ^2 tests for the nonagenarian controls and the patients with OHF analyzed in the present study.

	Frequency of genotypes and alleles and SD			
Genotypes/Alleles	**Nonagenarian controls (n = 207)**	**Patients with OHF (n = 364)**	χ^2	***P*-value**
TT + TC	0.773 ± 0.029	0.786 ± 0.022	0.126	0.723
CC	0.227 ± 0.029	0.214 ± 0.022		
T	0.512 ± 0.025	0.529 ± 0.019	0.297	0.585
C	0.488 ± 0.025	0.471 ± 0.019		

4 Discussion

There are well-known risk factors related to osteoporosis fractures, such as age, gender, race, geographical region, diet, lifestyle, hormonal status, BMD, bone quality, body mass index, and medical comorbidities (Dontas and Yiannakopoulos, 2007). In contrast, the effect of other factors, like genetic LNP, is not clear-cut yet.

In the present study, we investigated if differences with regard to *LCT* -13910C>T polymorphism could be found between 364 patients with OHF and 376 control individuals, all of them from Cantabria (Northern Spain), being the patients an older adult sample with 82 ± 9 years. These sample sizes were enough to obtain a statistical power greater than 90%.

The *LCT* -13910C>T polymorphism was studied since it was found to be related to LP in the European populations (Enattah et al., 2002) and is being widely used to determine this condition. The LP genotypes (TT and TC) were identified in 78.57% and 72.61% of the individuals from the case and control groups, respectively. This high frequency of LP is consistent with the values established for populations in central-western Europe (Gerbault et al., 2011). On the other hand, the frequency of CC genotype was 21.43% in patients with OHF and 27.39% in the control group. Contrary to expectation, the frequency of LNP was higher in the control group, i.e. individuals without OHF. This could be due to the age difference between both groups (82 ± 9 and 69 ± 7 yr for the case and control groups, respectively). To rule out this, we compared the *LCT* -13910C>T polymorphism between our patients and another control sample of 207 nonagenarians without OHF. The frequencies of LP/LNP were similar (Table 4). Therefore, the comparison of both groups remained statistically non-significant with independence of individuals' age.

Obermayer-Pietsch *et al.* ~~(Obermayer-Pietsch et al., 2004)~~ (Obermayer-Pietsch et al., 2004) were the first in reporting an association between the LNP genotype and reduced BMD and fractures in Austrian postmenopausal women (Table 1). These results have been confirmed by several studies. In 2005, Enattah *et al.* found that the CC genotype was associated with the risk of hip and wrist fractures in Finnish older adults (age > 85) (Enattah et al., 2005). Also, in Finland, Laaksonen *et al.* (Laaksonen et al., 2009) suggested that LNP genotype may be associated with increased risk for greater bone loss in young adult men (Laaksonen et al., 2009). Similarly, Bácsi *et al.* (Bácsi et al., 2009) observed that Hungarian postmenopausal women carrying CC genotype showed a decreased BMD in sites such as radius, Ward's triangle, total hip, and lumbar spine (Bácsi et al., 2009), and Marozik *et al.* (Marozik et al., 2013) found statistical differences in the frequencies of *LCT* -13910C>T genotypes in a case-control study for Belarusian osteoporosis women (Marozik et al., 2013).

In the present study, we were unable to confirm the results obtained in the previously mentioned investigations and no statistically significant differences for the *LCT* -13910C>T polymorphism in the patients with OHF and the control group were found. No differences were observed either in the crude analysis or in the sex-stratified analysis. In line with those results, the allele frequency distribution was also similar in a group of control nonagenarians without OHF. Our results are in concordance with those obtained by Agueda *et al.* ~~(Agueda et al., 2010)~~ (Agueda et al., 2010) for a younger Spanish cohort (55.5 ± 8.7 yr). They did not find any relation between *LCT* -13910C>T polymorphism and

the osteoporotic traits, like BMD and/or fractures, in a cohort of 964 Spanish postmenopausal women. Moreover, no associations were found in several studies for different cohorts and populations, such as Finland (Tolonen et al., 2011; Enattah et al., 2004, 2005), Austria (Obermayer-Pietsch et al., 2007; Gugatschka et al., 2007), France (Esterle et al., 2009), Estonia (Kull et al., 2009), United Kingdom (Smith et al., 2009), Netherlands (Koek et al., 2010), and Denmark (Bergholdt et al., 2018) (Table 1). In all these studies, the frequency of LNP individuals varied from 6.80% (Smith et al., 2009) to 43.84% (Obermayer-Pietsch et al., 2007) (Table 1). Even though the frequencies are different, the results of these studies agree that the LNP does not increase the risk of fractures.

Generally, people who have LNP tend to avoid the ingestion of milk and milk-based products to prevent clinical symptoms of intolerance (Obermayer-Pietsch et al., 2004, 2007; Enattah et al., 2004). Since milk is an important calcium (Ca) source, lactose intolerance may lead to low Ca intake and an indirect effect on gastrointestinal Ca absorption, being able to play a role in osteoporosis development. However, humans can adapt to a wide range of Ca intake. In this sense, Ca can be absorbed in the intestine by two mechanisms: non-saturable passive transport, which predominates when there is a high intake of Ca, and active transport, which is responsible for Ca absorption when its intake is low but is readily saturable (Nordin, 1990). The net amount of Ca absorbed by active transport increases with the intake, rapidly at first, and gradually decreases when it is saturated. Therefore, the maximum level of Ca absorption achieves with low intakes. In fact, the net absorbed Ca is zero for a daily intake of 200 mg, 150 for 550 mg, and 250 for an intake of 1500 mg. Despite lower intake of Ca, absorption remains high. When there is an increase in Ca intake, Ca absorption remains with very few changes since the exceed of Ca is eliminated with the urine, keeping the Ca balance. Therefore, differences between the intake of 500 and 1500 mg of Ca are relatively small and can be compensated by Ca elimination in the urine. In consequence, negative Ca balance and subsequent osteoporosis rarely occurs in young adults. However, in menopausal women and older adults, a change in the metabolic systems occurs, producing progressive bone loss and increasing the risk of fractures (Nordin, 1990).

Since maximum Ca absorption occurs with low intakes, the lack of LCT is a factor that has little influence in populations with habits of consumption of dairy products, such as the case of the one studied in this work. It is probable that the habitual consumption of traditional dairy products in Northern Spain where our population was settled, such as cheese and curds, as well as, low amounts of milk without negative effects in people *LCT* -13910CC, favors calcium intake and therefore has no influence on OHF.

The limitations of this study include the difficulty of extrapolating the results obtained to other populations since lifestyle may have an important influence on the incidence of OHF. On the other hand, it has been seen that hormonal therapies reduce the incidence of fractures related to osteoporosis in postmenopausal women (Levin et al., 2018). In our study, this data in the female samples were missing. Therefore, in the future, it would be very interesting to expand the study taking this into account.

In conclusion, the analysis of the *LCT* -13910C>T polymorphism in 364 patients with OHF (82 ± 9 years old) showed no association between the genotype of this polymorphism and the risk for suffering OHF in the older adult population of Northern Spain.

CRediT authorship contribution statement

Tamara Kleinbielen: Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Review and editing. **Leire Palencia-Madrid:** Formal analysis, Investigation, Methodology, Writing - original draft, Review and editing. **Carmen Garcia-Ibarbia:** Resources, Review and editing. **Fernando Ortiz:** Resources, Review and editing. **José A. Riancho:** Funding acquisition, Resources, Review and editing. **Marian M. de Pancorbo:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing - original draft, ~~Writing -~~ rReview and ~~-~~& editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gene.2021.145560.

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Highlights

- Hip fracture is a common health problem very frequent in the elderly population.
- Several studies reported that *LCT* polymorphism could be related to fracture risk.
- We investigate if *LCT* polymorphism is a potential risk factor in elderly population.
- DNA samples of 364 patients with hip fracture and 376 controls were analyzed.
- LNP~~L~~ older adults with dairy moderate intake not show an increased risk of OHF.

Appendix A Supplementary data

The following are the Supplementary data to this article:

Multimedia Component 1

Supplementary data 1

Queries and Answers

Q1

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact r.ravindranathan@elsevier.com immediately prior to returning your corrections.

Answer: Yes

Q2

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

Answer: Yes

Q3

Query: Please note that the reference style has been changed from a Numbered style to a Name–Date style as per the journal specifications.

Answer: It is well.

Q4

Query: Ref(s). 'NanoDrop 1000' is/are cited in the text but not provided in the reference list. Please provide it/them in the reference list or delete these citations from the text.

Answer: This is an equipment, so I have cited its business house.