

RESEARCH ARTICLE OPEN ACCESS

Frequency of rs1051338 and rs116928232 Variants in Individuals from Northwest Mexico

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Received: 10 January 2024 | **Revised:** 6 May 2024 | **Accepted:** 5 June 2024

Funding: The authors received no specific funding for this work.

Keywords: LAL | LALD | Mexico | rs1051338 | rs116928232

ABSTRACT

Background: *LIPA*, situated on chromosome 10q23.2-q23.3, encodes the enzyme lysosomal acid lipase (LAL) (EC 3.1.1.13). Genetic alterations in *LIPA* lead to lysosomal acid lipase deficiency (LALD), an inborn error causing lipid metabolism anomalies and impairing cholesterol and triacylglyceride degradation. Over 40 *LIPA* variants have been documented, yet this study focuses on just two. The rs1051338 variant (NM_000235:c.46A>C) affects the signal peptide in Exon 2, whereas rs116928232, located in Exon 8, alters the splice site (NM_000235:c.894G>A), impacting lysosomal acid lipase activity. Considering the diverse clinical manifestations of LALD and the rising hepatic steatosis prevalence in Mexican population, mainly due to diet, these variants were investigated within this demographic to uncover potential contributing factors. This study aimed to reveal the frequency of rs1051338 and rs116928232 among healthy mestizo individuals in Northwest Mexico, marking a significant genetic exploration in this demographic.

Methods: Three hundred ten healthy mestizo individuals underwent PCR-RFLP analysis for both variants, and Sanger sequencing was performed for variant rs116928232. Bioinformatic analysis was also performed to predict protein changes.

Results: Allele frequencies for rs1051338 (FA = 0.39, p value = 0.15) and rs116928232 (FA = 0.0016, p value = 0.49) aligned with reported data, while bioinformatic analysis allowed us to identify the protein alteration observed in both variants; finally, the variants showed no linkage between them (normalized D' = 1.03, p value = 0.56).

Conclusions: Allelic frequencies closely matched reported data, and protein structure analysis confirmed variant impacts on LAL enzyme function. Notably, this study marks the first analysis of rs1051338 and rs116928232 in a healthy Mexican mestizo population.

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1 | Introduction

Lysosomal acid lipase deficiency (LALD) is a congenital autosomal recessive inborn error that results in a metabolic deficiency of lysosomal acid lipase (LAL), this condition encompasses two distinct phenotypes: Wolman's disease (WD), marked by the absence of LAL production and frequently ending in fatality, and cholesterol ester storage disease (CESD), characterized by partial deficiency in LAL activity. Although the exact prevalence of LALD is unknown, it is estimated to range from 1:40,000–50,000 individuals to 1:300,000–350,000 individuals in European populations [1–3]. LAL is coded by *LIPA* (MIM*613497), located on chromosome 10q23.2-q23.3. The gene covers 36 kb, consisting of 10 exons, and produces two transcripts of 2660 and 2654 nucleotides. The enzyme comprises 399 amino acids, with the first 21 amino acids acting as a signal peptide. Its mature form contains 378 amino acids and weighs 43 kDa [4, 5].

It is interesting to note that LAL is synthesized in almost all human tissues, with higher expression in the liver, fibroblasts, macrophages, and lymphocytes. However, LAL mRNA expression varies depending on the tissue and the cellular demand [6]. The proenzyme is synthesized in the rough endoplasmic reticulum (RER) and later modified in the Golgi apparatus. Six asparagine glycosylation sites (N36, N72, N101, N161, N273, and N321) are added, which play an important role in its function and are highly conserved in the lipase family [5]. Once the LAL has been modified, it is released in vacuoles covered with mannose-6-phosphate and a clathrin envelope, which allows them to be recognized by lysosomes and endocytosed to fulfill their function [7, 8]. It is worth noting that for these enzymes to function, the lysosome is required to acidify its interior via ATP pumps to a pH of 3.5–4.5 [9].

Lysosomal acid lipase (LAL) is crucial for hydrolyzing lipoprotein cholesterol esters (CE) into free cholesterol (FC). Once FC exits the lysosome, it can be re-esterified in the endoplasmic reticulum, forming cytosolic lipid droplets. However, excessive FC accumulation within the lysosome can hinder lysosomal activity, including that of LAL. Regulation of cholesterol and free fatty acid biosynthesis involves a group of transcription factors called sterol regulatory element-binding proteins (SREBPs); these proteins activate the enzyme cascade necessary for synthesizing cholesterol, acylglycerols, triacylglycerols, and phospholipids. SREBP precursors are sequestered in the endoplasmic reticulum (ER) via close association with sterol regulatory element-binding protein cleavage-activating protein (SCAP), a membrane protein that modulates cholesterol levels within mammalian cell membranes [10–13]. Elevated cholesterol levels, particularly 27-hydroxycholesterol, bind to the hepatic X receptor (LXR), initiating the transcription of genes involved in reverse cholesterol transport. Among these genes is ATP-binding cassette transporter A1 (*ABCA1*), encoding the A1 ATP-binding cassette transporter primarily located in the cytoplasmic membrane, notably in the liver, this transporter facilitates the transfer of apolipoprotein A1 (Apo A-I) and high-density lipoprotein cholesterol (HDL-C); consequently, the decrease in 27-hydroxycholesterol levels contributes to reduced expression of *ABCA1*, likely leading to low plasma HDL-C levels [12].

The enzymatic activity deficiency of LAL results in significant lipid accumulation within cells, upregulating 3-hydroxy-3-m

ethyl-glutaryl-coenzyme-A-reductase (HMG-CoA-reductase) activity. This cascade increases 27-hydroxycholesterol synthesis and transcription factor activation, leading to elevated production of low-density lipoprotein receptors (LDLR). Additionally, it stimulates ApoA1 migration, reduces HDL-C expression in blood levels, and diminishes leukocyte adhesion [2, 14, 15].

In that perspective, our study focused on two variants associated with *LIPA*. The initial variant identified was NM_000235:c.46A>C (rs1051338), situated within Exon 2. It has been characterized as likely benign, resulting in a substitution of threonine to proline at amino acid 16 within the signal peptide (NP_000226:p.Thr16Pro). This change alters the cleavage site of the signal peptide, reduces the α -helical core to 11 residues, disrupts the hydrophobic core, and decreases the enzymatic activity of LAL [16–18]. The rs116928232 variant (NM_000235:c.894G>A) has been identified as pathogenic. It is situated in Exon 8 and impacts the –1 donor site of the splice site, it eliminates codons 254–277 in the mRNA of LAL and transcribes a truncated enzyme that lacks 24 amino acids (NP_000226:p.delSer275_Gln298), where recognition sites for N-glycosylation are located; these are the most frequently reported variants found in patients with LALD, representing 60% of the variants found in patients with CESD [19–22].

More than 40 variants associated with *LIPA* have been identified, leading to a clinically diverse range of presentations [22]. The aim of the study was to elucidate the frequency of the rs1051338 and rs116928232 variants of the *LIPA* among healthy mestizo individuals residing in the Northwest region of Mexico, thus representing a notable milestone in the field of genetics, as it constitutes the first examination of these variants in this demographic. The selection of the rs1051338 variant was motivated by its substantial correlation with overweight and obesity, as substantiated by previous investigations. Nevertheless, research pertaining to this variant within the Mexican populace is presently absent. The decision to scrutinize the frequency of the rs116928232 variant was prompted by a preceding study conducted by Vespasiani-Gentilucci et al. [21], which underscored its prevalence among individuals afflicted with LALD, a condition frequently characterized by hepatic steatosis and cryptogenic cirrhosis, present in as many as 70% of cases. Given the heterogeneous clinical manifestations of LALD and the burgeoning prevalence of hepatic steatosis among Mexican patients attributable to dietary influences, we elected to explore this variant within this population in order to elucidate potential additional etiological factors. The findings of this study can have a far-reaching impact on future genetic research, making it a crucial contribution to the field.

2 | Materials and Methods

2.1 | Patients

Between November 2018 and March 2019, we analyzed 310 healthy mestizo individuals from Northwest Mexico, all of whom were free from diagnosed metabolic diseases. Among them, 91 individuals were under the age of 18 years. The participants

provided signed informed consent or their legal guardians did so on their behalf, and the hospital Ethics Committee UMAE Hospital Gineco-Obstetricia Lic. Ignacio García Téllez (CMNO) approved the study with the registry number R-2019-1310-006. The inclusion criteria encompassed apparently healthy individuals from Northwestern Mexico (Aguascalientes, Colima, Guanajuato, Jalisco, Michoacan, Nayarit, Zacatecas, Sonora, Sinaloa, and Baja California Norte and Sur), excluding those with infectious liver diseases at the time of sample collection. Samples were eliminated if genetic typing was impractical or if individuals opted to withdraw from the study at any time, either voluntarily or as decided by their legal guardians. The DNA extraction from peripheral blood samples collected in BD Vacutainer K2 EDTA tubes was performed using the modified Miller technique (see Appendix 1). Afterward, the extracted DNA underwent quantification using spectrophotometry at a wavelength of 260/280 nm, ensuring adherence to a DNA purity range of 1.8–2.0. The average DNA concentration was 354 ng, with a purity of 1.82 (see Appendix 1).

2.2 | Genotyping

The primers for Exon 2 were custom-designed using Oligo 7, whereas the ones for Exon 8 were taken from Pullinger et al. [2] and verified by PCR in silico using the UCSC Genome Browser Gateway portal (<http://genome.ucsc.edu/cgi-bin/hgGateway>). PCR amplification of exon fragments of the *LIPA* was performed. For the rs1051338 variant in Exon 2, primers were designed to yield a 279 bp size fragment (5'GTTCAGAACTTTTGTGGG3'; 3'ATGGCTTCATGTAGCTCAC5') (see Appendix 2), and the restriction enzyme *Ava*II (Time-Saver, New England, BioLabs Inc., catalog # R0153S) was used for digestion at 37°C for 4 h. The presence of the adenine (A allele, wild type) leads to a cohesive cut, resulting in two fragments of 143 and 136 bp. In contrast, cytosine (C allele, polymorphic) does not yield any cuts. The PCR amplification of Exon 8 (for the rs116928232), yielded a 242 bp fragment (5'TGCTTTGAAGGGCAAATAC3'; 3'TTCTATTGGAAAGGGTTTGC5'). The restriction enzyme *Bst*NI (Time-Saver, New England, BioLabs Inc., catalog # R0168S) was used for the genotyping of rs116928232 at 60°C for 4 h. In the presence of guanine (G allele, wild type), two cohesive cuts produce three fragments of 156, 73, and 13 bp; nevertheless, in the case of the polymorphic allele (adenine, thymine, and cytosine), it yields two fragments of 169 and 73 bp, which were further subjected to Sanger DNA sequencing to determine the nucleotide change. The obtained sequence was then blasted against the sequence available at the National Center for Biotechnology (<https://www.ncbi.nlm.nih.gov/>). Therefore, Sanger sequencing was employed to identify the rs116928232 variant. This involved amplifying the exons of the *LIPA* through endpoint PCR using the previously mentioned primers. The amplification was confirmed by running electrophoresis on 8% polyacrylamide gels stained with nitrate silver. Purification was carried out using ExoSap-IT PCR (Applied Biosystems). Subsequently, Sanger sequencing was conducted utilizing the Big Dye Primer 3.1 kit (Applied Biosystems), followed by purification using Sephadex G-10 columns. The sequencing process was automated using the ABI Prism 310 equipment (Applied Biosystems). Finally, the resulting electropherograms were analyzed using SnapGene

Viewer software and aligned with the FASTA sequence from the NCBI database using the Clustal Omega platform (EMBL-EBI). For quality control of DNA extractions, samples underwent quantification and purity determination via spectrophotometry at a wavelength of 260/280. Prior to PCR-RFLP procedure, all conventional PCRs were run via electrophoresis on polyacrylamide gels and stained with silver nitrate, allowing bands to be compared with a 50 bp marker to ensure the amplicon is of the correct size and cut as expected. Nucleotide sequences for sequencing were confirmed using the FASTA format sequence, available electronically on NCBI.

2.3 | Structural Analysis

The first step involved retrieving the amino acid sequences of LIPA (P38571-1) from the UniProt protein database (<http://www.uniprot.org/>) in FASTA format. Next, the secondary structure of LIPA was predicted using the PSIPRED program (<http://bioinf.cs.ucl.ac.uk/psipred/>). Finally, the tertiary structures of the wild-type and mutant forms of LIPA variants (rs1051338 and rs116928232) were modeled. The I-TASSER server was used for protein structure modeling (<https://zhanglab.cmb.med.umich.edu/I-TASSER/>). To estimate the quality of the predicted models, a confidence score (C-score) was used. The modeled 3D structures were visualized using UCSF ChimeraX (<https://www.cgl.ucsf.edu/chimerax/>). These three-dimensional protein models were generated to illustrate the alterations resulting from polymorphic changes.

2.4 | Statistical Analysis

The sample size n was determined based on the genotypic frequency (C/C of 0.15) observed for the rs1051338 variant in individuals with Mexican ancestry from Los Angeles, California. Using the Epi Info software, a sample of 240 individuals was selected with a confidence level of 97%. The study collected data on individuals represented by consecutive codes and all the data were documented in a database. Birthplace, gender, and age were analyzed through descriptive statistical parameters. Genotypic and allelic frequencies were also calculated, and the study population was compared to the reference Ensembl genome browser using chi-square. The Hardy-Weinberg equilibrium was obtained through chi-squared adjustment, and the linkage disequilibrium of the variants was calculated with Haploview 4.1 software at the Broad Institute. All statistical tests were considered significant at a level of ≤ 0.05 as a bilateral significance.

3 | Results

This study analyzed a cohort of 310 healthy individuals from Northwestern Mexico. Among them, 61% (189/310) were female, whereas 39% (121/310) were male. The average age of the participants was 24.3 years (SD 13). The highest percentage of Mexican individuals were born in the state of Jalisco with 79% (245/310), followed by the state of Michoacan (26/310, 8%), Colima (13/310, 4%), Sinaloa (11/310, 4%), Nayarit (8/310, 3%), Aguascalientes (5/310, 2%), and Sonora (2/310, 1%).

In our analysis, we found that only one individual had a heterozygous genotype (GA, 0.0033) for the variant rs116928232. For the variant rs1051338, our analysis revealed the following genotype distribution: 123 wild-type homozygous individuals (AA, 0.40), 135 heterozygous individuals (AC, 0.43), and 52 polymorphic homozygous individuals (CC, 0.17). The allelic frequencies of the A allele (wild type) and C allele (polymorphic) were 0.61 and 0.39, respectively (Table 1). The *p*-value of Hardy–Weinberg equilibrium (HWE) for rs116928232 was 0.49 after the Yates correction for chi-square, and for rs1051338, it was 0.15. The allelic frequencies of the G allele (wild type) and the A allele (polymorphic) for rs116928232 were 0.9984 and 0.0016, respectively.

In the case of the heterozygous individual (genotype GA) for rs116928232 in Exon 8 PCR was conducted twice, each time resulting in a discernible shift in 8% polyacrylamide gels, confirming that the band pattern corresponded with the heterozygous genotype. The rs116928232 variant, located in Exon 8, is identified as a triallelic variant (A, C, T) [23]. To confirm the expected nucleotide alteration, Sanger DNA sequencing analysis was performed on the individual, revealing a dual signal on the electropherogram, consistent with the PCR-RFLP findings (see Figure 1).

With a total of 620 genotypes, we calculated the linkage disequilibrium coefficient to verify the nonrandom segregation of the alleles. The distance between the variants rs1051338 and rs116928232 was 25 kb, and neither variant tended to segregate together. As a result, they were deemed not linked, with each variant exhibiting a *p*-value of 0.56 and a normalized *D'* of 1.03. The frequencies of the haplotypes inferred by the software showed that the GA haplotype had the highest frequency (61.3%), which corresponded to the wild-type alleles for each variant.

The secondary structure of both wild-type LIPA and variants (rs1051338 and rs116928232) were analyzed as the first step for comparison. To analyze the complete sequencing of the LAL protein, the sequence was divided into two regions: the signal peptide region where the rs1051338 variant is found (23 amino acids +7 amino acids to meet the minimum requirement of 30 amino acids by the PSIPRED program), and the remaining amino acids of the protein (376 amino acids) region, where the rs116928232 variant is found. These two regions were separately entered into the PSIPRED program, and the leitmotifs changed when the mutant allele was expressed in homozygosis, as shown in Figures 2 and 3.

After obtaining the secondary structure in PSIPRED, we added this information to the advanced options of the I-TASSER program to generate the three-dimensional structure of the signal peptide and catalytic domains for the wild-type and two mutants. The structure with the highest quality was chosen using the *Z*-score; the outcomes can be visualized through the three-dimensional structures depicted in Figures 4 and 5.

4 | Discussion

According to various studies, the *LIPA* gene has over 40 variants, including the rs1051338 and rs116928232 variants. The rs1051338 variant has been reported in many populations with the C allele frequency ranging from 0.28 to 0.31 worldwide; the frequency is higher in America, ranging from 0.35 to 0.4, and specifically in Mexicans with AF of 0.39 [23–25]. In our study, we found the same allelic frequency of the C allele at 0.39 in the global sample and slightly higher in the state of Jalisco (0.40), which had the largest sample size. Our analysis provides an overview of the possible frequencies of this variant in the states analyzed (Table 2).

TABLE 1 | Genotypic and allelic frequencies of *LIPA* gene variants.

Variant	Genotypic frequency (<i>n</i>)	Allelic frequency (<i>n</i>)	<i>p</i> -value of HWE
rs1051338	A/A 0.40 (123)	A = 0.61 (381)	0.15*
	A/C 0.43 (135)	C = 0.39 (239)	
	C/C 0.17 (52)		
rs116928232	G/G 0.9967 (309)	G = 0.9984 (619)	0.49
	G/A 0.0033 (1)	A = 0.0016 (1)	
	A/A 0.00 (0)		

Abbreviation: HWE, Hardy–Weinberg equilibrium.

*Chi-Squared test.

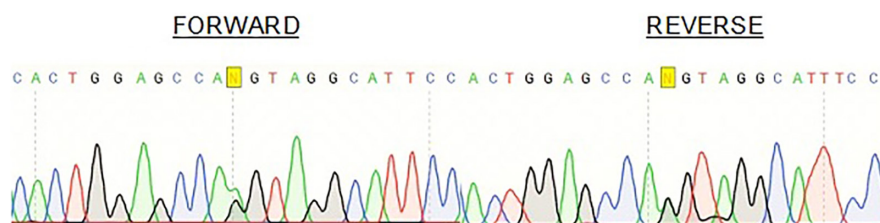


FIGURE 1 | Electropherograms of the heterozygous individual for the rs116928232 variant. There are two signals, guanine and adenine which corroborate the genetic component of “G/A.”

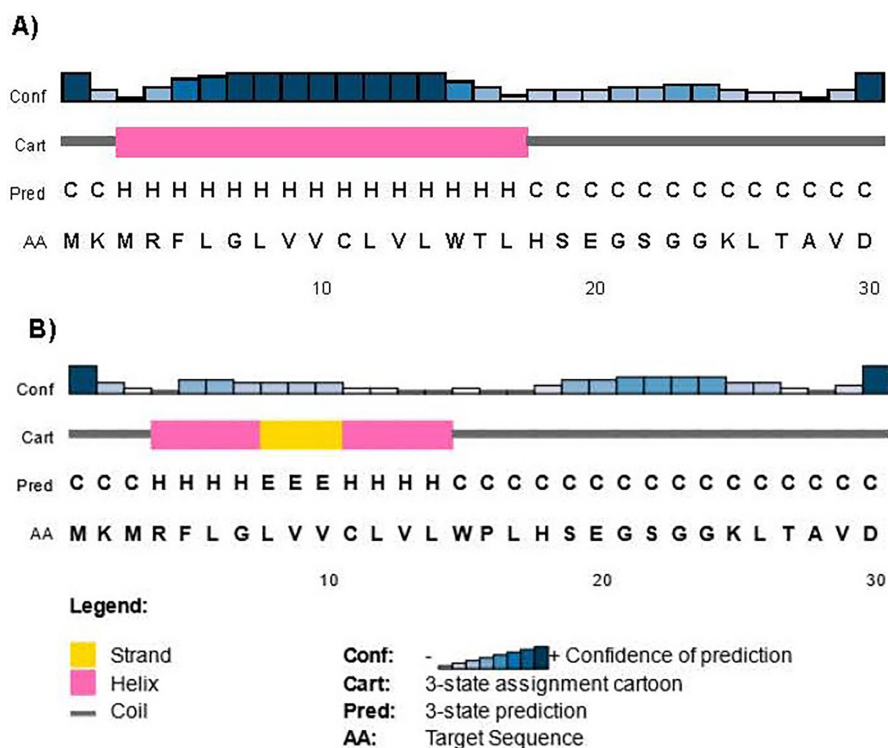


FIGURE 2 | Secondary structure analysis of the signal peptide in the rs1051338 variant. (A) Wild-type sequence of the LIPA protein is conferred by 50% of alpha helices and 50% of the random coil. (B) The LIPA sequence when a change of threonine is generated at position 16 by proline, is confirmed by 26.67% of alpha helices, 10% of beta sheets and 63.34% of random coils.

Access was obtained to unpublished data conducted by Cebolla et al., who examined the SNP rs1051338 in a cohort of 2042 Spanish individuals in 2019. Their findings revealed an allelic frequency of 0.74 for allele A and 0.25 for allele C, with a HWE *p*-value of 0.05. The genotypic frequencies were 0.56 for wild-type homozygotes (AA), 0.36 for heterozygotes (AC), and 0.07 for polymorphic homozygotes (CC) (Cebolla, J. 2019, unpublished data). The frequency of this variant in North American individuals with Iberian ancestry is reported to be 0.23 for the C allele [25], which is similar to what was found by the Spanish team. However, the discrepancy in the Mexican population (0.39 for the polymorphic allele) is due to the number of individuals analyzed and the admixture of the different ethnic groups established in the conquest of Mexico, especially in Jalisco [26]. However, our results coincide with the frequency of this variant in Mexican population reported in the NCBI database [23].

Several studies have linked the genetic variant rs1051338 to hypotension, coronary heart disease, and cardiovascular disease. Surprisingly, there is a notable absence of published studies examining this variant within the Mexican mestizo population, despite its potentially significant clinical implications. This gap in research is concerning, especially considering the findings of the Pan American Health Organization's 2016 report, which highlighted a surge in overweight and obesity rates among adults in the Americas, with Mexico notably standing out with high rates in both childhood and adulthood. Such trends underscore the pressing need to investigate the genetic factors contributing to these health challenges and their potential impact on the quality of life of Mexicans [27]. It is of interest that Mexicans population reports the highest frequency of this variant.

Numerous studies have explored the potential link between the rs1051338 polymorphism and a range of diseases. These studies suggest that this polymorphism may interfere with the pathway of cholesterol degradation, leading to a harmful impact [17, 18, 28–30]. In 2017, Morris et al. [17] discovered that rs1051338 reduced the concentration and function of the LAL protein in lysosomes in COS7 cells and macrophages. In our work, we present a 3D structural analysis of the rs1051338 polymorphism. It was observed that the interruption of the alpha helix caused by this polymorphism can affect the interaction with signal recognition particles. This, in turn, prevents the protein from reaching the endoplasmic reticulum and leads to its accumulation in the cytoplasm. The subsequent degradation of the protein is carried out by the proteasome. However, Morris et al. [17] also noted that in the presence of a ubiquitin–proteasome degradation pathway inhibitor, mutant protein levels in macrophage lysosomes resembled those of the wild-type LAL protein (Figure 4).

The rs116928232 variant has been described as a triallelic variant (A, C, T) located in exon 8 [23]. The exchange from guanine to adenine (G>A) affects the –1 donor site of the splice, which leads to the elimination of codons 254–277 of the mRNA of LAL [19]. This results in the translation of a truncated enzyme lacking 24 amino acids (p. Ser275_Gln298), where recognition sites for N-glycosylation are located [20]. The allelic frequency of the A allele (polymorphic) has been previously reported in various populations, such as Germans (allelic frequency of 0.0025), Asians (allelic frequency of 0.0005), Caucasians, and Hispanics (allelic frequency of 0.0017) [1, 31]. In this study, the allelic frequency of the A allele was found to be 0.0016, which is similar to that reported in Scott et al. and other platforms linked to

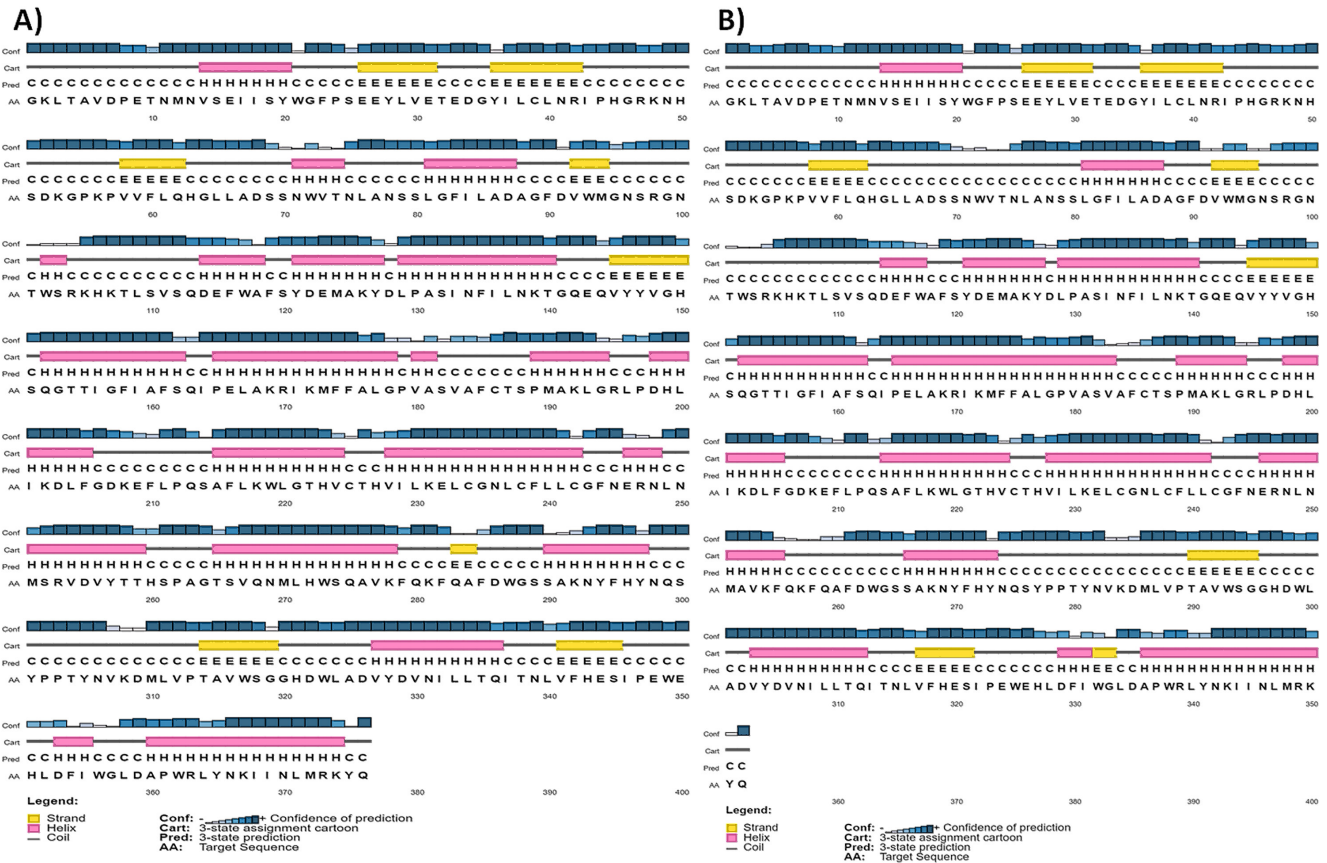


FIGURE 3 | Secondary structure analysis of the catalytic domain in the rs116928232 variant. (A) Wild-type sequence of the LIPA protein is conferred by 45.76% of alpha helices, 10.63% of strands, and 43.61% of random coil. (B) The LIPA sequence when 24 amino acid deletion is generated, is confirmed by 40.42% of alpha helices, 9.31% of strands and 50.27% of random coils.

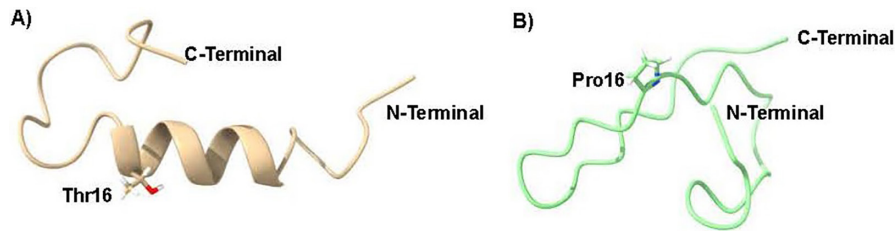


FIGURE 4 | Three-dimensional structure of the signal peptide. (A) The 3D structure of the signal peptide wild type is composed of an alpha helix from Leu8 to His18. (B) When Thr in position 16 is changed to a Pro all the alpha helix important for interaction with other proteins is eliminated.

the 1000 Genomes Project, with an allelic frequency of the A allele of 0.0013 for the Hispanic population of Los Angeles with Mexican ancestry [25, 32, 33]. Although we analyzed only 310 individuals, this result is consistent with previous reports.

One of the most frequently described variants is rs116928232, this defect related to residual activity has been reported in patients with no severe phenotype [34], which coincides with our findings. The structural analysis showed that removing 24 amino acids would not affect the enzyme's active site amino acids' positioning, which means that the enzyme could still have some activity left.

According to our research in the PubMed and Ensemble databases, we conducted a thorough search for articles on these two

variants. Our findings revealed that the rs1051338 variant has been linked to various diseases, whereas the rs116928232 variant has only been associated with lipid metabolism abnormalities (see Appendix 3). As the majority of Mexicans are mestizo and have a high incidence of obesity, studying both variants in this population is crucial. This study marks the first to establish a connection between the 3D structural analysis of both variants with predicted protein changes of the LAL protein.

5 | Conclusions

According to our findings, the allelic frequencies of rs1051338 and rs116928232 of the *LIPA* gene were 0.39 and 0.0016, respectively. We also observed that the *p*-values of Hardy-Weinberg

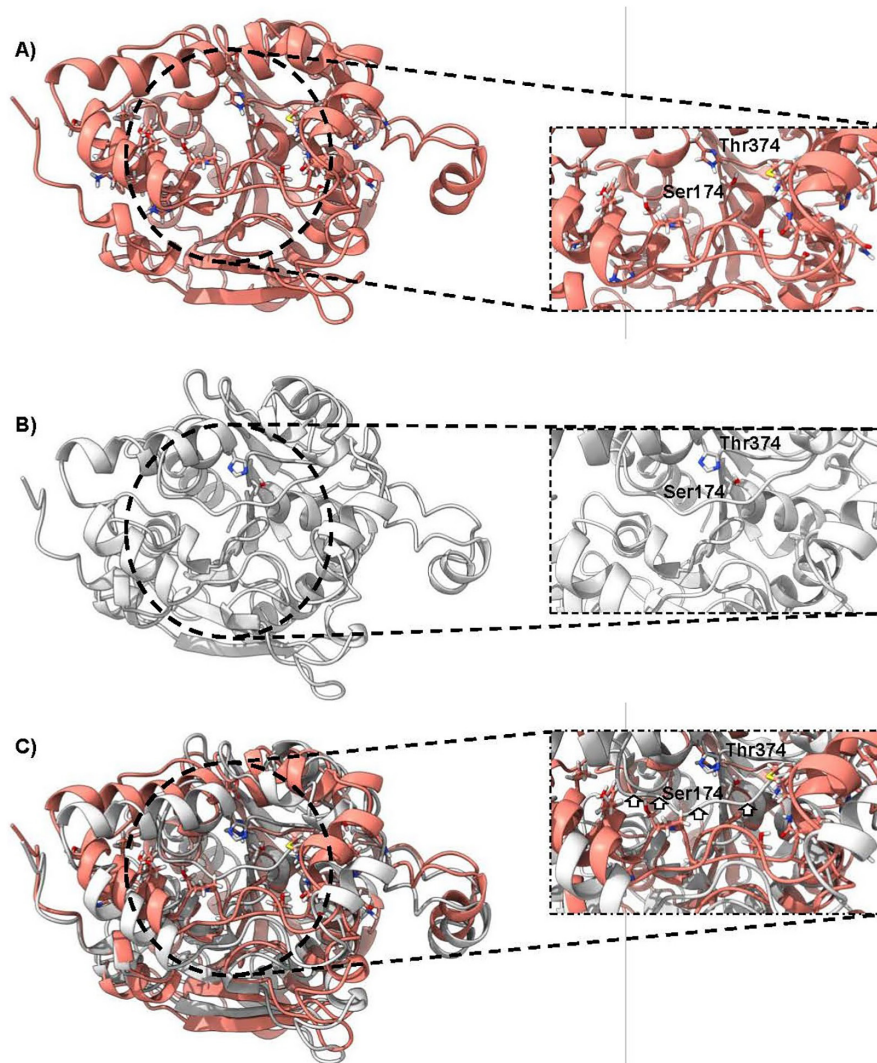


FIGURE 5 | Three-dimensional structure of the catalytic domain. (A) The amino acids in the wild-type 3D structure that make up the catalytic sites Ser174 and Thr374 and the side chain of amino acids that are eliminated (with a softer color) with the rs11698232 mutation are shown. (B) 3D structure of the tiny catalyst after the deletion of 24 amino acids, in addition, the amino acids Ser174 and Thr374 are shown. (C) The two overlapping wild and mutant structures show that no changes in the position of the amino acids Ser174 and Thr374 were generated; however, the mutant protein chain indicated with the white arrows (in the third image) could interact with the catalytic domain.

TABLE 2 | Demographic distribution of the rs1051338 variant in this study. The genotypic frequency is shown according to the state of origin of the 310 individuals analyzed, mostly born in the state of Jalisco.

State	Variant rs1051338						Total individuals
	Genotype						
	A/A		A/C		C/C		
	Individuals	Proportion	Individuals	Proportion	Individuals	Proportion	
Aguascalientes	3	0.0244	1	0.0074	1	0.0192	5
Colima	7	0.0569	6	0.0444	0	—	13
Jalisco	93	0.7561	109	0.8076	43	0.82690	245
Michoacan	10	0.0813	11	0.0814	5	0.0962	26
Nayarit	4	0.0325	3	0.0222	1	0.0192	8
Sinaloa	5	0.0407	4	0.0296	2	0.0385	11
Sonora	1	0.0081	1	0.0074	0	—	5
Total	N=123	1	N=135	1	N=52	1	N=310

equilibrium were 0.15 for rs1051338 and 0.49 for rs116928232; and the variants are not linked to each other (normalized D of 1.03, p value of 0.56). Our study is the first to investigate the frequency of the rs1051338 and rs116928232 variants on the Mexican population, which is mainly composed of mestizo individuals and has high rates of obesity and chronic degenerative diseases. Furthermore, the bioinformatic analysis carried out details the impact of these variants on the LIPA protein, and the possible consequences of these on individuals.

Acknowledgments

To all the individuals who were part of the study population.

Disclosure

There has been no significant financial support for this work that could have influenced its outcome; we know of no conflicts of interest associated with this publication, one of the authors is an employee of Takeda Pharmaceuticals outside the submitted work without stock options. As principal author, I confirm that the manuscript has been read and approved for submission by all the named authors.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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Appendix 1

DNA Extraction by Salt Precipitation Technique (Modified Miller)

1. Take 5 mL of peripheral blood in a tube with EDTA anticoagulant.
2. Place the sample in a 50 mL tube.
3. Add two volumes of 10:1 $\text{NH}_4\text{Cl}/\text{NH}_4\text{HCO}_3$ lysis solution, gently shake, and let it rest for 10–20 min in the freezer at -20°C .
4. Subsequently, centrifuge at 4500 rpm for 20 min.
5. Discard the supernatant in chlorine; add two volumes of the 10:1 solution, centrifuge again for 20 min at the same rpm. Repeat as necessary.
6. Re-suspend the pellet with a lysis buffer at pH 8.2 with vigorous shaking.
7. Allow to sit for 12–24 h at 37°C with 300 μL of proteinase K and 100 μL of 10% SDS.
8. Add 6M NaCl while vigorously shaking for 1–2 min until foam forms.
9. Centrifuge at 3500 rpm for 20 min.
10. Recover the supernatant and transfer it to another sterile tube.
11. Add two volumes of absolute ethanol and mix by inversion to precipitate the DNA.

12. Recover the DNA and place it in a sterile 1.5 mL microtube.
13. Wash with cold 70% ethanol.
14. Remove the ethanol from the tube and let it evaporate.
15. Once dry, add 200–300 μL of TE buffer pH 7.5.

The DNA extraction technique utilized was based on and adapted from the document by Miller SA, Dykes DD, Polesky HF, titled "A simple salting out procedure for extracting DNA from human nucleated cells," published in *Nucleic Acids Res.* on February 11, 1988 (Vol. 16, No. 3, pp. 1215).

Appendix 2

Primers of the *LIPA* Gene Using In Silico PCR

Exon	Forward (5'-3')	Reverse (3'-5')	Size (pb)	TM ^a
2	G TTCAGAACTTTTGTGGG	ATGGCTTCATGTAGCTCAC	279	59°C
8	TGCTTTGAAGGGCAAAATAC	TTTCTATTTGAAAGGGTTTGC	242	62°C

^aAnnealing temperature.

Appendix 3

Compilation of Published Articles Associated With the rs1051338 and rs116928232 Polymorphisms

	Country	N (samples)	Conclusions	Title	Author
rs1051338	Italy	164 consecutive patients with biopsy-proven NAFLD and cell cultures	5 NAFLD patients with LAL activity <0.30 nmol/spot/h Three patients were heterozygous and one patient was homozygous for the missense mutation c.46 A>C (rs1051338)	Lipid accumulation impairs lysosomal acid lipase activity in hepatocytes: Evidence in NAFLD patients and cell cultures	M. Gomasrashi et al. 2019
	Germany	286 individuals with probable Alzheimer's disease (AD) and 162 controls	Genotype frequency: Individuals with probable AD: CC 0.56, AC 0.37, AA 0.6 // Controls: CC 0.52, AC 0.37, AA 0.09 Not associated with AD	Association analysis of genes involved in cholesterol metabolism located within the linkage region on chromosome 10 and Alzheimer's disease	Riemenschneider M et al. 2004
	United Kingdom	26 controls and cell cultures	Coronary artery disease-associated coding variant rs1051338 causes reduced lysosomal LAL protein and activity because of increased LAL degradation, providing a plausible causal mechanism of increased coronary artery disease risk There was no difference in LAL protein or activity in whole macrophage extracts; however, we found reduced LAL protein ($p=0.02$) and activity ($p=0.026$) with the risk genotype in lysosomal extracts, suggesting that the risk genotype affects lysosomal LAL activity	Coronary artery disease-associated <i>LIPA</i> coding variant rs1051338 reduces lysosomal acid lipase levels and activity in lysosomes	Gavin E Morris et al. 2017
	EUA	Cell culture and transfection 104 controls	rs1051338, resulting in T16P with similar prevalence and increased risk for CAD FA: "C" 0.35 controls rs1051338 is dose-dependently associated with higher human monocyte <i>LIPA</i> mRNA expression	Functional characterization of <i>LIPA</i> (lysosomal acid lipase) variants associated with coronary artery disease	Trent D Evans et al. 2019
	China	A total of 202 cases of PTB and 218 healthy controls	rs1051338, the heterozygous (AC vs. AA) $p: 0.001$, OR: 1.998, 95% CI: 1.312–3.042 and homozygous (CC vs. AA) $p: <0.001$, OR: 4.078, 95% CI: 2.134–7.796 Codominant associated with increased risk for the disease. Under recessive (CC vs. AA + AC), $p: 0.001$, OR: 2.829; 95% CI: 1.543–5.185 and dominant model (AC + CC vs. AA) $p: <0.001$, OR: 2.331, 95% CI: 1.564–3.474 the genotypes distribution increased the individual risk, plus its alleles distribution ($p: <0.001$, OR: 2.004, 95% CI: 1.505–2.669) The findings of our study strengthen the hypothesis that <i>LIPA</i> rs1051338 and rs7922269 polymorphism associated with increased risk for pulmonary Tb in a sample of northern Chinese population	Lysosomal acid lipase gene single nucleotide polymorphism and pulmonary tuberculosis susceptibility	Deo Kabuye et al. 2021

(Continues)

APPENDIX 3 | (Continued)

Country	N (samples)	Conclusions	Title	Author
Italy	74 subjects with hypoalphalipoproteinemia or mixed hyperlipemia	Multivariate analysis highlighted independent association between rare allele and FL severity in subjects with NAFLD. The rs1051338 SNP may modulate FL severity and atherogenic dyslipidemia in patients suffering from NAFLD	Effect of a common missense variant in <i>LIPA</i> gene on fatty liver disease and lipid phenotype: New perspectives from a single-center observational study	Andrea Pasta et al. 2021
Germany	244 patients with AD (German), 285 controls	None of the observed polymorphisms showed a significant influence on the risk of AD. They found that <i>LIPA</i> Exon 2 polymorphism (rs1051338) influenced plasma 24S-hydroxycholesterol/cholesterol ratios in patients with AD where carriers of the C/C allele presented with higher ratios than heterozygote carriers of the <i>LIPA</i> allele. Even though the biological function and gene location of <i>LIPA</i> on chromosome 10 suggest that <i>LIPA</i> might be a candidate for an AD risk gene, results revealed that polymorphisms in <i>LIPA</i> did not influence the risk of AD in the study	Influence of lysosomal acid lipase polymorphisms on chromosome 10 on the risk of Alzheimer's disease and cholesterol metabolism	K-T von Trotha et al. 2006
Japan	2263 consecutive autopsies of older Japanese subjects	rs1051338 (p.T16P, OR: 2.50, 95% CI: 1.70–3.66, $p = 2.79 \times 10^{-6}$) This study suggests that <i>WIPF3</i> and <i>LIPA</i> , both of which are expressed in the macrophages are involved in pathological AAA. These results should be regarded as hypothesis-generating; thus, replication study is warranted	Association of nonsynonymous variants in <i>WIPF3</i> and <i>LIPA</i> genes with abdominal aortic aneurysm: an autopsy study	Yuko Maeda et al. 2016
EUA	10,142 women, 737 of whom developed primary colorectal cancer (CRC)	Chronic inflammation is one of the interconnected pathways that mediate the adiposity CRC association, but additional studies on larger populations are warranted to confirm our results Could contribute to the disease	Genetically determined elevated C-reactive protein associated with primary colorectal cancer risk: Mendelian randomization with lifestyle interactions	Su Yon Jung et al. 2021
rs1051338	Vital study ^a , CL08 study ^b 19 patients	rs1051338 was found in 1 patient; rs116928232 in 2 patients Enzymatic activity was not analyzed	Long-term survival with sebelipase alfa enzyme replacement therapy in infants with rapidly progressive lysosomal acid lipase deficiency: final results from 2 open-label studies	Suresh Vijay et al. 2021
EUA, Europe, and Australia	16,918 subjects with colorectal	They found association with p value of 0.1 and $FA = 0.3$ Could contribute to disease	Genetically predicted circulating C-reactive protein concentration and colorectal cancer survival: A Mendelian randomization consortium study	Xinwei Hua et al. 2021
Canada	Sequenced in 25 individuals, genotyped in a cohort of 1751 obese individuals	The C allele of SNP rs1051338 was associated with lower blood pressure (BP; systolic [SBP] $p = 0.004$; diastolic [DBP] $p = 0.006$)	Association of <i>LIPA</i> gene polymorphisms with obesity-related metabolic complications among severely obese patients	Frédéric Guénard, et al. 2012

(Continues)

APPENDIX 3 | (Continued)

	Country	N (samples)	Conclusions	Title	Author
rs116928232	UK	Stored DNA samples from 663 patients were genotyped for the E8SJM variant	The allele frequency 0.0023 (1:221 individuals) Only three were found to be c.894C>T heterozygotes Sequencing of the <i>LIPA</i> gene of these three participants showed that all three had one or more variants in Exon 2: rs1051338 and rs1051339	Estimation of the prevalence of cholesteryl ester storage disorder in a cohort of patients with clinical features of familial hypercholesterolemia	Ashfield-Watt et al. 2018
	EUA	Participants (N= 1357) were identified by lipid profiles	Six patients were heterozygous One patient was heterozygous frameshift mutation involving deletion of Exon 4 (p.Gly77Valfs*17 c.230-106_c.428 + 541del)	Identification and metabolic profiling of patients with lysosomal acid lipase deficiency	Clive R Pullinger, et al. 2015
	Italy	11 patients with LALD	Description of eight patients with enzymatic activity of <11%	Lysosomal lipase deficiency: molecular characterization of 11 patients with Wolman or cholesteryl ester storage disease	Tommaso Fasano et al. 2011

^aVital study (UK, EUA, France, Ireland, Egypt, and Turkey).

^bCL08 study (UK, Finland, EUA).