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Wolman's Disease and GNE Myopathy Frequency to Religion

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ABSTRACT

Complete absence or deficiency of Lysosomal acid lipase (LAL) leads to the rare infantile disorder Wolman's Disease [1]. Decreased function of GNE can lead to GNE myopathy [2]. Although both pathological phenotypes are rare, LAL deficiency only affects 1 in every 500,000 births and dysfunction of GNE affects 1 in every 1,000,000 births, there is a higher incidence in the religious groups of the Middle East; the incidence is projected to be as high as 1 in every 4,200 births [3,4]. The LIPA gene encodes for LAL while the GNE gene encodes for UDPN-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase [4,5]. LAL catalyzes the hydrolysis of cholesterol esters and triglycerides [6]. GlcNAc and ManNAc are responsible for the biosynthesis of sialic acid [7]. Presence of variations in GNE genes are known to impair the enzymes and lead to pathological phenotypes. This study reports the prevalence of mutations c.260G>T; p.G87V and c.894G>A; E8SJM in the lipase gene (LIPA) and c.2228T>C p.M743T in GNE gene amongst different religions. We sequenced 1,354 samples for the presence of the variations in LIPA exon 4 and 8 along with GNE exon 13(formerly exon 12). The sample population comprised individuals of varying Middle Eastern religions such as Mizrahi Jewish, Muslims, Christians, Baha'i, Catholic, Buddhist and Zoroastrian. The other religions that were not Mizrahi Jewish were classified under "Non-Jewish" to have a more even distribution. LIPA gene exon 4 variation p.G87V had a heterozygous frequency of 22 in 1,354(1.62%) with 20 of 22(90.9%) heterozygotes being Mizrahi Jewish while 2 of 22(9.09%) heterozygotes were Non-Jewish. Exon 8 splice junction mutation E8SJM, G>A transition at position 1 had a frequency of 2 in 1,354 (0.14%) with 2 of 2(100%) heterozygotes being Mizrahi Jewish. GNE exon 13 variation p.M743T (formerly exon 12 p.M712T) had a mutation frequency of 58 in 1,354(4.28%) with 5 of these 58(8.62%) samples being homozygous while 53 of the 58(91.37%) were heterozygous. In 58 samples that yielded a mutation, 54 of them were Mizarhi jewish (93.1%).

Introduction

Human GNE gene encodes for the bifunctional enzyme uridine diphosphate (UDP)-N-acetylglucosamine (GlcNAc) 2-epimerase/N-acetylmannosamine (ManNAc) kinase [5]. This enzyme catalyzes rate-limiting steps involved in the biosynthesis of 5-N-acetylneuraminic acid (Neu5Ac), or more commonly known as sialic acid [7]. The biosynthesis of sialic acid takes place in the cytosol where glucose is converted to UDP-GlcNAc, which is the substrate for the GNE enzyme [8]. The GNE gene is located on chromosome 9 and has 13 exons [9]. Mutations within this gene lead to the under production of sialic acid and cause a rare inherited disease GNE myopathy, which is characterized by skeletal muscle atrophy.

Neu5Ac is the most prevalent sialic acid in human cells and is an essential sugar chain for glycoproteins and glycolipids involved in many biological processes [10]. Currently, GNE has several mutations that can lead to dysfunction of the UDP-N-GlcNAc 2-epimerase domain of the enzyme in the rate-limiting step of converting UDP-GlcNAc to ManNAc [11]. The underproduction of Neu5Ac means that the addition of it to glycoproteins and glycolipids is also decreased leading to hyposialylation. The decreased addition of Neu5Ac is one of the causes of this myopathy [12].

GNE myopathy has previous names including hereditary inclusion body myopathy (HIBM), inclusion body myopathy type 2 (IBM2) or Nonaka myopathy [13]. GNE myopathy is a later onset disease, with clinical symptoms presenting from ages 20 to 40 [14]. These signs consist of foot drop, ataxia, and the inability to raise toes. These manifestations are the result of progressive skeletal atrophy. Currently, there is no cure for GNE myopathy. Early diagnosis is critical for limiting the progression of this disease. Following a diagnosis, treatments consist of physical therapy and supplementation of sialic acid or its precursor, but there are many treatments that are being actively researched such as gene theory [15].

Lysosomal acid lipase (LAL) is an enzyme that is encoded by the LIPA gene [4]. LAL functions in the lysosome and catalyzes the hydrolysis of cholesterol esters (CE) and triglycerides (TAG) [6]. LAL is essential for lipid metabolism as it regulates lipids and cholesterol levels by releasing CEs and TAGs in order to be degraded [6]. The LIPA gene is located on chromosome 10 and has 10 exons [17]. Variants of this gene could lead to LAL deficiency (LAL-D), a disorder that is characterized by impaired LAL production. LAL-D disrupts the degradation of CEs and TAGs, and allows infiltration of lipids into tissues throughout the body [18].

LAL-D has two known clinical manifestations that can result from several mutations on the LIPA gene. The phenotype of the disease is a result of which specific mutations have occurred. The mutations could result in Wolman's disease (WD) - the more debilitating early onset variant and Cholesteryl Ester Storage Disease (CESD) - the later onset variant that is not as severe [19] in all cases, patients that exhibit LAL-D show hepatic dysfunction, dyslipidemia, and elevated levels of Low-density lipoprotein (LDL) [20].

Dysfunction of LAL allows lipids to build up in the lymph nodes, spleen, liver, and bone marrow. In early life, WD patients are healthy but quickly develop malnutrition in the first months of life and often die within the first year of life. Early screening for WD is very important to incorporate treatments such as bone marrow transplant, enzyme replacement therapy, and gene therapy. The symptoms of CESD are profound in the later stages of life. CESD presents with hepatic complications that resemble what is seen when patients have chronically elevated lipid levels. When treating CESD the implementation of treatment should not be delayed as, it can progress to cirrhosis and severe hepatic complications.

Due to GNE myopathy and LALD only being diagnosed by mutations along their genes, prevalence rates are not accurate. However, previous studies have estimated that this GNE myopathy affects 1 in every 1,000,000 births while LALD affects 1 in every 350,000 births worldwide [3,4]. These diseases happen in all religious groups, but are especially common in religious groups that are in the Middle East at a rate of 1 in every 4,2000 births.

In this study, to refine the understanding of the prevalence of these missense mutations in the GNE and LIPA genes across religious groups from the Middle East, we analyzed 1,354 samples of individuals of religious groups from the Middle Eastern within the Los Angeles community for the following LIPA variations: LIPA gene variation c.894G>A and c.260G>T and GNE gene exon 13 variation: c.2228T>C.

Materials and Methods

In our study, we analyzed variations in the LIPA gene and GNE gene from 1,354 samples of individuals with religions from people of Middle Eastern background within the Los Angeles community. The 1,354 samples were collected from various Middle Eastern communities in Los Angeles with different religious and cultural backgrounds. The sample population comprised individuals of varying Middle Eastern heritages such Mizrahi Jewish, Muslims, Christians, Baha'i, Catholic, Buddhist and Zoroastrian. The other religions that were not Mizrahi Jewish were classified under "non-Jewish" to have a more even distribution.

The molecular assays defined were performed by Firma Laboratory INC specialized in performing high complexity clinical molecular genetic testing, regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), California Laboratory Field Services (LFS), and accredited by the College of American Pathologists (CAP).

Buccal epithelial cells were collected with the Hydra Flock 6" Sterile Elongated Flock Swab w/Plastic Handle & Dry Transport Tube (Puritan Medical Products, Glendora, United States,) cellular DNA was isolated using the Quick-DNA Kit (Zymo Research, Irvine, United States,) following manufacturer's instructions. DNA optical density was measured using the NanoDrop One

Spectrophotometer (Thermo Fisher Scientific, Waltham, United States) for concentration determination. After DNA quantitation, DNA was diluted using RNAse free H_2O to a concentration of 20ng/µL. Isolated DNA was stored at -20ºC as needed. PCR amplification was done on LIPA and GNE gene exons using BioRad CFX 96 Real Time System. PCR Primers were designed for the amplification of exon 4 and 8 coding regions of the LIPA gene (see Table 1) and exon 13 of GNE gene (formerly exon 12 p.M712T) (see Table 2).

Table 1: Primers designed for Amplification of the LIPA gene Exon 4 and 8.

LIPA Exon	Forward Sequence	Reverse Sequence
4	TATGTAAAACGACGGCCAGT-GGATCTTTTAC GAATGTTCTATTTAGC	TTTAAGGGAACAAAAGCTG-GTACCTCTTCTG CTGGAAGCC
8	TATGTAAAACGACGGC-CAGTTGCTTTGAAG GGCAAAATA	TTTAAGGGAACAAAAGCTG-GTTTCTATTTGGA AAGGGTTTGC

Table 2: Primers designed for Amplification of the GNE gene Exon 13.

GNE Exon	Forward Sequence	Reverse Sequence
13	TATGTAAAACGACGGCCAGT-GGATCTTTTAC GAATGTTCTATTTAGC	TTTAAGGGAACAAAAGCTG-GTACCTCTTCTG CTGGAAGCC

The amplification assay included a 3 min enzyme activation step at 94ºC followed by 35 PCR cycles, each of which involved three steps: denaturation of DNA template and primers for 30s at 94ºC, annealing for 30s at 60ºC, and extension for 1 min at 72ºC.

The amplicon was electrophoresed for 30 minutes at 90V and viewed in a 4% Nusieve® 3:1 plus agarose, to confirm that amplification. The gel was electrophoresed with a 3µL Genemate Quanti-Marker 1kb and 3µL amplicon. The Electrophoresis gel signal was detected on an Epi Chemi II Darkroom (UVP, United States) and viewed using LabWorks (Figure 1).

After amplification was confirmed, the amplicons were then cleaned and concentrated using a ZR DNA Clean & Concentrator™ (Zymo Research, Irvine, United States) kit according to the manufacturer's instructions and eluted with triple-distilled water to a final volume of 30µL. Optical densities and DNA quantification using the Thermo Scientific NanoDrop One Spectrophotometer. Gene LIPA exons 4 and 8 along with GNE exon 13 (formerly exon 12 p.M712T) were sequenced using the Sanger method. A 15µL reaction mix was prepared for cycle sequencing, comprised of 5µL of 5X sequencing buffer, 2µL of forward and reverse universal primers (1.0µM), 1µL of Big Dye Terminator® (Thermo Fisher, West Hills, United States), and 7µL of 5ng/µL amplicon product. Cycle sequencing was attained with an enzyme activation step of 1 min at 96ºC followed by 25 PCR cycles, each included denaturation for 1 min at 96ºC, annealing for 5s at 50ºC, and extension for 1.5 min at 60ºC. Cleaning of the mix using the ZR DNA Sequencing Clean Up Kit™ (Zymo Research) and eluted with 10µL of HiDi™ Formamide. Eluted samples were diluted 1:10, with a total volume of 10µL and loaded for Sanger sequencing on the AB (Applied Biosystems) 3130xl Genetic Analyzer.

Electropherograms were visually examined for peak shape and height to confirm nucleotide identity using the Sequencher 4.9 software. Results were aligned and compared to reference assembly human DNA by the Human Genome Sequencing Number NM_001127605 for LIPA and NM_001374798 for GNE.

Results

Mutations of the LIPA and GNE gene can be found across all populations of people but are most prevalent in the religions of people that have Middle Eastern descent. This study follows the mutation c.260G>T and c.894G>A in the LIPA gene and c.2228T>C in the GNE gene. Variant c.260G>T was found on exon 4 and results in a glycine to valine amino acid change (Figure 6). Variant c.894G>A was found on exon 8 and resulted in splice junction mutation causing the mRNA to lack amino acids (Figure 7). Variant c.2228T>C resides on exon 13 (formerly exon 12) of the GNE gene and constitutes a methionine to threonine amino acid change Figure 8a and Figure 8b). To evaluate the prevalence of the variants c.260G>T, c.894G>A, and c.2228T>C 1,354 samples collected from the different religious communities in the Los Angeles area were analyzed.

Mizrahi Jews accounted for 54.5% of the study population while 45.5% consist of other religions that are classified under "Non-Jewish" (Figure 2). For the LIPA gene sequencing results showed 24 out of 1,354 heterozygotes (1.77%). This correlates with the already predicted estimate of heterozygotes within this population group.[9] Out of 24 heterozygotes 22 were Mizrahi Jewish (91.6%) and 2 "Non-Jewish" (8.4%) (Figure 3). The GNE gene variant p.M743T (atg>acg) yielded a mutation frequency of 58 in 1,354 (4.28%) with 5 of these 58 (8.62%) samples being homozygous while 53 of the 58 (91.37%) were heterozygous (Figure 4). In these 58 samples that yielded a mutation 54 of them were Mizarhi jewish (93.1%) and the remaining 4 (6.89%) were "Non-Jewish" (Figure 5). The prevalence of heterozygous exon 4 variation, p.G87V (ggc>gtc**)** was 1.62% in this study population. The prevalence of heterozygous exon 8 variation p.E8SJM (cag>caa**)** was 0.147% in this study population.

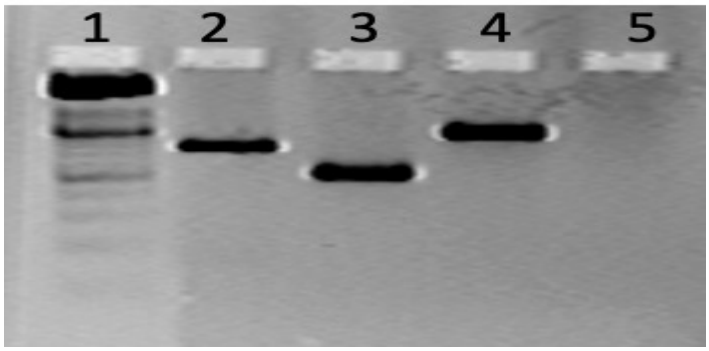


Figure 1: Amplicons from the LIPA exon 4, LIPA exon 8 and GNE exon 13 were amplified and electrophoresed to confirm amplification. From left to right the columns contain the Genemate Quanti-Marker 1kb (1) LIPA E04 (2),LIPA E08 (3), GNE E12 (4), and Negative Control (5).

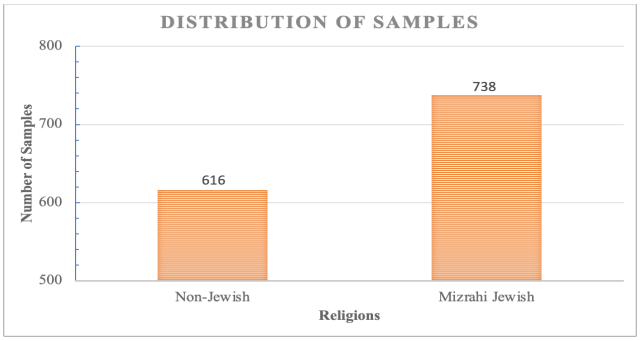


Figure 2: Distribution of samples based on religion within this study.

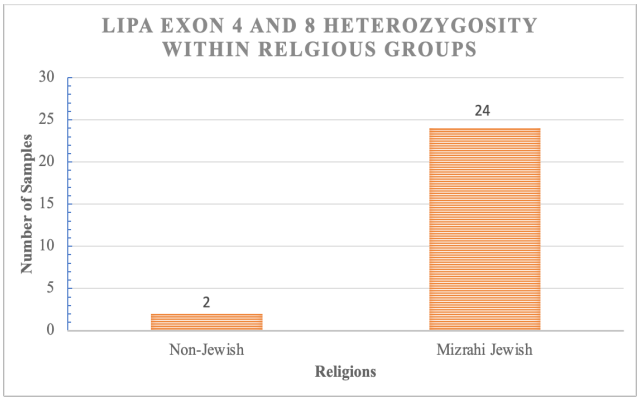


Figure 3: LIPA exon 4 and 8 Heterozygotes that were found within the study based on religion.

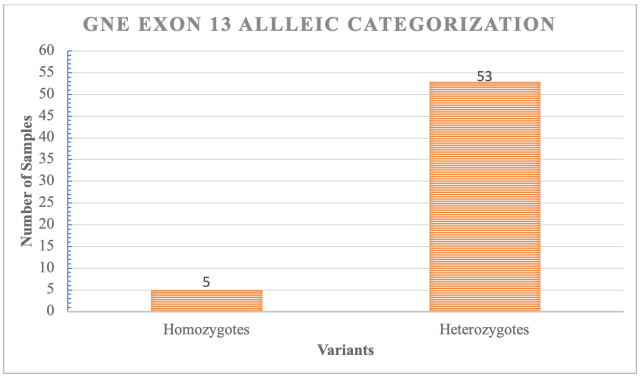


Figure 4: GNE exon 13 heterozygotes and homozygotes found within this study.

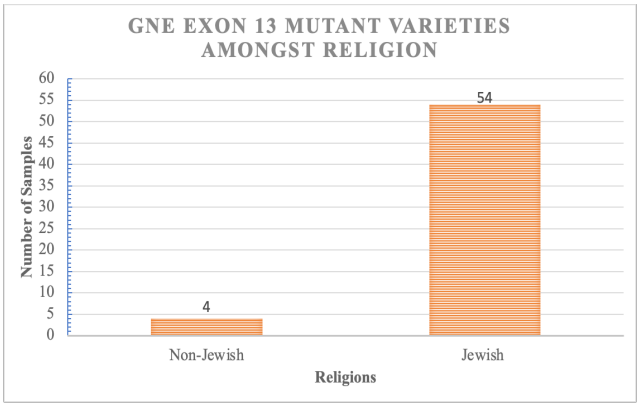


Figure 5: GNE exon 13 mutant varieties categorized by religion.

Figure 6: Electropherogram of exon 4 depicting the heterozygous exonic variation (p.G87V ggc>gtc). The arrow marks the variation.

Heterozygous LIPA Variation p.G87V (ggc>gtc)

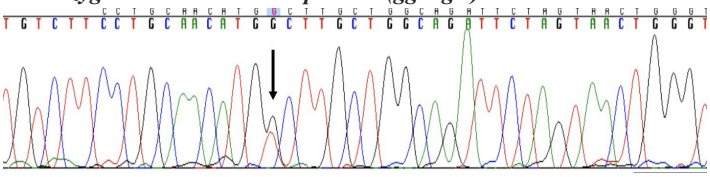


Figure 7: Electropherogram of exon 8 depicting the heterozygous exonic variation (p.Gln298 cag>caa).The variation is marked by the arrow.

Heterozygous LIPA Variation p.E8SJM (cag>caa)

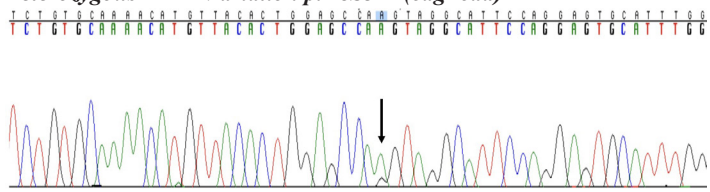


Figure 8a: Electropherogram of exon 13 depicting the homozygous exonic variation (p.M743T atg>acg).The variation is marked by the arrow.

Homozygous GNE Variation p.M743T (atg>acg) (formerly p.M712T)

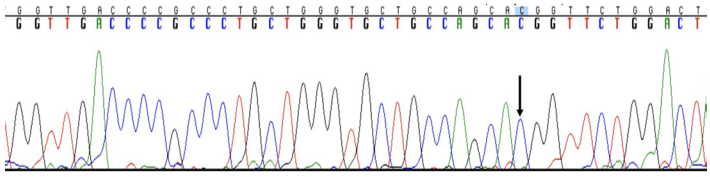
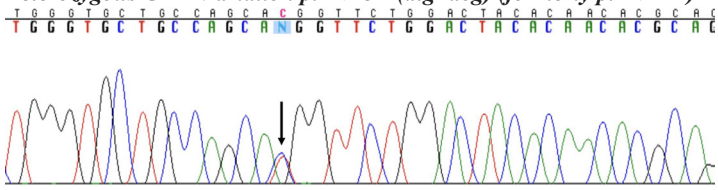


Figure 8b: Electropherogram of exon 13 depicting the heterozygous exonic variation (p.M743T atg>acg).The variation is marked by the arrow.

Heterozygous GNE Variation p.M743T (atg>acg) (formerly p.M712T)



Discussion

Previous studies, which aimed to validate the frequency of mutation within the LIPA and GNE genes amongst this subset of people, have produced comparable results as those found in this study. In a previous study on the mutations along the LIPA gene, 162 samples were sequenced and 5 of the 162 yielded a mutation (3.09%) [4]. This frequency is very comparable to the 24 of 1354 (1.77%). Variant p.M743T (previously known as p.M712) had a study conducted to estimate the mutation frequency. In this study 792 samples were sequenced and 31 of the 792 yielded a mutation (3.91%) [21]. The frequencies from these studies are comparable to the ones found in this study for LIPA 24 of 1,354 (1.77%) and GNE 58 of 1,354 (4.28%). Both studies only focused on Iranian populations but not specifically the religious groups that arise from the Middle East. In this study, religious groups from the Middle East were included to better understand how truly prevalent these mutations are Mizarhi jewish were significantly more prone to the variants mentioned. From this larger study population, more individuals are carriers of the variants that could ultimately lead

to the fatal Wolman's Disease and slowly progressive GNE myopathy. This study suggests genetic screening should be more readily available for people of Middle Eastern descent in order to improve the likelihood of early diagnosis and halt the progression of these diseases.

References

1. Cohen JL, Burfield J, Valdez-Gonzalez K, et al. Early diagnosis of infantile-onset lysosomal acid lipase deficiency in the advent of available enzyme replacement therapy. Orphanet J Rare Dis. 2019; 14: 198.
2. Mullen J, Alrasheed K, Mozaffar T. GNE myopathy: History, etiology, and treatment trials. Front Neurol. 2022; 13: 1002310.
3. Carrillo N, Malicdan MC, Huizing M. GNE Myopathy: Etiology, Diagnosis, and Therapeutic Challenges. Neurotherapeutics. 2018; 15: 900-914.
4. Valles-Ayoub Y, Esfandiarifard S, No D, et al. Wolman disease (LIPA p.G87V) genotype frequency in people of Iranian-Jewish ancestry. Genetic Testing and Molecular Biomarkers. 2011; 15: 395-398.
5. Celeste Frank V, Vilboux T, Ciccone C, et al. Mutation update for GNE gene variants associated with GNE myopathy. Human mutation. 2014; 35: 915-926.
6. Zhang H. Lysosomal acid lipase and lipid metabolism: new mechanisms, new questions, and new therapies. Curr Opin Lipidol. 2018; 29: 218-223.
7. Pham ND, Pang PC, Krishnamurthy S, et al. Effects of altered sialic acid biosynthesis on N-linked glycan branching and cell surface interactions. J Biol Chem. 2017; 292: 9637-9651.
8. Carrillo N, Malicdan MC, Huizing M. Figure 1. [the Biosynthesis of Sialic Acid]. Nih.gov, University of Washington, Seattle. 2023; 15: 900-914.
9. Jing M, Xiao-Jing W, Xu W, et al. A case report: identification of a novel exon 1 deletion mutation in the GNE gene in a Chinese patient with GNE myopathy. Medicine. 2020; 99: e22663.
10. Shyamasree G. Sialic acid and biology of life: An introduction. Sialic Acids and Sialoglycoconjugates in the Biology of Life, Health and Disease. 2020; 1-61.
11. Pogoryelova O, González Coraspe JA, Nikolenko N, et al. GNE myopathy: from clinics and genetics to pathology and research strategies. Orphanet J Rare Dis. 2018; 70: 13.
12. Carrillo N, Malicdan MC, Leoyklang P, et al. Safety and efficacy of N-acetylmannosamine (ManNAc) in patients with GNE myopathy: an open-label phase 2 study. Genetics in medicine: official journal of the American College of Medical Genetics. 2021; 23: 2067-2075.
13. GNE Myopathy. Genome.gov. 2019.
14. GNE Myopathy - NORD National Organization for Rare Disorders. NORD National Organization for Rare Disorders, NORD, 14 May 2020.
15. ALL. Orphanet: GNE Myopathy. Orpha.net, 2018.
16. Schulze RJ, Sathyanarayan A, Mashek DG. Breaking fat: The regulation and mechanisms of lipophagy. Biochim Biophys Acta Mol Cell Biol Lipids. 2017; 1862: 1178-1187.
17. Von Trotha KT, Heun R, Schmitz S, et al. Influence of lysosomal acid lipase polymorphisms on chromosome 10 on the risk of Alzheimer's disease and cholesterol metabolism. Neurosci Lett. 2006; 402: 262-266.
18. Leopold C, Duta-Mare M, Sachdev V, et al. Hepatocyte-specific lysosomal acid lipase deficiency protects mice from diet-induced obesity but promotes hepatic inflammation. Biochim Biophys Acta Mol Cell Biol Lipids. 2019; 1864: 500-511.
19. Boldrini R, Devito R, Biselli R, et al. Wolman disease and cholesteryl ester storage disease diagnosed by histological and ultrastructural examination of intestinal and liver biopsy. Pathol Res Pract. 2004; 200: 231-240.
20. Strebinger G, Müller E, Feldman A, et al. Lysosomal acid lipase deficiency - early diagnosis is the key. Hepat Med. 2019; 11: 79-88.
21. Khademian H, Mehravar E, Urtizberea Ja, et al. Prevalence of GNE p.M712T and hereditary inclusion body myopathy (HIBM) in Sangesar population of Northern Iran. 2013; 84: 589-592.