

# Obesity and Niemann-pick-diseases 2: Pathogenesis and Bioinformatics Correlations

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## ABSTRACT

Interest to understand the molecular pathogenesis of obesity is on the rise, since the disease is becoming of global health concerns with consequences of moribundity, other complications and mortality. However, little is known about the pathogenesis association between obesity and the Niemann-pick diseases. This review therefore critically discusses the classical types of obesity with focus on its genetic links with the Niemann-pick diseases in terms of pathogenesis, associated proteins, signaling pathways and bioinformatics analysis. We analyzed the sequence similarity network between the reported genes found implicated in the two diseases pathways respectively. Two nodes of MC/4R-ERK and APPL1 were found connected, an indication of similarity sequence wisely and probably sharing similar cellular function in relation to obesity and Nieman's Pick disease which could be in a similar way with NPC2.

**Keywords:** Obesity; Niemann-pick-diseases; Genetics; Pathogenesis; Signaling pathways; Bioinformatics

## INTRODUCTION

According to the World Health Organization (WHO), obesity and overweight are regarded as abnormal or excessive fat accumulation that presents a risk to the human health. The key measure of obesity is the body mass index (BMI), which is calculated according to individual's weight (in kilograms) divided by the square of the individual's height (in meters). Perhaps, any individual with a BMI of 30 or more is generally considered obese. On the other hands, individual with a BMI equivalent to or more than 25 is considered overweight. Obesity and overweight are major risk factors for several sequelae of chronic diseases, including cardiovascular diseases, diabetes, and cancer. Indeed, they were once considered a problem only in high income countries, nowadays, obesity and overweight are on the rise in the low-and-middle-income countries (LMIC), particularly in urban settings. In 2016, WHO global health observatory reports that 39% of women and 39% of men aged 18 and over were obese and/or overweight. The trend is not only in adults, perhaps, 18% of children and adolescents aged 5-19 were obese or overweight.

Obesity being a chronic metabolic disease is characterized by excessive triglycerides storage in adipose tissue, which is achieved

by adipocyte hyperplasia (increased number) or hypertrophy (increased size) or in most cases both. Indeed, adipocyte hypertrophy is believed to occur before adipocyte hyperplasia and to be the main mechanism of fat mass expansion in obesity [1].

A study by Dinsa and co-workers highlighted the overall association between socioeconomic status and obesity globally. Indeed, in developed countries, obesity is widely considered a condition that affects people of lower socioeconomic status more so than those of higher socioeconomic status. In LMIC, however, the debate continues as to whether obesity primarily affects the poor or the rich with most studies reporting more prevalence among the rich [2].

Niemann-Pick disease on the other hand, has since been used to designate a heterogeneous group of autosomal recessive lysosomal lipid storage disorders, with common features of hepatosplenomegaly and sphingomyelin storage in reticuloendothelial and parenchymal tissues, with or without neurological involvement. Indeed, the disease has been classified into four different subgroups namely, type A, B, C, and D [3]. Niemann-Pick disease type C encompasses disorders characterized by unique abnormalities of intracellular transport of endocytosed cholesterol with se-

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questration of unesterified cholesterol in lysosomes and late endosomes [4]. Niemann-Pick disease type C is believed to share some protein interaction with obesity related signaling pathways, and hence could play a key role in the etiology of complex disorders such as obesity [5]. The present study therefore aims to provide an overview on the association in the pathogenesis of obesity and Niemann-Pick disease type C. The references were extracted from the relevant biomedical databases, i.e., PubMed, Embase, Scopus, and Web of Science databases using the following keywords; obesity, overweight, Niemann-Pick diseases, pathogenesis, signalling pathways, body mass index, and bioinformatics.

## BODY MASS INDEX (BMI) AND OBESITY

Even though BMI does not measure body fat directly, however, it has been shown that BMI is moderately correlated with more direct measures of body fat obtained from bioelectrical impedance, skinfold thickness measurements, underwater weighing, dual energy x-ray absorptiometry (DXA) [6,7]. Moreover, BMI appears to be strongly correlated with various adverse health outcomes consistent with these more direct measures of body fatness [8,9]. Therefore, according to these descriptors, the Centers for Disease Control and Prevention (CDC) defined BMI and obesity according to the parameters as reported in Table 1. Furthermore, the CDC classified obesity into three different subcategories also according to BMI of individuals, namely; class I-III, indeed, class III is usually referred to as severe obesity.

**Table 1:** Relationship between BMI and obesity, and classification of obesity.

BMI (Kg/m <sup>2</sup> )	Description/status		
<18.5	Underweight	BMI (Kg/m <sup>2</sup> )	Obesity category
18.5-24.9	Healthy weight	30-34.9	Class I
25.0-29.9	Overweight	35-39.9	Class II
≥ 30.0	obese	≤ 40	Class III

## CAUSES AND RISK FACTORS ASSOCIATED WITH OBESITY

Obesity results from a combination of causes and several contributing factors, including individual behaviors and genetic makeup. Behaviors are related to the individual's dietary intake, sedentary lifestyles, physical activity or inactivity, medication use, and other exposures. Other factors include environment, education and skills, and food marketing and promotion. Obesity is of serious global health concern because it is associated with poorer mental health status, reduced quality of life, and one of the leading causes of death worldwide.

### Behavior

Healthy diet pattern and regular physical activity are among the individual behaviors with direct consequences on person's obesity or weight status. Energy balance of the number of calories consumed from foods and beverages with the number of calories the body uses for activity plays a role in preventing excess overweight. Even though, the individual genetic makeup play role in obesity, it is however believed that obesity is likely to occur in individuals who consumes more calories than necessary [10]. A healthy diet pattern emphasizes on eating fruits, whole grains, vegetables,

lean protein, low-fat and fat-free dairy products and clean drinking water [11]. To maintain a healthy body weight balance, it is recommended that, adults do at least 150 minutes of moderate intensity activity or 75 minutes of vigorous intensity activity, or a combination of both, along with 2 days of strength training per week. Having a healthy diet pattern and regular physical activity is also important for long term health benefits and prevention of chronic diseases such as type 2 diabetes and heart diseases.

### Environment

Individuals may make decisions regarding their lifestyles based on their environment or community. An individual may choose not to walk or bike because of a lack of sidewalks or safe bike trails. Community, home, childcare, school, healthcare facilities, and workplace settings can all influence individual's daily behaviors including physical activity. Therefore, it is important to create environments in these locations that make it easier to engage in physical activity and eat a healthy diet in order to enable individuals to control their body weight and obesity associated risk factors. Indeed, increased automation at job places, home, even in the transportation coupled with extensive urbanization are a greater associated environmental risk factors in obesity [10].

### Genetics and role of family history

The variation in how people respond to the environment that promotes physical inactivity and intake of high-calorie foods suggests that genes do play a role in the development of obesity. Genes give the body instructions for responding to changes in its environment. Studies have identified variants in several genes that may contribute to obesity by increasing hunger and food intake. In most cases, inherited obesity within a family is not caused by a specific variant of a single gene (monogenic obesity). Most obesity, however, results from complex interactions among multiple genes and environmental factors that remain poorly understood, this feature is commonly referred to as multifactorial obesity [12].

Family health history has been a great tool in identifying people at high risk of obesity-related diseases such as diabetes, cancers, and cardiovascular diseases. Family health history reflects the effects of shared genetics and environment among close relatives. Families may not change their genes, but they could change their environment to encourage healthy eating habits and physical activity. Indeed, those changes can improve the health of family members, and improve their quality of life, by-and-large could change the health history of their next generation [5,10,12].

### Other diseases and drugs

Other diseases may lead to obesity or weight gain. These may include polycystic ovary syndrome, and Cushing's disease. Drugs such as antidepressants and steroids may also cause weight gain and obesity. The science continues to emerge on the role of other factors in energy balance and weight gain such as chemical exposures and the role of the microbiome.

## CONSEQUENCES OF OBESITY

### Health consequences

Individuals with obesity often encounter other health related challenges, and in most cases, they are at high risk for many deleterious diseases notably, high blood pressure (hypertension), high levels of low-density lipoprotein (LDL) cholesterol, low levels of high-density lipoprotein (HDL) cholesterol, or high levels of tri-

glycerides (dyslipidemia), Type 2 diabetes, gallbladder disease, coronary heart disease, stroke, osteoarthritis, cancers of endometrial, breast, colon, kidney, gallbladder, and liver. Perhaps, including sleep apnea and breathing problems. Other complications are related to mental illness such as generalized anxiety disorders, and major depressive disorders, body pain and difficulty with physical functioning, low quality of life, and increased mortality.

### Economic and Societal Consequences

Medical costs associated with obesity may have both direct and indirect consequences. Direct consequences are associated to medical costs related to obesity which include diagnostic, treatment and preventive services. On the other hand, indirect costs are related to morbidity and mortality including low productivity. Productivity measures include decreased productivity of employees while at work, and/or costs related to employees being absent from work for obesity-related health problems.

## PATHOGENESIS OF OBESITY AND NIEMANN-PICK-DISEASES

Obesity is a risk factor for a large range of various diseases including but not limited to type 2 diabetic mellitus (T2DM), cardiovascular diseases (CVD), insulin resistance (IR) and dyslipidemia. This is due to its role in many signaling pathways that govern to complications in various diseases. In adipose tissue (AT) expansion, an increase of infiltration leukocytes and activation of many cytokines such as tumor necrotic factor (TNF- $\alpha$ ) was observed. AT, being recognized as a major storage tissue is found participating in an immune response as an endocrine organ. Therefore depending on the energy balance, the AT can perform various functions including lipolysis which produce fatty acid and glycerols as well as glycolysis which esterify both compounds via acyl-CoA formation. Thus, there is a need of energy balance i.e the energy intake should not be greater than its expenditure and if otherwise it will result in the AT responds by recruiting preadipocytes which in a long run will result to a stromal and vascular growth. However, one of the major hallmarks of obesity is chronic inflammation which is characterized by increase in pro-inflammatory cytokines such as TNF- $\alpha$  which can be suppressed using glycine by inducing the activation of (NF- $\kappa$ B), as such most of inflammatory related disease are found implicated in the obesity inflammation cross-talk, such was found as a major cause of female infertility due to excess free fatty acid that intoxicate reproductive tissues and lead to a serious cellular damage with a low grade inflammatory state. Obesity affect females not only in infertility related pathways but also on age related signaling pathways whereby Nogging was found expressed in progenitor cells but refused in adipocytes which is believe to possibly allow for lipid accumulation, as such this promote age related obesity in both genders. Obesity continues to be a public health problem in this generation and also increases the risk for the development of heart failure through the analogy of the term obesity paradox. In line with this, Alpert et al. stated that some certain neurohormonal and metabolic alterations normally occur in obesity may to some extent alter the cardiac structure and function. Among which include the activation of sympathetic nervous systems and renin-angiotensin-aldosterone as well as hyperleptinemia due to leptin resistance, insulin resistance with hyperinsulinemia and possibly a cardiac lipotoxicity. It's also known that obesity contribute greatly to the sudden cardiac death. For this reason, there is a speculation that this association may be related to delayed ventricular repolarization (VR).

With reference to Neimann pick disease type C (NPC) which is a fatal neurodegenerative condition which is yet to have FDA-approved therapy was believed to share a protein interaction with obesity related signaling pathways due to its heterogeneity complex with many other neurological implications. As such, Shin et al. carried out a genome-wide transcriptome analysis to identify the key pathways involved in early NPC. Their results described a novel activation pattern of interferon downstream signaling in pre-symptomatic NPC, as well as atypical pattern of interferon downstream signaling that involves both IFN- $\beta$  which was found linked with obesity as well. However, despite the fact that there is accumulation of evidence that both NPC1 and NPC2 are involved in cellular cholesterol trafficking, the exact mechanism that link it to brain remain unknown. Another study revealed that NPC2 transports cholesterol to the membrane through collision, which implies interaction between NPC2 and unknown membrane receptor protein. Tissue distribution analysis revealed that NEGR1 was found highly expressed in brain especially in cerebral cortex. As such a new finding revealed that NEGR1 may directly interact with NPC2 and the overexpression is an indication of abnormal cholesterol accumulation suggesting that NEGR1 may be the membrane binding partner of NPC2 protein that help in cholesterol transportation especially in brain.

## OBESITY AND NIEMANN-PICK-DISEASES ASSOCIATED PROTEINS

There are many proteins reported to be directly or indirectly be associated with obesity and Niemann-pick-diseases. This is governed by their expressions or silencing due to some expressional effect or otherwise. Such as in Nafamostat mesilate used for the treatment of some diseases like cancer, the physiological process is enhanced by the demethylated fat mass and obesity-associated protein (FTO). In humans, the variations of FTO gene have been linked to obesity and have an effect on neuronal functions through dopamine receptor D2. Moreover, neuroplasticity-associated protein's level could be decreased in the presence of excessive endoplasmic reticulum stress (ERS) which could be induced by dietary obesity. In addition, Nasser et al. found that, FTO polymorphism to be a potential predictor of type 2 diabetes by significantly affecting the development of insulin among Iraq population. On the other hand, we believe physical exercise play a great role in controlling obesity, however the effect of that need the help of some genes to activate certain pathways for the effect to fully manifest. In that regard, acute exercise was proven to play such a role by increasing serum adiponectin level as well as adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1 (APPL1) content in hypothalamus, which followed by reduced food intake in obese mice therefore controlling the food intake in mouse model. A newly raft-associated protein called neuronal growth regulator 1 (NEGR1) was found associated with human obesity, its defect in a mouse model shows an increased cholesterol levels and triglyceride, this possibly explaining the missing link between NEGR1. Through such a link, a cholesterol intercellular interplay between the NEGR1 and Niemann-pick-disease type C2 (NPC2) protein occur, which its defect is linked to a fatal human neurodegenerative disease. GARP complex is also involve in this intercellular trafficking while transporting cholesterol through targeting NPC2 to lysosomes. On the other hand, absence or deficiency of some proteins helps to induce obesity. Such as phospholipid transfer protein (PLTP) deficiency attenuates high fat diet and indirectly induced obesity

by increasing insulin sensitivity. An inflammation related gene expression studies revealed that, the level of Annexin A1 (Anx1) were higher in individuals with obesity also its level with IL-6 was found correlated.

## OBESITY SIGNALING PATHWAYS

As obesity was found to relate with many diseases, it also, share many signaling pathways through the process of gene-regulations and expression. A new finding identify IFN $\beta$ -macrophage pathway as a mechanistic link between obesity and accelerated atherogenesis. Moreover, in obese patients GPVI signaling pathway activation was found to increased which simultaneously increased the levels of obese platelets. While trying to regulate monogenic form of obesity Kühnen et al. identified Melanocortin signaling pathway as a potential for weight regulation. This will help to minimize rate of feeling hunger when fully exploited. However, apart from weight loss, feeding behavior needs to be regulated as well, in this regard via MC3/4R-ERK signaling pathway Hypothalamic nesfatin-1 was found mediates the feeding behavior in rats. Moreover, through hypothalamic-pituitary-adrenal (HPA) axis, obesity is frequently accompanied by neuroendocrine changes. Looking at the intricate relationship between the two, tackling one of them may affect the other. In line with that, growing evidence suggests the role of gut microbiota in obesity and brain functioning through the modulation of the inflammatory response and HPA axis. On the other hand, just like in murine, human obesity also share similar status of metaflammation and cyclic geonidine monophosphate (cGMP) signaling pathway. Correspondingly, the cGMP cascade was found dysregulated in gonadal but not in inguinal fat of induced obese mouse. while trying to understand different pathways that link diabetics with other diseases as essential key towards its treatment, Ca<sup>2+</sup>/cAMP signalling pathways believed to play an essential role in cell physiology, that results to a debate for the possible involvement of Ca<sup>2+</sup>/cAMP signalling in the clinical link between diabetes and a higher risk for the development of several types of diseases.

## GENE INTERACTION ANALYSIS USING BIO-INFORMATICS APPROACH

Based on the recent development in trying to find a link between the two pathologically related diseases, we decide to look into the combination of the reported genes that play roles in both obesity and/or Neimann-pick diseases, and find out their relationship from their sequences by designing a phylogenic tree as shown in Figure 1. We extracted their sequence from NCBI data base using their names and accession number assigned for sequence easy identification and specificity (Table 2).



**Figure 1:** Network analysis and Edge closeness view.

**Table 2:** Genes playing role in the signaling pathways and gene-regulation function related to obesity and/or Neimann-pick diseases.

List of genes	Played roll in Obesity	Played role in Neimann-Pick Disease
NEGR1	Yes	Yes
NPC2	*	Yes
NPC1	*	*
GARP	Yes	*
PCTP	Yes	*
Annexin A1	Yes	*
IL-6	Yes	*
IFN	Yes	*
MC/4R-ERK	Yes	*
HPA	Yes	*
cGMP	Yes	*
CAMP	Yes	*
TNF1alpha	Yes	*
NF-kB	Yes	*
APPL1	Yes	*

Note: \*stands for not yet discovered

## CONSTRUCTION OF SEQUENCE SIMILARITY NETWORK (SSN)

An SSN for all the mentioned genes was constructed by using the option D of the Enzyme Function Initiative (EFI)-Enzyme Similarity Tool (EFI-EST). This option enables the use of IDs from different data-base, as such we used UniProt IDs of all the proteins for sequence retrieval. The similarities between these sequences was calculated and finally the SSN was generated based on the sequence similarities. Moreover, the created network was filtered out and visualized using by using Cytoscape version 3.7.2. Two nodes were normally connected with edges if they shared a sequence identity of  $\geq 35\%$  at an e-value threshold of  $\leq 10^{-5}$ . Finally, as described in Figure 1, we were able to get 2 nodes connected to each other with edges out of the 13 nodes in the SSN and the 2 nodes were analyzed (Figure 1).

## MULTIPLE SEQUENCE ALIGNMENTS AND PHYLOGENIC ANALYSIS

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length=15.14665758 is shown. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pair-wise deletion option). There were total of 12681 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.



**Structural analysis**

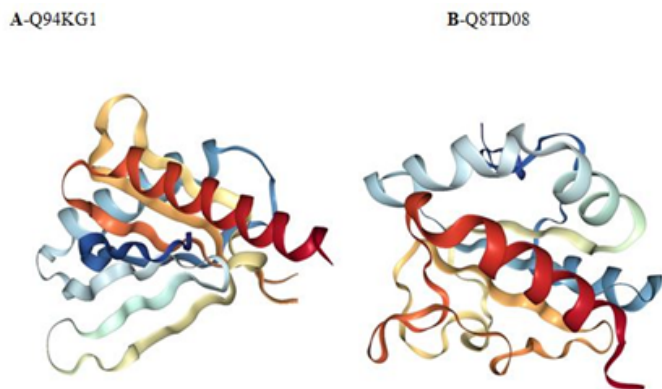
The Protein Data Bank (PDB) was searched throughout December 2019, using the unique UniProt IDs of HPA and APPL1 respectively. This is done in-order to determine the 3D structures that are available for the two correlated genes. The structure with the best resolution was selected and presented. 3D of the two selected structures were compared by installing a local version of TM-align software. This software provides us with the best protein structural alignments that combine TM-score matrix. Moreover, TM-score has more sensitivity in terms of topology of proteins than to a variation in terms of local structure. As such based on that, a TM-Score of 1 indicates a perfect match (Figures 2-7).



**Figure 2:** GNT generated and visualized using Cytoscape for network visualization.

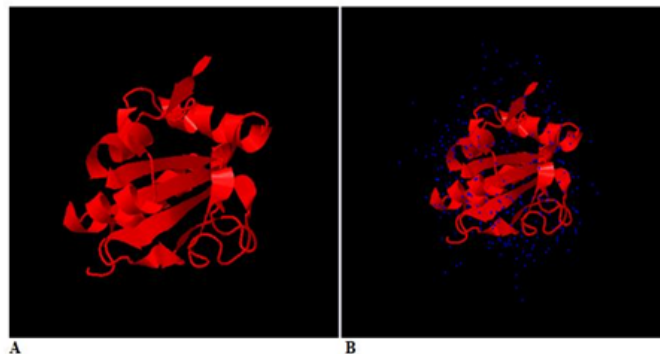


**Figure 3:** Layout based on age-weighted force directed for all nodes (Edge Betweenness).

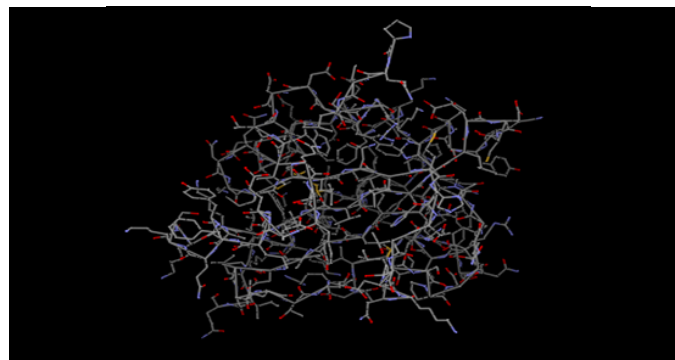


**Figure 4:** APPL1 and HPA 3D structure A and B respectively,

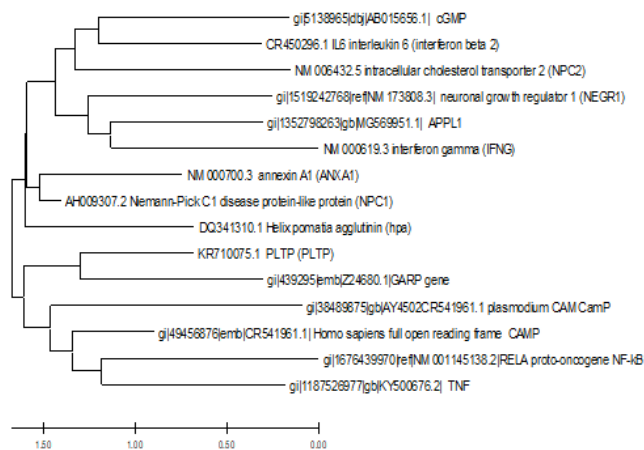
viewed from Protein Data Base (PDB).



**Figure 5:** TM-align between APPL1 (1) and ERK (2). Protein 1 in blue protein 2 in red. A shows superposition of the two proteins while B indicates superposition of two proteins with ligands and solvents.



**Figure 6:** 3D molecular structure showing the superposed full-atom structure in the aligned region.



**Figure 7:** Phylogenetic analysis showing multiple sequence alignments among the reported genes based on their sequence similarity. Which indicates the closeness of the respected genes.

**CONCLUSION**

As highlighted, there are several correlations in the pathogenesis of obesity and Neimann-pick diseases at molecular levels. Perhaps, to provide an overview about the list of genes identified playing role in the two diseases directly or indirectly, based on the recently published data, we grouped those genes and constructed SSN to see if they are related to one another sequence wise so

that we may have a deep insight about their relationships through signaling pathways and network function. The resulted network consists of 13 nodes and an edge connected to the 2 nodes. The nodes represent a group of sequences that share identity. We also proceed to check the Genome Neighborhood Network (GNN) of the SSN data generated. Based on the minimum co-occurrence value of 20% and neighborhood size of 10, the two genes APPL1 and ERK were found connected with a large Hub-node size and strong Edge connection when visualized from Cytoscape using layout option as well as Network analysis from the tool option. This indicates correlation between the two genes that are both implicated in obesity signaling pathway. On the same vein, APPL reduce the fasting glucose of obese mice and increased leptin-induced hypothalamic p-ERK, while feeding behavior needs to be regulated as well, in this regard via MC3/4R-ERK signaling pathway hypothalamic nesfatin-1 was found to mediate the feeding behavior in rats through HPA axis. This complex of pathways and interaction of these two genes might be a derivative link that may connect the pathology of obesity with Nieman's pick diseases. Moreover, considering the latest finding with NEGR1 that shows a linkage between the two diseases through NPC2, a phylogenetic analysis from a relative sequence alignment and close relationship between the two reported genes (NEGR1 and NPC2) and our predicted gene APPL1. The sequence similarity might also be a reason for their molecular function in the cholesterol trafficking. However, while constructing the phylogenetic tree, ERK gene was excluded for the analysis due to its central role in many signaling pathways so as to improve the reliability of the result and have more clear alignments between the remaining genes. Furthermore, the structural analysis between the two genes shows a TM-score of 1, indicating a perfect match between the two aligned genes, predicting the importance of this ERK-APPL1/HPA pathway as a potential target for Nieman's pick disease as demonstrated in diabetes using mice models. Moreover, closeness between NEGR1 and APPL1 from phylogenetic tree may also give some explanation in-vivo towards understanding the pathology of Nieman's pick diseases in relation to diabetes.

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## DECLARATION OF CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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