In Silico Analysis of Missense Deleterious Mutations of Human *MBOAT4* Gene and Predicting Their Structural Effects on Protein Dynamics

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ABSTRACT

Introduction: Obesity is a multifactorial disease marked by an abnormally high level of fat deposition in various body tissues (1). Obesity is not only a cosmetic concern in life; it is also a health-associated issue that increases the risk of other problems and diseases, such as heart disease, diabetes, high blood pressure, and certain cancers (1). MBOAT4 (membrane bound O-acyltransferase domain containing 4) gene product, *i.e.*, ghrelin O-acyl transferase (GOAT), that catalyzes the acylation reaction necessary for the activation of GOAT (2-4). Acylated GOAT (MBOAT4 gene) has the highest tissue expression in the stomach compared to other tissues and plays a significant role in the regulation of appetite and metabolism (5-6). Therefore, GOAT has been expected to be a novel antiobesity target because it is responsible for acyl-GOAT production (2-5). SNPs (single nucleotide polymorphisms) are the most common genetic differences observed in populations, and they aid in predicting an individual's response to specific drugs, susceptibility to environmental factors such as toxins, and proclivity to develop diseases. SNP-level investigation can be utilized to track the inheritance of disease-associated genetic variation within families (6-7).

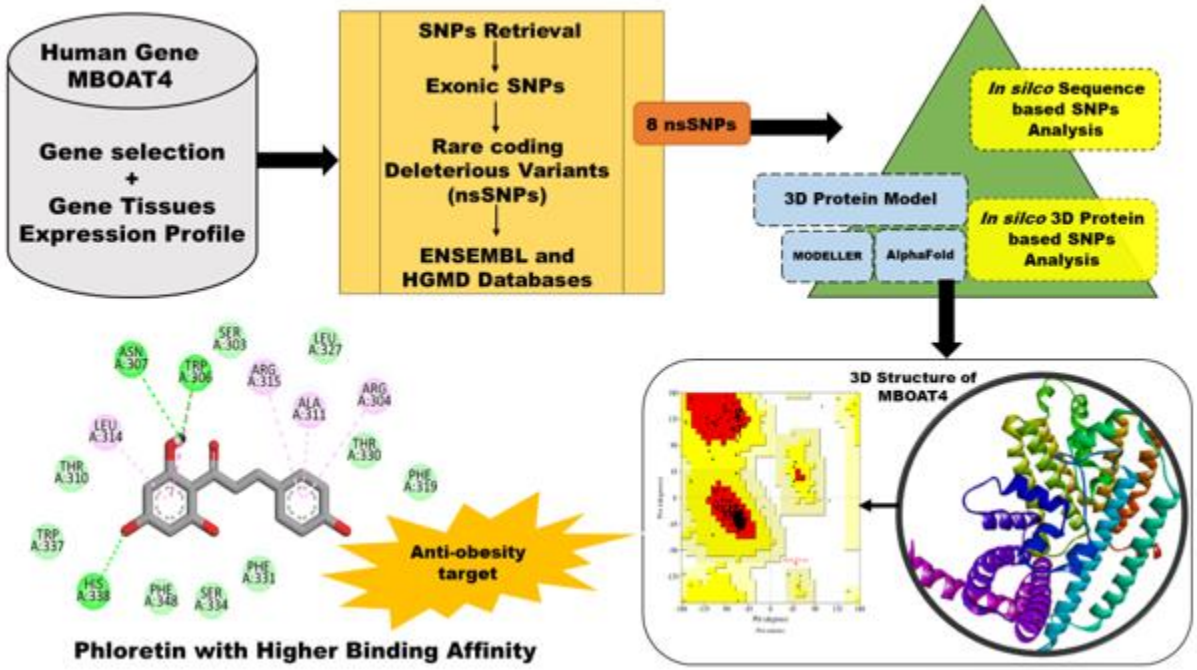
Objectives: This study used various *in silico* tools to investigate gene (MBOAT4) SNPs, the effect of region-specific non-synonymous variations, and the mutational impact on protein functioning and stability. This investigation identifies potential therapeutic targets to manage the challenge of obesity and corresponding protein cavities as well as to explore their binding interactions with intervening compounds.

Methodology: In this work, we extracted around 625 exonic SNPs from the ENSEMBL database and one phenotype-based missense mutation associated with obesity (A46T) from the HGMD (Human Gene Mutation Database) (7-8). The SNPs from the MBOAT4 gene were differentiated on the basis of potentially harmful missense SNPs through the recruitment of a MAF (minor allele frequency: < 0.01) cutoff in correlation with some bioinformatics-based supervised machine learning tools (7). Various machine learning *in silico* tools were recruited to investigate the potentially deleterious missense mutations, which decrease the stability of protein molecules, across the entire mutations dataset (7).

Results: We found 8 rare-coding, harmful missense SNPs. The consensus classifier (*PredictSNP*) tool predicted the SNP (G57S, C: rs561065025) as the most pathogenic of the rest. Several trained *in silico* algorithms predicted a decrease in protein stability [ΔΔG (kcal/mol)] function in the presence of these eight single-point, rare-coding pathogenic mutations in the MBOAT4 gene. After structural protein identification, the stereochemical quality check, i.e., validation and assessment, of the 3D model was performed. Initially, a blind cavity docking approach was used to search the druggable cavities and molecular interactions with citrus flavonoids of the *Rutaceae* family, ranked with energetic estimations. Significant interactions were also observed with Phloretin 3',5'-Di-C-Glucoside at R304, W306, N307, A311, L314 and H338 (GEMDOCK: -95.82 kcal/mol and AutoDock: -7.80 kcal/mol).

Conclusion: The MBOAT4 gene and its molecular interaction analysis could serve as an interventional future anti-obesity target. The findings of the current study will be beneficial in future prospects for large population-based studies and drug development, particularly in generating personalized medicine.

Keywords: MAF, MBOAT4, Phloretin 3',5'-Di-C-Glucoside, SNP.



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