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INPS and I-Mutant 2.0: Predicting the Impact of Variations on Apoe Protein Stability

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Abstract: Cardiovascular diseases are associated with apolipoprotein E (ApoE), a crucial lipid transport protein. APOE gene mutations can have a substantial impact on protein stability, which can alter treatment approaches and disease risk. This study uses I-Mutant 2.0 and INPS, two computational techniques, to assess the effect of 31 single nucleotide variants (SNVs) on ApoE stability. In I-Mutant 2.0, mutations are classified as either destabilizing or stabilizing, while INPS gives numerical ΔΔG values. The findings suggest that 77.4% of mutations result in reduced stability, with the largest destabilizing effects occurring in L38P, W58S, and L72P. G9V and A85V were the most stabilizing mutations, with just 7 mutations (22.6%) projected to increase stability. Even though the two tools have a 71% consistency rate, there are some differences, especially between L38P and R49P, which show how different the computational techniques are. According to the results, stabilizing mutations may offer therapeutic insights, while destabilizing variants may be linked to ApoE malfunction and the advancement of disease. For ApoE-related disorders, future research should combine clinical data, molecular simulations, and experimental validation to improve mutation

impact predictions and develop tailored treatment.

Keywords: ApoE gene, Cardiovascular Disease, INPS, I-Mutant 2.0, Protein Stability.

Introduction

An essential protein in lipid metabolism, apolipoprotein E (ApoE) is involved in both neural repair and the transport of cholesterol. ApoE2, ApoE3, and ApoE4 are the three main isoforms that differ by single amino acid alterations that are encoded by the APOE gene(1). Finding mutations in the gene that produces the ApoE4 protein could impact the protein's stability and structure. Given that this gene is one of the genes most closely linked to cardiovascular disorders and Alzheimer's disease (AD), this is seen as a highly significant issue (2). These mutations may impact the ApoE protein's stability, which may impact the protein's typical activity and contribute to the development of the disease (3; 4). The best techniques for evaluating protein stability are X-ray crystallography and nuclear magnetic resonance (NMR). Although these techniques are costly in terms of time and money, they can be applied experimentally (5).

Protein stability is one of the most important factors that may affect the ability of ApoE to interact with lipids and their receptors. Protein conformational changes resulting from mutations may cause misfolding, aggregation, or loss of function—all of which are indicators of many diseases (6). For example, in neurodegenerative conditions, ApoE4 is less stable than ApoE3, all of which is due to domain interactions that support pathological processes. Therefore, to understand the disease mechanism and possible treatment options, the use of tools to predict changes in stability resulting from mutations is a powerful way to explore such issues. Therefore, INPS-Mutant 2.0 These two algorithms or tools are among the most common computational prediction methods to determine how variations affect protein stability clearly (7). Sequence or structure can provide information through which I-Mutant 2.0 can predict, as the principle of this tool is based on support vector machine (SVM), where it can predict changes in protein stability when a point mutation is detected (8). It will give ΔΔG (delta delta G) values, which will indicate whether the protein is stable or unstable due to a mutation. Similarly, INPs, this technique relies on machine learning to predict changes in protein stability based on the information between sequence and structure (9). It is necessary to understand the effect of mutations in the ApoE gene because through it drugs can be developed, as computational methods help in that. This study aimed to study the prediction of the extent of the effect of ApoE changes on protein stability, and a report can be given about the potential pathogens using I-Mutant 2.0 and INPs.

Materials and methods

Database Extraction for SNPs

The sequence of Apo E protein and its related SNPs list were retrieved from UniProtKB. The information collected from NCBI at (http://www.ncbi.nlm.nih.gov/projects/SNP) helped in most computational studies.

Impact of Non-Synonymous Mutations on Protein Stability (INPS)

Non-Synonymous Mutations' Effect on Protein Stability, or INPS According to (10), A machine learning method that can predict how variations affect protein stability is INPS, by combining sequence and composition data. INPS is a good tool for protein engineering and analysis of disease-associated mutations because it can perform diagnostic functions to produce more accurate predictions than I-Mutant 2.0, which primarily uses SVM models (10).

I-Mutant 2.0 prediction method

Using structural and sequencing data, I-Mutant 2.0 is an SVM-based technique that forecasts changes in protein stability upon point mutations (11). It gives ΔΔG (delta delta G) values, which show if a protein is stabilized or destabilized by a mutation. This instrument is frequently used to investigate the structural effects of mutations in ApoE and other disease-related proteins.

Table 1: List of Bioinformatics tools in this study

Algorithm	web
INPS	http://inpsmd.biocomp.unibo.it
I-Mutant 2.0	http://folding.biofold.org/imutant/imutant2.0.html.

Results Table 2: List of nsSNP analysis by INPS and I-Mutant 2.0 tool.

Gene	Nucleotide change	Amino acid change	INPS prediction	I-Mutant 2.0 prdiction
1	C>T	P4S	-0.00688175	Decrease
2	G>T	G9V	0.932133	Increase
3	G>C	R15P	-0.398151	Decrease
4	G>C	G21A	0.30218	Increase
5	G>A	G22S	0.280489	Decrease
6	G>A	G26E	0.295372	Decrease
7	C>T	R36W	0.438601	Decrease
8	G>A	R36Q	-0.324858	Decrease
9	G>T	A37S	-0.0312911	Decrease
10	T>C	L38P	-0.699889	Increase
11	C>A	L39M	0.142472	Decrease
12	C>G	S44C	0.571318	Decrease
13	C>T	P46S	0.0423885	Decrease
14	C>G	P46R	0.457244	Decrease
15	G>C	R49P	-0.398151	Increase
16	G>A	V50M	0.186403	Decrease
17	G>A	G53R	0.63639	Decrease
18	G>A	S56N	-0.188673	Decrease
19	G>C	W58S	-1.02871	Decrease
20	G>T	W58C	-0.737804	Decrease
21	C>T	R60W	0.438601	Decrease
22	G>A	V63I	0.230379	Decrease
23	A>G	E67G	-0.236884	Decrease
24	C>T	P68S	0.0423885	Decrease
25	T>C	L72P	-0.623685	Decrease
26	C>A	P79T	0.178888	Decrease
27	G>T	R80L	0.456892	Decrease
28	G>A	A81T	0.176687	Decrease
29	C>T	T84I	**1.0735**	Decrease
30	C>T	A85V	0.908045	Increase
31	G>A	G100R	0.63639	Decrease

The set of data includes predictions from two computational methods, I-Mutant 2.0 and INPS, as well as 31 single nucleotide variants (SNVs) in the ApoE gene. One important aspect affecting ApoE's function and illness connections is protein stability, which these methods predict would increase or reduce. INPS Predictions: Quantitative values; higher stability is suggested by positive scores, whereas destabilization is indicated by negative scores. I-Mutant 2.0 Forecasts: An "Increase" or "Decrease" category output according to ΔΔG values. Strong Destabilizing

Effects of Mutations Indicating possible adverse impacts on ApoE function, the L38P, W58S, W58C, and L72P mutations received negative INPS scores and were projected as "decrease" by I-Mutant 2.0. The biggest destabilizing effects are seen in L38P and W58S, which could have a major effect on the structure and function of ApoE. The exception is T84I, which has a high INPS score of 1.0735 but is expected to lose stability as a result of I-Mutant 2.0. I-Mutant 2.0 anticipated that only seven mutations would improve stability. These might improve ApoE activity or change how it interacts with receptors and lipids. According to INPS, G9V and A85V are very stabilizing, which would suggest that ApoE has structural reinforcing effects. Results from L38P and R49P are contradictory; I-Mutant 2.0 predicts a stability increase, whereas INPS suggests instability.

Discussion

The majority of mutations tend to reduce the stability of proteins; according to I-Mutant 2.0, 24 out of 31 (77.4%) were classed as destabilizing(12).The infrequent stabilizing effects in ApoE are suggested by the fact that only 7 mutations (22.6%) exhibit improved stability(13). The pattern that many mutations result in protein instability is supported by the majority of INPS values being negative. Possible Mutations Linked to Disease Destabilizing mutations (such L38P, W58S, and L72P) may cause ApoE to misfold, aggregate, or malfunction more frequently, which could lead to cardiovascular diseases. By improving ApoE function, stabilizing mutations (such as G9V and A85V) may provide information for therapeutic protein engineering. To verify their effect on ApoE function, predicted destabilizing mutations must be experimentally validated. Molecular dynamics simulations could evaluate conformational flexibility and improve stability predictions(15-17).In neurodegenerative and cardiovascular disorders, clinical data integration may establish a connection between certain mutations and patient outcomes (18-20).

Conclusion

Using INPS and I-Mutant 2.0, this study examined 31 ApoE mutations and found that the majority of them reduce protein stability, which may have an effect on ApoE's function in the pathophysiology of disease. There are some differences between the two tools, despite their general agreement, which emphasizes the necessity of complementary computational methods and experimental validation. With implications for disease research, medication development, and therapeutic interventions, these findings advance our understanding of ApoE stability variations.

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