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Computational Studies and Scaffold Search for APOE4 as Coronary Artery Disease Target by Virtual Screening

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Abstract: Apolipoprotein E (APOE) polymorphism is involved in the pathogenesis of atherosclerosis and conveys a higher risk of coronary artery disease (CAD). The structural features of the isoforms (APOE2, APOE3, and APOE4) differ by only single amino acid that explicate their unique functions as lipid transporter with a role in cholesterol metabolism. It is therefore hypothesized that the cysteine/arginine change at position 112 results in structural differences within APOE3 and APOE4 leading to variation in binding affinities of ligands. We report for the first time computational and structural studies that reveal selectivity amongst ligands for APOE binding, with possible links to CAD pathogenesis. Molecular dynamics study allowed to understand the APOE conformational flexibility and its stability followed by Molecular docking studies that identified scaffold of Ligand 11802 by screening of 22,203 molecules from ChemDiv Library which showed the highest affinity towards APOE4. The ligand showed the presence of chemical moieties, similar to that present in known APOE4 stabilizers in Alzheimer's Disease, which opened a possibility for the ligand as a potential therapeutic agent that could affect the behaviour of APOE4 in CAD pathogenesis. Further, ligand-binding preferences of each isoform with LDL receptors (LDLR) allowed understanding of the in-vivo mechanism in CAD pathogenesis.

Keywords: Cardiovascular, Apolipoprotein, Molecular Docking, SAR, Atherosclerosis

1. Background

Coronary Artery Disease (CAD) is a cardiovascular disorder characterized by atherosclerosis in coronary arteries that has remained as one of the most complex diseases with a high morbidity and mortality worldwide [1, 2]. Recent studies have suggested that apolipoprotein (APOE) polymorphism is involved in the pathogenesis of atherosclerosis and thereby conveys a higher risk of CAD [3]. The human APOE gene locus was the first polymorphic gene to be described as metabolically involved in variation of major plasma lipoprotein fractions and their components accounting for 8.3% of the total genetic variance for low-density lipoprotein cholesterol (LDL-C). This leads to modest associations of APOE4 isoform with plaque and coronary heart disease outcomes [4]. The APOE gene encodes a 34.15 kDa plasma glycoprotein (299-amino acids) having receptor-binding region at N-terminal domain (1-164 residues) and lipid-binding region at C-terminal domain (165-299 residues) [5–7]. APOE affects the cholesterol levels in the atherosclerosis process and in premature cardiovascular disease (CVD) which make it a promising genetic marker with significant influence on cholesterol metabolism that increases the risk for developing neurological and cardiovascular disease [8, 9]. The three predominant isoforms of APOE exists as one of the following: epsilon 2 (ε2), epsilon 3 (ε3) and epsilon 4 (ε4) [7, 10]. In human blood circulation, this glycoprotein is a ligand for the LDL receptor (LDLR), the LDL receptor-related protein (LRP) and the very low-density lipoprotein receptor (VLDL) and involved in cholesterol metabolism [11, 12]. Studies reported that E4 variant of APOE is frequently associated in familial mixed hypertriglyceridemia (Type V) and higher LDL-C levels that promote hyperlipidaemia, increased risk of stroke and cardiovascular disease (CVD) as

well as Type 2 diabetes mellitus (T2DM) [4, 13, 14]. The role of APOE4 in Alzheimer's disease (AD) is already established and investigations on APOE genotypes in coronary heart disease (CHD) risk population indicate a strong likelihood that APOE4 can be a bridge between AD and CHD serving as a promising therapeutic target [15].

The APOE has single amino acid substitution at 112 and 158 position resulting as the following three isoforms: APOE2 (Cys112/Cys158), APOE3 (Cys112/Arg158) considered as the 'parent' protein and APOE4 (Arg112/Arg158) which has profound influence on lipid profiles and is well linked to AD and CVD [14, 16]. The functional consequences of the APOE variants at both the cellular and molecular levels is due to the sequence dissimilarity at 112 and 158 positions that cause intramolecular domain interaction owing to difference in their isoelectric points. The three isoforms differ sequentially by one charge unit and hence differ in the binding affinities to LDL receptors and lipoproteins particles [7, 17]. The presence of 112-Cysteine residue confers oligomerization properties to APOE3, which allows to form a disulphide linkage which is absent in APOE4. The Cys/Arg substitution facilitates a salt bridge formation between Arg61 and Glu255 in APOE4 which makes the molecule assume a compact tight structure, unlike that in APOE3 which is comparatively stable and open for easy binding of phospholipid-rich high-density lipoproteins [18]. The receptor binding affinity of APOE4 although remains unaffected, but APOE (E4/E4) homozygotic individuals have been reported for higher risk for coronary heart disease and a significantly greater risk for developing AD [19] which brings out the need for further investigations.

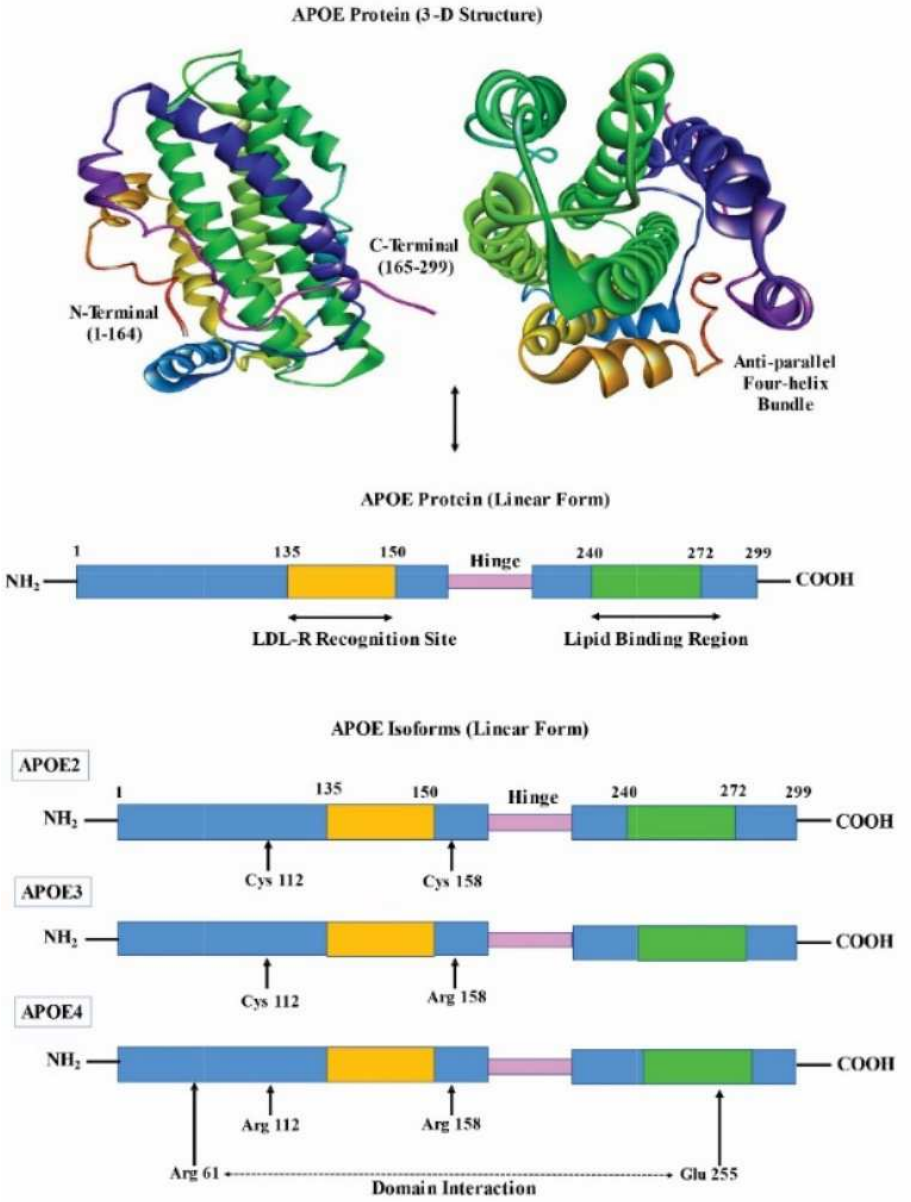

Figure 1. *3D structure and a schematic representation of the structural and functional domains of human apolipoprotein E (APOE) isoforms. APOE contains two functional domains: the N-terminal domain (1-164) containing the receptor-binding region (residues 135 - 150) and the C-terminal domain (165-299) containing the lipid-binding region (residues 240 - 272). The APOE4 isoform exhibits inter domain interaction between Arg61 and Glu255 residue.*

The arginine residue substitution at 112th position in APOE4 results in salt bridge formation between glutamine (109) and arginine (112) that cause side chains of arginine (61) to get exposed and interact with glutamine (255) as shown in Figure 1. The detrimental effects of APOE4 in cardiovascular, neurological, and infectious diseases correlate with its structural features i.e., its domain interaction that distinguish it from APOE3 and APOE2 [20]. APOE4 has a strong and established genetic association for Alzheimer's disease (AD) through intermolecular interaction presenting substantial changes in binding conformations as reported from molecular dynamics simulation (MDS) [21]. Williams et al., 2015 presented the first structural model of an APOE4 misfolded intermediate state, that served to elucidate the molecular mechanism underlying the role of APOE4 in AD pathogenesis [22]. However, the detailed molecular and dynamic properties of the protein interaction with different ligands or any therapeutic candidates in relation to CAD are still unknown and exact mechanisms to explain the physiological and pathological role of this relationship is unclear till date. A more comprehensive study is therefore important for the understanding of the pathological mechanisms of APOE4 towards CAD, which may provide significant molecular insights on therapeutic target sites and how APOE4 can interact with different molecules to contribute for CAD pathogenesis. In the present study, using molecular docking and dynamics simulation, the characteristics of APOE3 and APOE4 are studied.

The present study identified potential drug scaffolds for APOE4 targeted CAD and use them to probe the differential binding of ligands to APOE proteins. This was achieved by screening a large number of compounds in ChemDiv® Library against the 3D structure of APOE4. Selection of the library compounds was based on their known association to variety of targets related to cardiovascular diseases. The complex was then subjected to interaction profiling to examine the differential behaviour of molecular interactions between the screened compounds and the functional residues of APOE4. This study provides key understanding of the differences in structural features of APOE4 with respect to CAD pathogenesis.

2. Materials and Methods

2.1. Preparation of Target Structure

The targeted APOE4 protein structure included in this study had been modelled and validated both on geometric and energetic scale and also minimized in our previous study [23], whereas the APOE3 (2L7B) structure was retrieved from PDB database. The homology model built was submitted to Protein Model DataBase (http://srv00.recas.ba.infn.it/PMDB/main.php) and was assigned a PMDB ID: PM0084216 [24]. APOE4 structure was prepared by adding hydrogen atoms with hydrogen bond network optimization at physiological pH.

2.2. Molecular Dynamic Simulations of Protein Structures

Initial molecular dynamics of protein structures of APOE3 and APOE4 was performed using GROMACS v2020.3 [25]. Molecular dynamics help to explore conformational flexibility of the two isoforms and also to further optimize modelled APOE4 structure. System was prepared by protonation of the structures at physiological pH and were further enclosed in a simulation box with a distance of at least 10 Å from each side of the cubic box. System was solvated with the TIP3P water model and neutralized by adding counter ions. Entire system was parameterized by the OPLS all-atom force field. Both systems were well minimized and further equilibrated for 5ns in NVT and 5ns in NPT ensemble. Production dynamics was run under NPT thermodynamic ensemble for 50ns in unrestrained mode.

2.3. Selection of Ligands

Molecules from ChemDiv®'s Cardiovascular Library, ChemDiv®, Inc. USA that contains 23,203 compounds (https://www.chemdiv.com/cardiovascular-library/) has been included in the study. This library consists of various knowledge-based and 2D similarity-based scaffolds covering diverse target and biochemical space, making it a suitable for novel starting structures.

Ligand Preparation: Molecules were prepared by using MMFF94 optimization to generate low energy conformers and further treated with AutoDock Vina Meeko ligand preparation program (https://github.com/forlilab/Meeko). Meeko adds hydrogens at physiological pH and computes gasteiger charges [26, 27]. It also prepares the PDBQT files required to run AutoDock vina.

2.4. Molecular Docking Studies

Molecular docking is widely used for predicting the binding affinities for a number of ligands. In the present study, docking was performed with Vina 1.2.3. [27]. All the ChemDiv® compounds were geometry optimized and energy minimized by RDKit python package using MMFF94 force field (https://www.rdkit.org/) prior to docking [28]. Binding site for docking, provided as a search volume, was derived from the co-crystallized stabilizer structures from PDB (PDB IDs: 6NCN and 6NCO). Dimensions of the grid box were 28 x 26 x 28 with grid spacing of 1Å. Lowest energy conformations were chosen for further investigation. This was applied to all the ChemDiv compounds and the selected conformations were analysed with receptor structure for interaction analysis. Docking for APOE3 as the receptor molecule was also performed using AutoDock Vina, with the grid dimension as 28 x 26 x 28 with grid spacing of 1 Å [27]. The ligand conformation which showed the lowest docked energy (binding affinity) was chosen and compared with that of APOE4, as shown in Table 1 and Table 2. Several

compounds in the library constituted of macrocyclic rings, which were handled by meeko package of AutoDock Vina. Macrocyclic structures were broken and their flexibility was modelled on the fly by AutoDock Vina to obtain low energy poses.

2.5. Protein-Protein Interaction Study of APOE and LDLR

To understand the effect of conformational changes in APOE isoforms, on their binding with the LDL receptor, a protein-protein docking was performed using ClusPro server (https://cluspro.bu.edu/) and interface of the complexes was analysed using PDB ePISA server [29]. The PDB structures of APOE3 (2L7B) and low energy structure of molecular dynamics trajectory of APOE4 were used for protein-protein docking.

3. Results and Discussion

3.1. Molecular Dynamics Simulation

MD studies on the protein structures allowed to observe the structural differences and compare conformational flexibility of APOE3 and APOE4. This also helped in minimizing the modelled structure of APOE4 using an explicit solvent system. Comparative analysis of molecular dynamics trajectories showed higher conformational flexibility in APOE4 than APOE3, as shown in Figure 2.

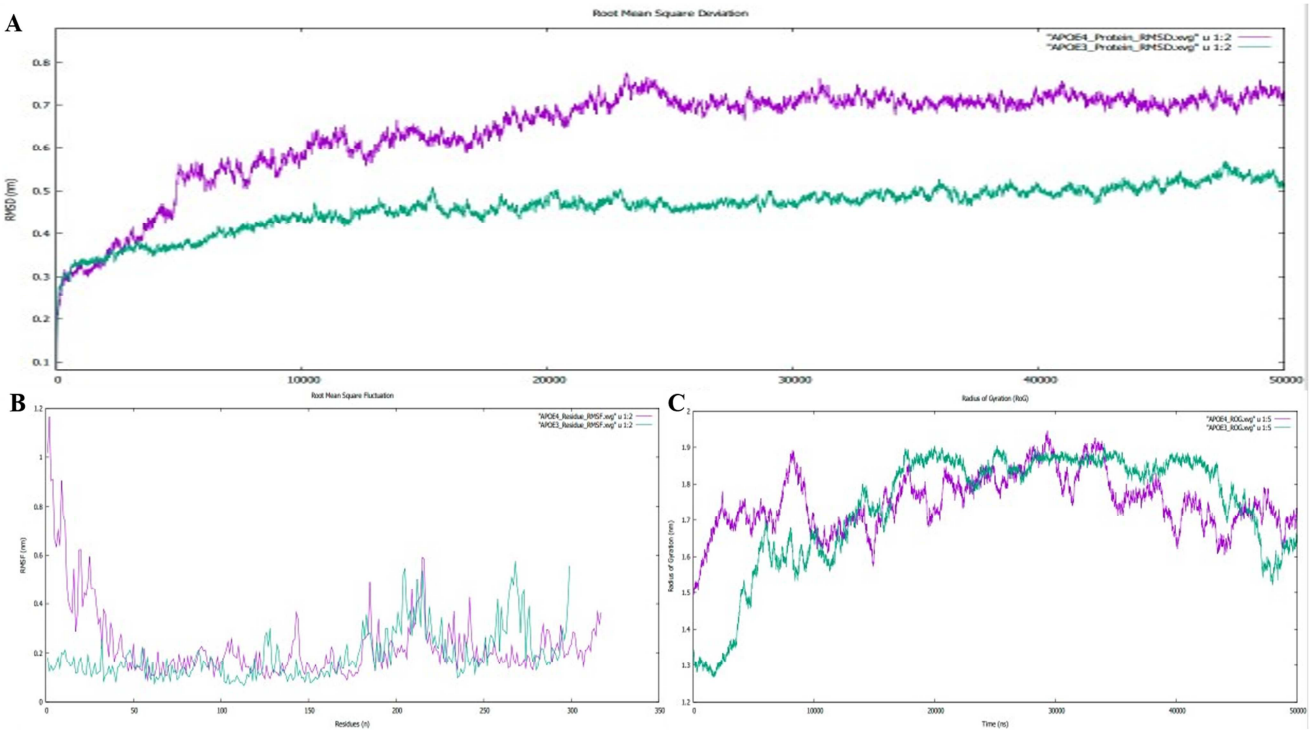

Figure 2. *(A) RMSD plot shows the change in structure of APOE4 over the time of simulation, while APOE3 appears to be relatively stable. (B) RMSF plot shows the average deviation of residues during the course of MD simulation (C) Radius of gyration during the course of simulation is slightly larger for APOE4 than APOE3.*

The differences in all atom root mean square deviation (RMSD) of APOE from its initial structural conformation to its final position is observed in Figure 2 that reflected light on the stability of the protein relative to its conformation. RMSD for APOE4 showed maximum fluctuation up to 7 Å in 50 ns simulation, while in APOE3, it showed RMSD value of only up to 5 Å throughout the simulation. It can be said that Cys-112-Arg substitution influences the key structural features of APOE4 to push itself away from the core. The residue wise root mean square fluctuation (RMSF) showed slight differences between the isoforms shown in figure 2. Change in radius of gyration (RoG) during molecular dynamics also shows a similar trend of average RoG being higher for APOE4. Using an explicit solvent system, the APOE4 modelled protein was minimized and MDS comparative analysis showed higher conformational flexibility in APOE4 than APOE3.

3.2. Molecular Docking Studies

Molecular docking included virtual screening of 22,202 compounds in ChemDiv®'s Cardiovascular Library, against human APOE4. The screened compounds were ranked according to their binding affinity. The same set of compounds was also screened against APOE3 isoform and the differences in binding patterns between APOE3 and APOE4 was investigated, as shown in the Tables 1 and 2.

Table 1. *Molecular Docking outcome of top 10 compounds based on AutoDock Vina binding affinity score. Binding energy was expressed in terms of kcal/mol.*

APOE3		APOE4	
Ligand ID	**Binding Affinities (kcal/mol)**	**Ligand ID**	**Binding Affinities (kcal/mol)**
ligand_18403	-16.172	ligand_11802	-18.818
ligand_7981	-15.659	ligand_21084	-17.95
ligand_18716	-15.113	ligand_3621	-17.545
ligand_19443	-14.926	ligand_19011	-16.949
ligand_19010	-14.85	ligand_18716	-16.58
ligand_18186	-14.67	ligand_6344	-15.866
ligand_7987	-14.522	ligand_3265	-15.807
ligand_19044	-14.466	ligand_19008	-15.253
ligand_792	-14.045	ligand_19010	-15.031
ligand_14302	-13.851	ligand_19029	-14.894

Table 2. *Differential binding affinity of APOE4 ligands as compared to their affinity for APOE3.*

Ligand ID	**APOE4**	**APOE3**
	Binding affinities (kcal/mol)	
ligand_11802	-18.818	-10.706
ligand_21084	-17.95	-10.082
ligand_3621	-17.545	-7.5
ligand_19011	-16.949	-11.512
ligand_18716	-16.58	-15.113
ligand_6344	-15.866	-8.2
ligand_3265	-15.807	-6.3
ligand_19008	-15.253	-13.439
ligand_19010	-15.031	-14.85
ligand_19029	-14.894	-11.496

Molecular Docking provided molecules with highest binding affinity as shown in Table 1. Table 2 shows the top 10 binding affinities of ligands for APOE4 and their corresponding binding affinities for APOE3. From the 22,202 screened compounds, Ligand 11802 (Figure 3) was found to have highest affinity for APOE4 (-18.8 kcal/mol) while comparatively less binding energy towards APOE3 (-10.7 kcal/mol), presenting a difference of around 8 kcal/mol. While the highest affinity for APOE3 was shown by ligand 18403, (-16.172 kcal/mol) it showed affinity of only –5.964 kcal/mol for APOE4. It is observed from Table 1 that APOE3 and APOE4 has different binding preference for ligands, evident from the ranked binding affinities of different ligands. The highest difference is observed for ligand 3621 of around 10 kcal/mol, where the ligand binds to APOE4 with -17.545 kcal/mol and that to APOE3 with -7.5 kcal/mol.

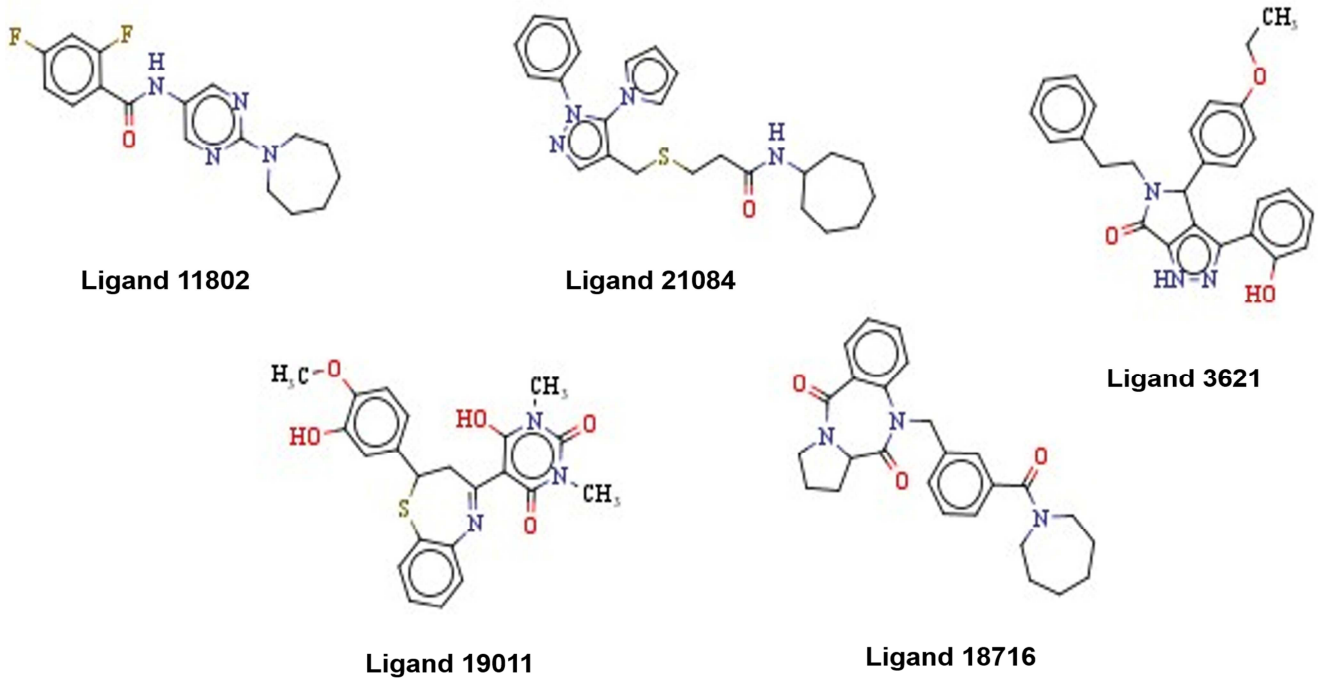

Figure 3. *2D representation of top 5 APOE4 ligands from molecular docking. Molecular structures of these compounds show the presence of phenyl ring. Four of the top 5 compounds show the presence of 7 membered macrocyclic ring structures which contribute to the hydrophobic interactions with the target.*

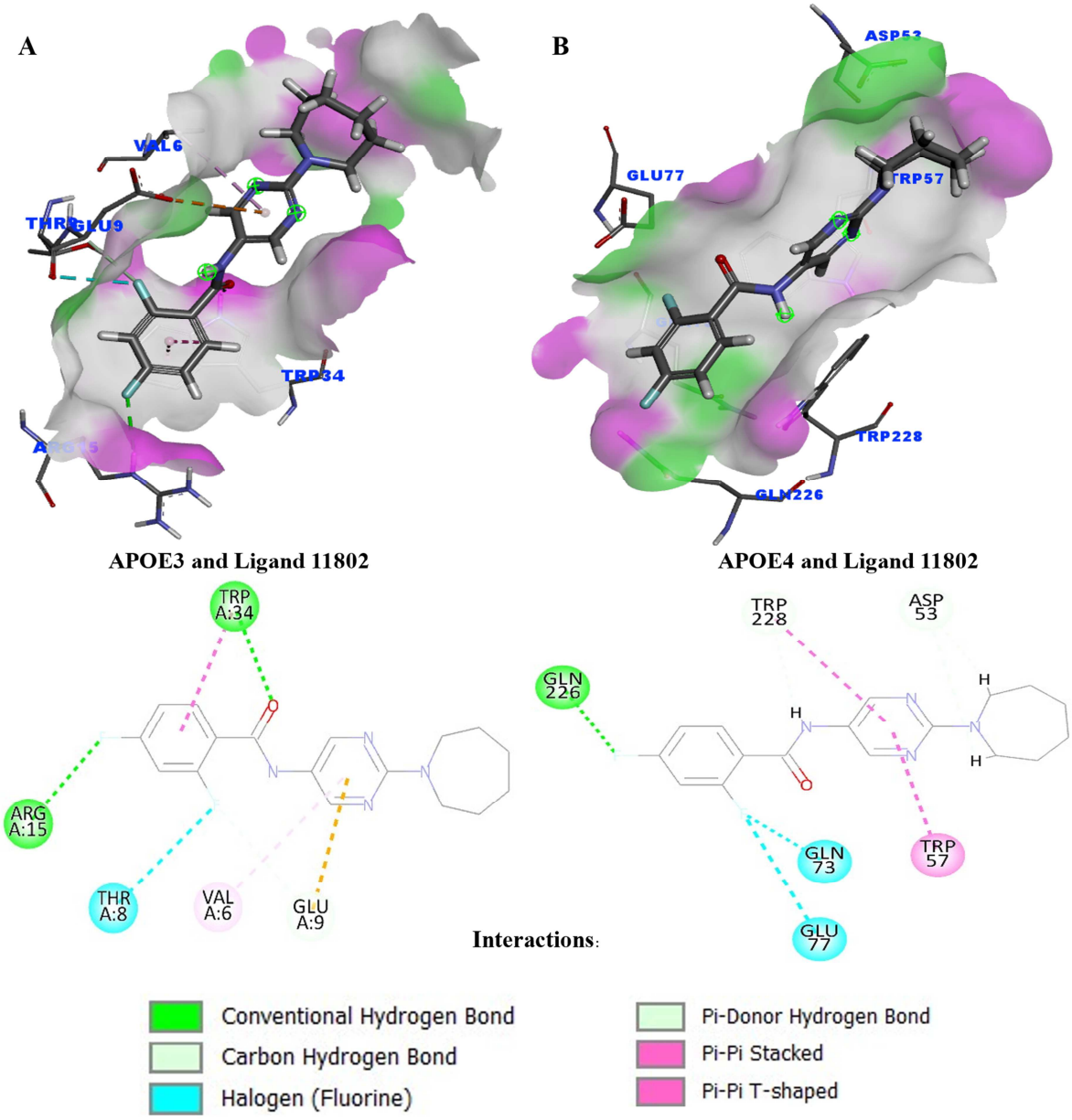

Figure 4. *APOE3 (A) And APOE4 (B). Top: 3D representation of interactions between ligand 11802 and APOE isoforms shows more surface area of the binding pockets covered in APOE4. Bottom: 2D representation shows the hydrogen bond interactions between the docking partner and APOE isoforms. APOE3 interactions included electrostatic and hydrophobic interactions which involved Pi-Alkyl hydrophobic interactions. APOE4 exhibited hydrogen bond interactions and was supported by several halogen, Pi and hydrophobic interactions.*

Figure 4 shows the inter molecular interactions of ligand 11802 with APOE. The binding of the ligand to APOE3 is supported by two hydrogen bond interactions and one Van der Waals interactions. Hydrogen bonding with Trp34 and Arg15 was observed. One of the hydrogen bonds was made by fluorine. The ligand made several contacts with APOE4 which involved hydrogen bond with Gln226. It also showed several hydrophobic interactions and halogen bonds additionally making Van der Waals interactions with Trp228 and Asp53.

3.3. Analysis of Binding Site

Identification of ligand binding site is important to understand the intermolecular interactions in a protein as well as to understand the receptor-drug interaction in disease pathogenesis. Binding site for docking, provided as a search volume, which was derived from the co-crystallized stabilizer structures from PDB (PDB IDs: 6NCN and 6NCO) [6]. In the present study, the binding site on both APOE3 and APOE4 was mapped to the same location, but it was found that ligand chose to make significantly different contacts which could be result of structural differences in APOE3 and APOE4 (Figure 5). Interaction analysis of other top compounds also showed similar binding patterns where binding to the target was supported by numerous Van der Waals and hydrophobic (Pi interaction) interactions. Comparatively the number of Van der Waals interaction seem to suggest important contributions to the receptor binding.

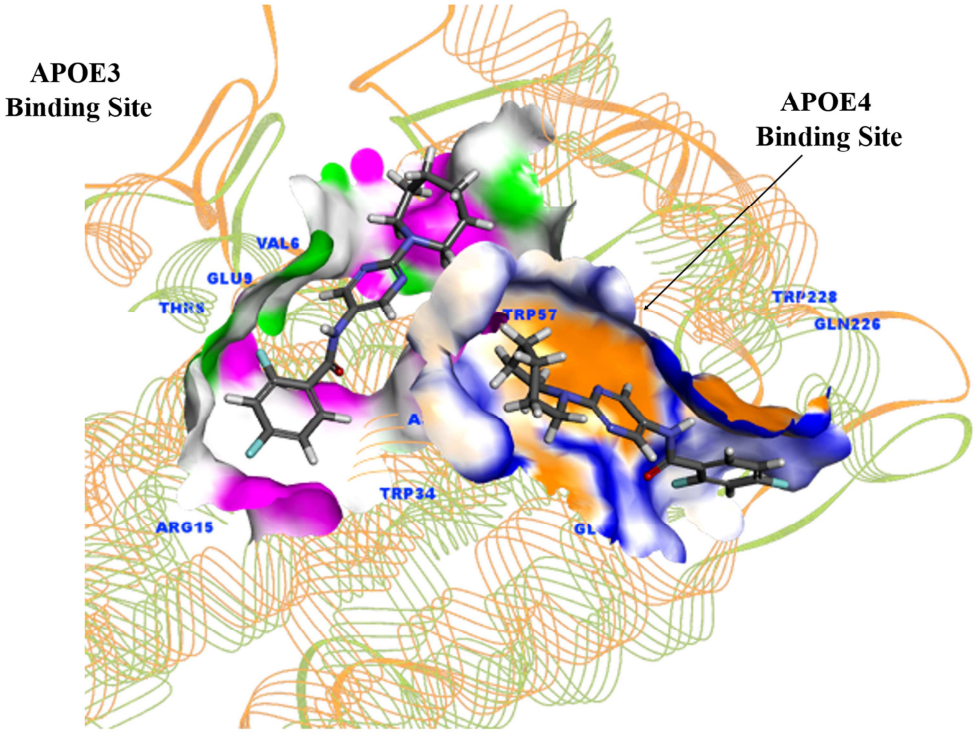

Figure 5. *Binding Sites of APOE3 and APOE4. Structural superimposition of APOE3 and APOE4 bound by the same ligand 11802 at two different pockets.*

Petros et al., 2019 have reported structure activity relationship (SAR) of phenyl ring in APOE4 activity [6]. The extension of the phenyl ring with various substituents like hydroxyl groups, halides can lead to increased activity of a compound against APOE4, potentially by stabilizing it [6]. The compounds selected from the docking study also show the presence of similar chemical moieties which might be responsible for their affinity towards APOE4 (Figure 3). The amino acid residues in APOE protein-ligand interactions were analysed using Discovery Studio, as shown in Figure 4 A and B. These differences in the binding sites may hint towards altered ligand binding preferences of APOE4 due to change in a single amino acid (Cys-112-Arg). The docked complexes of APOE3 and APOE4 with Ligand 11802 indicate their binding preferences. These altered ligand binding preferences of APOE4 may be due to the change in a single amino acid at 112th position in the N-terminal region. Studies earlier reported that one third of the APOE sequence consist of charged residues like arginine, lysine, glutamine and aspartate that play a role in imparting stability and the flexibility during the binding [18] and it is observed from the type of interactions of the participating amino acids (Figure 4). The receptor binding site in the N-terminal region (1 - 167) on the lipidated APOE binds readily to the LDLR, but, in isolation, it binds weakly to lipids [30]. Protein lipidation modulates binding affinities to biological membranes influencing the protein hydrophobicity resulting in changes to their conformation, affecting folding and stability, membrane association, localization [31]. Therefore, the change in binding preferences of the APOE isoforms supports the fact that APOE in lipidated state play a critical role in body's physiological function and disease pathogenesis including atherosclerosis. The 112th arginine substitution may be indicative of this difference in the binding affinities of APOE3 and APOE4 which is due to altered ligand binding preferences as a consequence of change in structural conformity. From the figure 4, it is observed that the Gln226 forms a hydrogen bond while Gln73 and Glu77 forms two halogen bonds in APOE4 and Trp at multiple positions engage in Pi interactions. The electrostatic interactions including the unfavourable bumps in APOE3 accounts for the unique topology which allows extensive intra-domain interaction shielding the major LDLR binding region in the N-terminal domain. Studies reported that structure of APOE3 is adapted to ensure the optimal receptor-binding activity by the fully lipidated APOE during lipoprotein transport in circulation [30].

3.4. Protein-Protein Interactions of APOE Isoforms and LDL Receptor

Protein-Protein docking of APOE isoforms was performed to understand binding with LDL receptor. While APOE3 showed a higher affinity with the LDLR, APOE4 showed no significant interactions. ClusPro docking score was calculated from the balanced electrostatic and Van der Waals interactions. This difference in binding mode of APOE4 also suggests significant differences in binding of APOE4 to the LDLR, reducing its LDLR binding affinity. Docked complexes were subjected to interface analysis using PDB ePISA server; Interactions and Solvent accessibility at the interface was studied. APOE3 binds to LDLR with a solvent accessible area of 948.2 Å^2 with a solvation energy of -149.2

kcal/mol while APOE4 binds to LDLR with a more solvent exposed area of 1252.5 Å^2 and lesser solvation energy, suggesting that the binding between APOE4 and LDLR is weaker in comparison to APOE3. This is further observed in the interactions at the interface, where APOE3 interacts with 16 hydrogen bonds and 13 salt bridges and APOE4 binds to LDLR with 15 hydrogen bonds, but only a single salt bridge between them as described in Table 3. Different orientations of APOE isoforms with respect to LDLR are as shown in Figure 6.

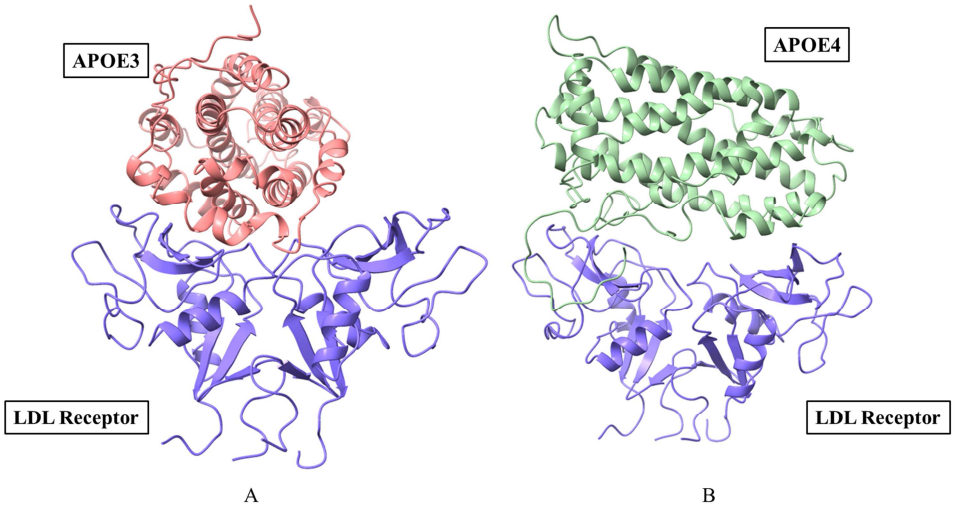

Figure 6. *Binding modes of APOE3 and APOE4 observed from protein-protein docking. (A) APOE3 binds to LDLR with more close contacts and buried surface area, while (B) APOE4 shows weak binding with large surface area exposed to water at the interface.*

Table 3. *Hydrogen Bonding and Salt Bridge interactions between APOE isoforms and LDLR.*

Interactions		APOE3			APOE4		
		LDLR residues	**Distance (Å)**	**APOE3 residues**	**LDLR residues**	**Distance (Å)**	**APOE4 residues**
Hydrogen Bonds	1	A:GLN 236[NE2]	3.03	B:GLU 9[OE1]	A:ARG 231[NH2]	2.80	C:MET 1[O]
	2	A:TYR 238[HH]	1.86	B:GLU 9[OE1]	A:ARG 231[NH1]	2.63	C:MET 1[O]
	3	A:GLN 236[NE2]	2.98	B:GLU 9[OE2]	A:TYR 252[HH]	1.83	C:LYS 2[O]
	4	A:ARG 248[NH2]	2.77	B:GLU 50[OE1]	A:ARG 231[NH1]	2.77	C:VAL 3[O]
	5	A:ARG 248[NH1]	2.70	B:GLU 50[OE1]	A:ARG 229[N]	3.10	C:ALA 6[O]
	6	A:ARG 248[NH2]	2.71	B:GLN 55[OE1]	A:ARG 229[NH1]	2.80	C:GLN 17[OE1]
	7	A:ARG 248[NH1]	2.75	B:TRP 210[O]	A:ARG 229[NH2]	2.74	C:GLN 17[OE1]
	8	A:ARG 231[NH2]	2.87	B:LEU 214[O]	A:GLN 236[NE2]	3.26	C:ASP 248[OD1]
	9	A:ARG 231[NH1]	2.54	B:ARG 215[O]	A:GLN 236[NE2]	2.88	C:GLU 249[O]
	10	A:ARG 231[NH1]	3.77	B:ALA 216[O]	A:ARG 248[NH2]	2.82	C:VAL 301[O]
	11	A:ARG 231[NH1]	2.71	B:GLU 219[OE1]	A:ARG 248[NH1]	2.68	C:VAL 301[O]
	12	A:ARG 229[NH2]	2.77	B:GLU 219[OE2]	A:ALA 233[N]	3.66	C:SER 308[O]
	13	A:GLN 192[OE1]	2.58	B:ARG 215[NH1]	A:PRO 225[O]	2.94	C:LEU 8[N]
	14	A:ALA 253[O]	2.81	B:ARG 38[NH2]	A:TYR 238[OH]	1.67	C:LYS 251[HZ2]
	15	A:GLU 254[OE2]	2.05	B:ARG 38[HE]	A:GLU 254[OE2]	3.12	C:ALA 310[N]
	16	A:ALA 259[O]	3.54	B:ARG 32[NH1]			

Interactions		APOE3			APOE4		
		LDLR residues	**Distance (Å)**	**APOE3 residues**	**LDLR residues**	**Distance (Å)**	**APOE4 residues**
Salt Bridges	1	A:ARG 248[NH2]	2.77	B:GLU 50[OE1]	A:GLU 254[OE1]	3.39	C:LYS 251[NZ]
	2	A:ARG 248[NH1]	2.70	B:GLU 50[OE1]			
	3	A:ARG 231[NE]	3.05	B:GLU 219[OE1]			
	4	A:ARG 231[NH1]	2.71	B:GLU 219[OE1]			
	5	A:ARG 229[NH1]	2.75	B:GLU 219[OE1]			
	6	A:ARG 231[NE]	3.08	B:GLU 219[OE2]			
	7	A:ARG 231[NH1]	3.55	B:GLU 219[OE2]			
	8	A:ARG 229[NH1]	2.88	B:GLU 219[OE2]			
	9	A:ARG 229[NH2]	2.77	B:GLU 219[OE2]			
	10	A:GLU 254[OE1]	2.86	B:ARG 38[NH2]			
	11	A:GLU 254[OE1]	3.48	B:ARG 38[NE]			
	12	A:GLU 254[OE2]	2.89	B:ARG 38[NH2]			
	13	A:GLU 254[OE2]	2.84	B:ARG 38[NE]			

The pathological adaptation of APOE4 variant towards AD development through intramolecular interaction is

established in MDS studies earlier, which suggested APOE4 is thermally unstable isoform and undergoes the formation of an isoform-specific misfolded intermediate state. This misfolded state was speculated to modify the lipid transport efficiency via an isoform-specific mechanism of interaction with lipids and lipoprotein receptors that might play a crucial role in the onset of AD by affecting the kinetic of aggregation or by promoting the intracellular hyperphosphorylation and consequent self-assembly of target (tau) protein [22]. The same phenomenon is also expected to play a role in CAD, as the present study identified that instability of APOE4 through ligand interactions due to the change of the Cys-112-Arg residue. Studies affirmed that Arg112 in APOE4 influence its association with high level of plasma cholesterol, LDL, and apolipoprotein-B. The lipid binding region of APOE4 thus get altered that switch the lipid binding preference from small phospholipid-rich HDL (high density lipoprotein) to large triglyceride-rich VLDL. Frieden & Garai, 2012 speculated that the structural differences observed as a consequence of the cysteine/arginine change at position 112 may involve the highly charged helix 4 of the N-terminal domain, resulting in structural differences between APOE3 and APOE4 [5]. The structural and the functional aspects of the protein are related to the single amino acid change in the N-terminal domain that influence the major lipid-binding determinants in the C-terminal domain. Both the N- and C-terminal are independently folded domains but influence the properties of each other through intramolecular domain interaction, which contribute to the detrimental effects of APOE4. The substitution of Cys-112-Arg in the N-terminal domain of APOE4 influences the lipid-binding property of the C-terminal domain, resulting in alteration in the protein conformation and directs the APOE4 affinity for binding to VLDL promoting differential lipid efflux. In absence of this substitution, i.e., in case of APOE3, the preference is for HDL that enhances the ability to release lipids [20, 31].

The understanding for the molecular basis of the structural variation between human APOE isoforms was attempted by many studies. Nguyen et al. revealed that the overall stability of APOE exerts a major influence on its lipid- and lipoprotein-binding properties [32]. The Cys-112-Arg substitution exerts a direct inter domain destabilizing effect that enhances its lipid-binding capabilities relative to those of APOE3. The study indicated that the direct helix bundle destabilization induced by the presence of Arg112 is the major contributor for the pathological properties of APOE4 isoform [31]. Arg61, within the N-terminal domain known to affect lipoprotein preferences and is believed to form a salt bridge with Glu255 in APOE4. Since the function of APOE is to transport lipid and cholesterol, conservation of the residues is expected. Residues in the C-terminal (278–299) region in different mammals were found to have little conservation and were intrinsically disordered. This region is the part of lipid binding region, and the difference is related to dietary lipid intake or differences in lipid metabolism, especially in different mammalian species. Regions in the C- and N-terminal domains and specifically those around Trp264 and Ser94, are involved with lipid binding [18]. This is observed in the molecular docking of the present study that revealed the altered ligand binding preferences of APOE4 due to change in a single amino acid at 112th position.

The present study paves way for experimental studies to validate and allow development of different scaffolds to build therapeutic agents that could affect the behaviour of APOE4 relative to APOE3. Therefore, it can be concluded that the understanding of the different binding efficiency and the differential functional effects due to structural differences between APOE3 and APOE4 holds a rationale towards the underlying mechanism behind APOE4 in the development of CAD pathogenesis.

4. Conclusion

Present study has utilized a combination of molecular docking and other computation methods as a way to explore structural differences and probe the differential binding of ligands to APOE4, a crucial isoform of APOE known to be associated with AD as well as CAD. This work shows that with only a difference of a single amino acid, the two isoforms under investigation possess considerably different conformational flexibility, and lead to significant differences in their structural features. This is further demonstrated by selectivity amongst ligands for APOE binding. Previously hypothesized domain interactions in case of APOE4 could further be explored to substantiate the details of these mechanisms by running large scale molecular dynamics involving domain movements. These differences in binding sites are also demonstrated through distinct sets of scaffolds having favourable affinities to same binding site. This would lead to a novel target site to be used for developing new drugs specific towards APOE4 isoform. Scaffold of Ligand 11802 having highest affinity towards APOE4, can further be used to develop novel drug molecule in treatment of CAD. As the selected molecules share structural features with known stabilizers of APOE4, they may also work in similar manner by stabilizing the APOE4 structure thus, allowing it to have normal binding to its receptor like APOE3. Thus, the present study reported, for the first time, on the APOE ligand binding preferences using computational and structural studies, that holds strong relation to CAD pathogenesis.

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