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Research Article

Structural Analysis of Selected Missense Variants of NR1D1-F443L and R500H: Pharmacophore based Implications for REV-ERBα Ligand Recognition

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ABSTRACT

Objective: *Rev-erb-α, also known as nuclear receptor subfamily 1 group D member 1 (NR1D1), is an orphan nuclear receptor that plays an important role in regulating circadian rhythm, metabolism, inflammation, and energy homeostasis. Two missense variants F443L where Phenylalanine was changed to Leucine at position 443 and R500H where Arginine was replaced to Histidine at position 500 were selected since they aremapping to the highly conserved C-terminal Ligand-Binding Domain (LBD) of the human sequence. The structural impact of these missense variants on ligand recognition and pharmacological modulation of rev-erbα has been studied by computational methods. Pharmacophore modeling was performed to identify the hits that bind to these missense variants.*

Methods: *Missensevariants were generated using ChimeraX. Both the variants including wild type variety of Rev-erbα were docked against selected Rev-erbα agonists. Using Pharmit server pharmacophore modeling was performed with F443L variant to identify highest scored hits and their ADMET properties were studied.*
Results: Stl1267showed highest affinifty (-7.6 kcal/mol) when compared to other rev-erb agonists against wild type and same affinity with missense variety 8d8i F443L.

Conclusion: *F443L and R500H do not simply cause a loss of protein–ligand interactions but instead remodel the interaction landscape of the REV-ERBα ligand-binding pocket. Stl1267 appears relatively resistant to the effects of both mutations, whereas SR9011 is particularly sensitive to F443L. Pharmacophore modeling suggests the possible hit PubChem ID-138708911 with favourable ADMET properties.*

Keywords: *missense variants, pharmacophore modeling, reverb alpha, pharmacokinetic profile, ChimeraX*

INTRODUCTION

REV-ERBα belonging to nuclear receptor subfamily 1, group D, member 1 or NR1D1is a favourable target for treating metabolic disorders, inflammation, and other diseases. Itis a ligand-dependent transcriptional repressor that affects lipid metabolism, glucose homeostasis, and inflammation [1]. It is an orphan receptor [2], however subsequent identification of heme asits endogenous ligand showed a way to discover the mechanism of action of reverb alpha and its role in various disorders. Various synthetic modulatorshave been identified from then such as triazolopyridazines[3], tetrazole biphenyls[4], biphenyl thiazolidinediones etc demonstrating their potential role in managing metabolic diseases. It has been observed from the mutagenesis experiments that several ligand-pocket residues are involved in receptor modulation that helps in identifying novel REV-ERBα modulators[5]. Modifications to amino acid side-chain characteristics and the spatial configuration of the ligand-binding pocket is seen in missense variants which affects the receptor functioning and modifies ligand recognition between the ligand-binding domains (LBD) and associated regulatory proteins. Therefore studying NR1D1 missense variants provides insight into the structural aspects that regulate REV-ERBα pharmacology. However, structural and experimental evidence must be established to identify the functional consequences. An aromatic phenylalanine is replaced with an aliphatic leucine at ligand-pocket residue in F443L and was studied to examine the

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changes in local electrostatic properties, hydrogen-bonding potential. A study on the role of mutations in NR1D1 and other various circadian-relevant genes in Autism Spectrum Disorder (ASD) patients showed the presence of missense variants more in ASD patients than normal human beings. This proves thatREVERB proteins were likely to play a role in brain development, and disrupted molecular clock mechanisms may potentially contribute to the underlying causes of autism spectrum disorder. A novel heterozygous mutation c.1499 G > A (p.R500H) has been identified in individuals with ASD, which was absent in control subjects [6]. These findings indicate that impaired NR1D1 function may contribute to the development and underlying mechanisms of ASD. Pharmacophore modeling helps in molecular recognition by analyzing the spatial arrangement of chemical features such as hydrophobic regions, aromatic centres, hydrogen-bond donors, and hydrogen-bond acceptors. Integrating computational methods clarifies to some extent about amino acid substitutions and their influence on the shape or physicochemical environment of the LBD. However, pharmacophore models are further validatedthrough direct evidence of altered biological activity.

Although the selected variants have no established appropriate clinical pathogenicity, F443L variant is listed as a variant of uncertain significance in a variant database [7] and R500H was reported in human genetic literature [8]. In this investigation, F443L and R500H missense variants were examined regarding their effect on ligand recognition through structure-guided computational methods. The crystallographic structure of REV-ERBα ccorystallised with STL1267 (PDB ID: 8D8I) is used as a reference. The ligand–protein interactions, docking orientations, pharmacophore characteristics and the variant-induced changes in the ligand-binding pocket of wild-type and variant structures were compared. By combining structural analysis with pharmacophore modelling, this work establishes an understanding of role of NR1D1 missense substitutions in the ligand recognition and supports which helps in developing critical REV-ERBα modulators.

MATERIALS AND METHODS

Methodology

The present study employs a computational approach to analyzethe influence of amino acid substitutions on ligand-binding interactions in wild-type and variant protein structures. Pharmacophore modeling was adopted to identify the spatial distribution of molecular features potentially relevant to ligand recognition within both wild-type and variant binding sites. The methodology involves protein structure retrieval and preparation, variant construction, ligand preparation, molecular docking analysis, interaction analysis, and comparative pharmacophore study followed by ADMET study of identified hits.The 3D structure of PDB ID 8D8I was used as the structural template. The downloaded structure was examined to identify the protein chains, bound ligand, and resolved amino acid residues. The availability of residues corresponding to positions 443 and 500 was assessed before introducing the F443L and R500H substitutions. Appropriate protonation states were assigned and hydrogen atoms were added. Residue connectivity and structural integrity was checked and the prepared structure was saved and considered as wild type REV-ERBα structure. Missing residues or unresolved side chains were evaluated before further analysis.

Generation of F443L and R500H missense variants

Using the prepared wild-type as the structural template, The F443L and R500H variants were generated. UCSF ChimeraX[9] is used to introduce the amino acid substitutions. In the F443L variant, phenylalanine at position 443 was replaced with leucine, whereas in the R500H variant, arginine at position 500 was replaced with histidine.Following mutation generation, side-chain conformations were optimized using the available rotamer library. The resulting structures were inspected for steric clashes, unfavourable side-chain orientations, and potential changes in the local protein environment. Energy minimization was performed where appropriate to relieve local structural strain while preserving the overall protein fold. The wild-type and variant structures were subsequently saved as separate coordinate files for comparative analysis.

Ligand preparation,Molecular docking and Protein–ligand interaction analysis

The native synthetic REV-ERB agonist STL1267 in PDB 8D8I, was used as a reference ligand for binding-site identification and comparative structural analysis. Ligand structures were assigned appropriate bond orders, stereochemistry, hydrogen atoms, and protonation states. Geometry optimization was performed and the prepared ligand files were then converted into the .pdbqt format for molecular docking. For comparative docking, the same ligand preparation procedure and chemical states were maintained across the wild-type and mutant protein systems. Blind docking was performed to evaluate the all possible binding poses and predicted binding affinities of the selected ligands against wild-type REV-ERBα and its F443L and R500H variants. PyRx [10] was used as the docking engine. The same blind docking was applied to the wild-type and variant proteins to facilitate a meaningful comparison. Multiple poses were generated and ranked according to the predicted binding score. The top-ranked poses were examined for their location within the binding pocket and the predicted protein–ligand complexes were analysed using molecular visualization Biovia Discovery studio software [11]. Interactions like hydrogen bonds, hydrophobic contacts, π–π stacking, π–alkyl interactions, and other relevant non-covalent interactions were studied. Particular attention was given to residues involved in ligand stabilization and to changes in interaction patterns following the F443L and R500H substitutions. Comparative analysis was conducted to determine whether the variants were associated with the loss of existing interactions, the formation of new contacts, or changes in ligand orientation. Structural observations were interpreted

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alongside the docking scores rather than on the basis of docking scores alone.

Pharmacophoremodeling

Using Pharmit server[12] pharmacophore modelling was performed to investigate the spatial arrangement of chemical features potentially responsible for ligand recognition by wild-type and variant REV-ERBα. The analysis was designed to determine whether the F443L and R500H substitutions could alter the distribution or accessibility of pharmacophoric features within the ligand-binding environment. Pharmacophoric features, including hydrogen-bond donors, hydrogen-bond acceptors, hydrophobic regions, aromatic centres, and other chemically justified features, were identified from ligand structures and their interaction patterns. The wild-type, F443L, and R500H systems were compared with respect to their predicted binding scores, ligand orientations, interacting residues, and pharmacophore feature arrangements. The analysis focused on whether each substitution was associated with changes in pocket geometry, interaction networks, or the accessibility of ligand-recognition features. A variant showing a more favorable docking score was considered to have a potentially more favorable predicted interaction with the ligand under the selected computational conditions. Such a result was not considered sufficient, by itself, to establish enhanced biological activity or increased receptor agonism.

RESULTS AND DISCUSSION

Molecular docking was performed to evaluate the binding of GSK4112, SR9009, SR9011, and STL1267 against wild-type REV-ERBα and its F443L and R500H variants. Among the tested ligands, STL1267 exhibited the most favourable docking scores in the wild-type (−7.6 kcal/mol) and F443L (−7.6 kcal/mol) systems, followed by R500H (−7.4 kcal/mol)(Table 1). In the R500H variant, GSK4112 and SR9009 showed slight improvements, while STL1267 showed a modest reduction in predicted binding favorability. Distinct differences in the interaction profiles of wild-type (WT) REV-ERBα and the F443L and R500H variants with GSK, SR9009, SR9011, and STL1267 were observed. In the WT complexes, ligand recognition was predominantly supported by van der Waals and hydrophobic interactions involving residues within the ligand-binding domain, including PHE477, THR476, ASN599, GLY475, and SER603. Hydrogen-bonding interactions involving PHE477, THR476, and ASN599 were frequently conserved, suggesting their importance in stabilizing ligand binding. The local hydrophobic environment of the binding pocket was influenced by the replacement of the aromatic phenylalanine residue with leucine. Key hydrogen-bonding contacts involving PHE477, THR476, and ASN599 were retained for several ligands.This shows that the F443L mutation may modulate ligand recognition without completely disrupting the established binding network. Similarly, in R500H the substitution of arginine with histidine changes the physicochemical properties of the local environment and was associated with altered van der Waals and hydrophobic contacts. Notably, the R500H–STL1267 complex showed a π-anion interaction involving GLU534 indicating ligand stabilization. Several interactions involving GLY475, ASN600, SER603, and ARG596 were also observed in the mutant complexes (Table 2).

Table 1: Docking scores of REVERB agonists with wild and missense variants

	8d8i WT	8d8i F443L	8d8i R500H
GSK4112	-6.1	-6.2	-6.2
Sr9009	-6.5	-6.8	-6.7
Sr9011	-7.3	-6.9	-7.3
Stl1267	-7.6	-7.6	-7.4

Table 2: Interaction amino acids of REVERB agonists with wild and missense variants

	WT_GSK	WT_sr9009	WT_sr9011	WT_stl1267	F443L_GSK	F443L_sr9009	F443L_sr9011	F443L_stl1267	R500H_GSK	R500H_sr9009	R500H_sr9011	R500H_stl1267
Vanderwaals	GLU A:478	ALA A:474	GLU A:478	ASN A:600	THR A:470	GLY A:475	THR A:470	ASP A:549	THR A:470	THR A:470	THR A:470	THR A:301
	GLY A:475	GLY A:552	GLY A:475	THR A:476	SER A:551	LEU A:472	ASP A:549	ASN A:599	ALA B:2056	SER A:603	SER A:603	PHE A:300
	LEU A:472	LEU A:472	LEU A:472	ILE B:2059	ASP A:549	SER A:551	SER A:551	GLU A:604	GLY A:475	ASN A:600	ASN A:600	THR A:533
	ILE B:2055	ILE B:2059	ASP A:549	ASP A:549	ALA B:2056	GLU A:604	LEU A:472	PHE A:477	LEU A:472	GLU A:478	MET A:601	
	ASP A:549	ASP A:549	ASP B:2053	PHE A:477	ILE B:2055	ASN A:600	GLU A:604	THR A:476	ILE B:2055	ARG A:596	GLU A:604	
	ASP B:2053	ILE B:2055	THR A:470	ASN A:599	GLU A:478	THR A:470	ASN A:600	ILE B:2059	GLU A:478	ALA A:548	LYS A:605	
	THR A:470	SER A:551	SER A:551	GLU A:604	LEU A:472		MET A:601	ASP A:549	ILE B:2059	GLY A:475	HIS A:602	
	SER A:551	SER A:603	SER A:603		ARG A:596				ASP A:549	LEU A:472	LEU A:472	
		THR A:470	MET A:601						ASN A:600	ALA A:474	GLU A:478	
		ASN A:599	HIS A:602						SER A:603		GLY A:475	
			ASN A:600						ARG A:596		ASP A:549	
Hydrogen bonding	PHE A:477	PHE A:477	PHE A:477	SER A:603	PHE A:477	SER A:603	SER A:603	SER A:603	PHE A:477	PHE A:477		
	THR A:476	THR A:476	THR A:476		ASN A:599	ASN A:599	THR A:476		THR A:476	THR A:476		
	ASN A:599	GLY A:475	ASN A:599		GLY A:475	GLY A:475	GLY A:475		ASN A:599	ASN A:599		
					THR A:476	THR A:476	PHE A:477					
							ASN A:599					
Alkyl/pi-alkyl	ALA B:2052	ALA B:2052	ALA B:2052	ALA A:474	ALA A:474	ALA A:474	ALA A:474	ALA A:474	ALA A:474	ALA B:2056	ALA A:474	HIS A:296
	ALA B:2056	ALA B:2056	ALA B:2056	ALA B:2056	ALA B:2052	ALA B:2052	ALA B:2056	ILE B:2055	ALA B:2052	ILE B:2055	ALA B:2052	ARG A:483
	LYS A:473	LYS A:473	LYS A:473	LYS A:473		LYS A:473	ILE B:2055	LYS A:473			ALA B:2056	LEU A:532
	ALA A:474		ALA A:474	ILE B:2055				ALA B:2056				LEU A:527
Pi signa				ALA B:2052				ALA B:2052				LEU A:537
Amide pi stacked					LYS A:473	ALA B:2052	LYS A:473		LYS A:473	LYS A:473	LYS A:473	
							ALA B:2052			ALA B:2052	ALA B:2052	
Pi anion												GLU A:534

Therefore, only ligand-dependent changes were observed rather than a consistent improvement or reduction in docking scores upon amino acid substitutions. However, STL1267 remained the strongest-scoring ligand across all three protein systems. The observed differences suggest that F443L and R500H may influence ligand recognition differently depending on the chemical structure of the ligand. However, the relatively small docking-score changes do not independently establish altered binding affinity or receptor activity; further structural interpretation and experimental validation are required.

Pharmacophore modeling is performed to identify more potentially binding ligands compared to STL1267. Pharmacophore query string is generated by the server based on the input files of mutant proteins which was edited accordingly (filters applied include Molwt<=500; Rot bonds<=10; LogP<=5; HBA<=5; HBD<=5 and number of hits per molecule as one.) (Table 3) for pharmacophore based search.A total of 35,000 hit molecules are obtained from PubChem after screening of different databases. Other databases screened including CHEMBL36, ChemDiv, ChemSpace and ZINC (Table 4). After removing the duplicates, the resulted molecules were subjected to docking using inbuilt docking module in the server supported by AUTODOCK VINA [13], to obtain binding scores. PubChem-138708911 exhibited the most favourable predicted score (−10.64 kcal/mol) (Table 5). There is a recurrence of same hits with all the WT and mutant varieties which clearly supports for comparative analysis. ADMET property [14, 15] prediction of top one hit is given in the given below Table 6. However, these scores do not establish that the compounds bind experimentally or act as REV-ERBα agonists or antagonists. Further evaluation

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of binding-site interactions, chemical properties, predicted ADMET profiles, and, ultimately, biological activity is required before identifying validated hits.

Table 3: Pharmacophore query parameters

Parameter	X	Y	Z
Aromatic	28.41	-22.183	0
Aromatic	32.115	-20.006	0
HydrogenAcceptor	23.673	-16.134	0
HydrogenAcceptor	26.895	-17.258	0
Hydrophobic	28.41	-22.183	0
Hydrophobic	32.115	-20.006	0
Hydrophobic	24.786	-15.968	0
Hydrophobic	26.865	-14.011	0

Table 4: Number of hits obtained after pharmacophore based screening of databases

Database	No. of hits-F443L_stl1267
ChemBL36	2667
ChemDiv	964
ChemSPace	2258
ZINC	4060
PubChem	35000

Table 5: Top five hits and their binding scores obtained after docking

Compound ID	Binding score (kcal/mol) (F443L_stl1267)
PubChem-138708911	-10.64
PubChem-139435425	-10.14
PubChem-163435085	-10
PubChem-161956886	-9.57
PubChem-146540777	-9.4 with R500H mutant var.

Table 6: ADMET property prediction of top one hit

Pubchem ID	138708911
MolWt	534.53
Num. rotatable bonds	12
Num. H-bond acceptors	10
Num. H-bond donors	3
Log P o/w (iLOGP)	1.74
Drug likeliness	No
Synthetic accessibility	4.81
Predicted LD50 (mg/Kg)	1200
Hepatotoxicity	Inactive
Carcinogenicity	Inactive
Immunotoxicity	Inactive
Mutagenicity	Inactive
Cytotoxicity	Inactive

CONCLUSION

The comparative computational investigation of wild-type REV-ERBα and the F443L and R500H variants revealed that F443L variantprovided an opportunity to investigate the contribution of an aromatic binding-pocket residue, whereas R500H enabled evaluation of a potentially different local structural and chemical effect. Docking study, protein–ligand interaction analysis, and pharmacophoremodeling helped identify variant-associated changes in ligand-recognition features. Molecule with Pubchem ID138708911 showed more biding affinity than STL1267 binding score (-10.67 kcal/mol) which indicates the probable existence of potential REV-ERBα modulators. Feasible ADMET properties were also observed with this molecule; however, biological significance must be established apart from the actual computational results for proper experimental validation.

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