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Original Research Article

Investigation of single nucleotide polymorphisms of human vitamin D receptor gene and their subsequent effects on the receptor structure and function by in silico methods

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ABSTRACT

Background: Vitamin D receptor (VDR) polymorphism play vital role in genetic regulation of bone mass. It has been identified that the occurrence of osteoporosis mainly caused by mutations in functional regions of the VDR gene which can be highly disturb the metabolism of minerals especially the calcium ions. Our goal in this study is to use in silico methodologies and publicly accessible web databases to evaluate the impact of missense SNPs in the human VDR gene.
Methods: We used SIFT, VEP, PROVEAN, SNPs & GO, and PANTHER to predict the functional effects of mutations. I-Mutant 2.0 and Project HOPE were used to estimate the impacts on the protein's stability and three-dimensional structure. GeneMANIA has been used to evaluate how VDR gene would interact with 20 other genes.
Results: We estimate the effects of an amino acid substitution on protein structure and function depending on sequence homology, physical properties of amino acids and comparative physical properties respectively and also predicts the possible effect of an amino acid substitution on protein activity.
Conclusions: Overall, this is a thorough study that gives a quick overview of all the information on the clinically important missense SNPs of VDR gene.

Keywords: dbSNP, HOPE, I-Mutant 2.0, In silico, Osteoporosis, PROVEAN, SIFT, Single nucleotide polymorphism, SNPs & GO, Vitamin D receptor

INTRODUCTION

Vitamin D receptor (VDR) protein, encoded by VDR gene is a nuclear steroid receptor in Human.[1] VDR gene is situated at chromosome 12 cen-q12.[2] The gene is composed of 100 kb of genomic DNA and comprises 8 protein coding exons (exon 2-9), six untranslated exons 1a–1f which comprises three domains called a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain.[3,4]

Scientific reports explained the complex associations of vitamin D signaling with bone health and metabolism.[5] This action of vitamin D is mediated through VDR that specifically binds to the active form of vitamin D i.e., 1,25-dihydroxyvitamin D3 or calcitriol for the regulation of skeletal development, maintenance of skeletal architecture, hormone secretion and immune function.[6] Calcitriol or the active form of vitamin D plays an important role in calcium metabolism of osteoclasts and osteoblasts. There are multiple evidences that calcitriol working as a hormone enhances the amount of calcium absorption in the gastrointestinal tract and helps in osteoid tissue calcification.[7]

VDR needs to form a heterodimer with the retinoid X receptor to bind with vitamin D ligands that ultimately translocases to the nucleus and binds with the Vitamin D response element (VDRE) in the promoter regions of Vitamin D target genes.[8] The promoter hyper methylation of the VDR gene is one of the major regulators of its expression and function.[9] As VDR plays a significant role in mediating the effects of Vitamin D, investigating its (VDR) function is essential for the better knowledge of the pathophysiology of musculoskeletal diseases such as osteoporosis.[10] Patients of rheumatoid arthritis were detected with increased serum level of vitamin D concentrations and VDR promoter hypermethylation.[11] Current research indicates a connection between Caudal-type homeobox protein 2 (Cdx2) genotype-specific VDR expression and variable VDR promoter methylation in osteoporosis patients, indicating the intricate interplay between genetics, epigenetics, and environmental factors in the pathogenesis of osteoporosis.[9] Diseases like systemic sclerosis have been linked to decreased blood vitamin D levels.[12]

Beside other genetic consequences, Single Nucleotide Polymorphisms (SNPs) in VDR gene has been found to be associated with osteoporosis. The VDR gene has been found to contain more than 200 SNPs, yet it is unclear how these variations affect the function of the VDR protein.[13] Several VDR SNPs significantly alter its function, which results in osteoporosis development.[14] Some of the frequently researched VDR SNPs such as FokI (rs2228570), ApaI (rs7975232), BsmI (rs1544410), and TaqI (rs731236) are found to be related to osteoporosis.[15] *ApaI, TaqI and BsmI* were found to be significant determinant risk factors for osteoporosis progression and BMD regulator in the Saudi population.[13,16] These three polymorphisms have also been shown to be associated with post-menopausal osteoporosis risk in Belarusian women.[17] With the increasing aged population, osteoporosis has become a major health issue worldwide especially in developing country like India. Study shows that there is a sharp increase of osteoporotic patients in India between 2012 to 2022.[18,19]

In this period several studies have been organized to be find out the association between SNPs of VDR and osteoporosis. In North Indian postmenopausal women, BsmI and FokI is significantly linked to osteoporosis.[20] On the other hand, TaqI and ApaI may be significant genetic indicators of osteoporosis pathophysiology in a group of south Indian women.[21] There is a report that different VDR polymorphisms may associate differently with osteoporosis risk in different ethnic populations.[22] Almost all the available SNPs of VDR gene and their functional effects were thoroughly studied by in silico approach.

The objective of this study was to investigate the missense SNPs available in the database for VDR gene and to find out their functional significance by *in silico* methods.

METHODS

This original research article is based on the data available in different biological databases. The study was conducted in the Laboratory of Molecular Cell Biology and Genetics, Department of Zoology, University of Gour Banga, Malda, West Bengal.

Data collection and analysis

This original research article is based on the data available in different biological databases. For in-silico analysis various bioinformatics software are used. The study is conducted in the Laboratory of Molecular Cell Biology and Genetics, Department of Zoology, University of Gour Banga, Malda, West Bengal with an institutional ethical clearance (Ref. No.: UGB/REC/03/2024, Dated: 05/09/2024). No human subject, biological samples or hospital data is used for this study.

Exploring the protein and SNP database of VDR gene

The National Centre for Biotechnology Information (NCBI) dbSNP database was used to get the reported SNPs of VDR gene (NCBI Gene ID: 7421) in July 2024 (https://www.ncbi.nlm.nih.gov/snp). "VDR" was used as search term to find out the missense SNPs. Accession numbers of the missense SNPs found in this process are used for future analysis. Missense SNPs are particularly filtered for this study because they may lead to substitutions of amino acids in the sequence of the wild type protein, affecting the structure and function of the protein. The missense SNPs were sub-categorized into benign, likely benign, likely pathogenic, and pathogenic based on clinical significance. UniProt database (https://www.uniprot.org/) was used to find out the amino acid sequence of the protein (UniProt accession number: P11473) encoded by the VDR gene.

Prediction of the structural and functional impact of amino acid change on VDR protein

The structural and functional effects of missense SNPs on VDR protein were predicted by several freely available online software tools.[23,24] Then the SNP accession numbers of all the SNPs were uploaded one by one into Variant Effect Predictor (VEP) (http://www.ensembl.org/Tools/VEP) and enabled “SIFT” (Sorting Intolerant from Tolerant) (http://siftdna.org/www/SIFT_dbSNP.html) and “PolyPhen” (Polymorphism Phenotyping v2) (http://genetics.bwh.harvard.edu/pph2/) and run the program to obtain the result. SIFT and PolyPhen estimates the effects of an amino acid substitution on protein structure and function depending on sequence homology, physical properties of amino acids and comparative physical properties respectively. These two algorithms provided the respective predictions of the functional significance of each SNP. VEP provides a SIFT and PolyPhen prediction and score. Depending on the SIFT prediction it is selected both deleterious and tolerated

variants for the next level of analysis. The particular amino acid substitution for each SNP uploaded to three other software viz. PROVEAN (Protein Variation Effect Analyzer) (http://provean.jcvi.org/ index.php), SNPs & GO and PANTHER (http://www.pantherdb.org/ tools/csnpScoreForm.jsp) to get their predictions and scores. SNP & GO (http://snps.biofold.org/snps-and-go/snps-and-go.html) predicts about the relatedness of the SNP with a pathogenic condition. PROVEAN and PANTHER predicts the possible effect of an amino acid substitution on protein activity. The effect of these SNPs on protein stabilization was determined by I-Mutant 2.0 (http://folding.biofold.org/i-mutant/i-mutant2.0.html).
The effect of the amino acid substitutions on the 3D structures of the proteins was predicted by using an automatic program Project HOPE (http://www.cmbi.ru.nl/hope/method/).

Predicting the genetic interactome of VDR

To predict the genetic interactome of VDR as a candidate gene, GeneMANIA (https://genemania.org/) web server was used. In the search pane, "VDR" was put as the search term and a complex genetic network was retrieved. The network is based on various genes interacting with VDR in terms of physical interactions, gene co-expression, predicted, co-localization, pathway, genetic interactions and shared protein domains. A rank wise distribution of the genetic partners depending on the functional association 25, 26, 27 with VDR was also retrieved.

Gene-gene interactions

The interaction of VDR gene with another 20 genes is shown in figure 4 in the form of a highly connected network. GeneMANIA software tool shows that RXRB, MED1, CYP3A4, BAG1 are ranked in first, second, third and fourth position respectively in terms of the interaction with VDR gene. RXRB (retinoid X receptor beta) and

VDR are members of the nuclear receptor superfamily and they function as heterodimers to regulate transcription of various genes.[25] MED1 (mediator complex subunit 1) implement its role via KDM4B–CCAR1–MED1 signaling axis which induces euchromatinization near the promoters of osteoclast-related genes through H3K9 demethylation.[26] CYP3A4 (cytochrome P450 family 3 subfamily A member 4) gene is associated with low BMD.[27]

RESULTS

Prediction of the impact of missense SNPs on VDR protein function and stability

A total of 25538 SNPs were listed for the human VDR gene in the NCBI dbSNP database. 459 of these SNPs were identified as missense. 04 benign, 01 likely benign, 02 likely pathogenic, and 11 pathogenic missense SNPs were retrieved from NCBI utilizing category-wise dual filtration for clinical significance. SNPs were removed from the above list that NCBI deemed "merged" and those that did not have any changes to the amino acids, and then moved on to the remaining 18 missense SNPs (04 benign, 01 likely benign, 02 likely pathogenic, and 11 pathogenic) for additional study (Figure 1). Here rs2228570 is categorized as benign, it is also helpful in drug response. 13 missense SNPs identified as harmful and 5 were identified as tolerated by SIFT analysis.

Table 1 provides a summary of the information obtained from putting 18 SNPs through all the online applications. Figure 2 show the SNP IDs included in each functional category determined from SIFT, PolyPhen, PROVEAN, SNPs & GO, and PANTHER. I-Mutant 2.0 software tool results revealed that only one SNP has an enhancing influence on VDR stability, the other 17 SNPs have a reducing effect (Table 1). The protein has several domains and SNPs are situated on them. The details of all SNPs situated in different domain are enlisted in Table 2.

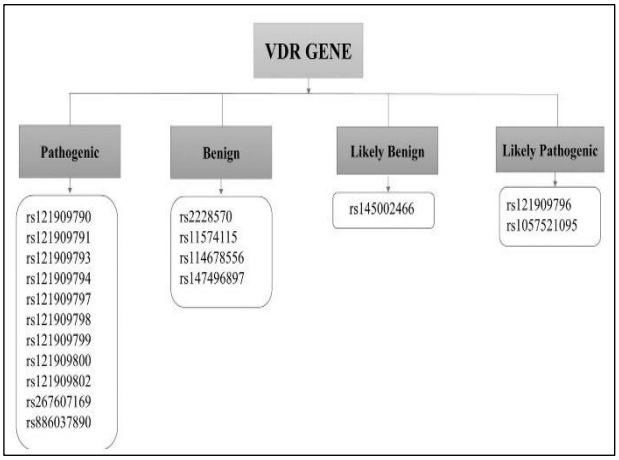

Figure 1: Different missense SNPs of VDR gene.

Table 1: Outcome of SIFT, POLYPHEN, PROVEAN, SNPs & GO, PANTHER and I-MUTANT software.

SNP ID	Nucleotide change	Amino acid change	SIFT	POLYPHEN-2	PROVEAN	SNPs & GO	PANTHER	I-mutant
			Result & score	Result & score	Result & Score		Result	Result Score
Pathogenic								
rs121909790	C>G,T	G33D	Deleterious & 0	Probably damaging & 1.000	Deleterious & -5.69	Disease	Probably damaging	Decrease & 9

Continued.

SNP ID	Nucleotide change	Amino acid change	SIFT	POLYPHEN-2	PROVEAN	SNPs & GO	PANTHER	I-mutant
rs121909791	C>T	R73Q	Deleterious & 0	Probably damaging & 1.000	Deleterious & -3.54	Disease	Probably damaging	Decrease & 9
rs121909793	C>T	R80Q	Deleterious & 0.013	Probably damaging & 0.996	Deleterious & -3.51	Disease	Probably damaging	Decrease & 9
rs121909794	C>T	R50Q	Deleterious & 0	Probably damaging & 1.000	Deleterious & -3.51	Disease	Probably damaging	Decrease & 8
rs121909797	C>A,G,T	G46D	Deleterious & 0	PD 1.000	Deleterious & -5.69	Disease	Probably damaging	Decrease & 8
rs121909798	G>A,C	H305Q	Tolerated & 0.155	Probably damaging & 0.977	Neutral & -2.37	Neutral	Probably damaging	Decrease & 6
rs121909799	A>C	I314S	Tolerated & 0.122	Probably damaging & 0.739	Deleterious & -3.21	Disease	Probably benign	Decrease & 4
rs121909800	G>A,T	R391C	Deleterious & 0	Probably damaging & 1.000	Deleterious & -6.76	Disease	Probably damaging	Decrease & 3
rs121909802	C>A,T	E329K	Deleterious & 0.004	Probably damaging & 1.000	Deleterious & -3.6	Disease	Probably damaging	Decrease & ,1
rs267607169	C>T	V346M	Deleterious & 0.001	Probably damaging & 0.998	Deleterious & -2.61	Disease	Probably damaging	Decrease & 6
rs886037890	T>G	H397P	Deleterious & 0(VEP)	Probably damaging & 0.999	Deleterious & -5.98	Disease	Probably damaging	Increase & 6
Benign								
rs11574115	G>A	T362I	Tolerated & 0.309	Benign & 0.009	Deleterious & -4.14	Neutral	Possibly damaging	Decrease & 3
rs114678556	C>A,T	R358H	Tolerated & 0.336	Benign & 0.045	Neutral & -1	Disease	Probably damaging	Decrease & 5
rs147496897	G>A,T	R18W	Deleterious & 0.002	Probably damaging & 0.998	Neutral & -1.84	Disease	Possibly damaging	Decrease & 8
Drug response								
rs2228570 (also Benign)	A>C,G,T	M1T	Deleterious & 0.013	Benign & 0.289	Neutral & 0.36	Neutral	Probably damaging	Decrease & 7
Likely benign								
rs145002466	G>A	T59I	Tolerated & 0.164	Benign & 0.217	Deleterious & -3.03	Disease	Probably damaging	Decrease & 7
Likely pathogenic								
rs121909796	C>A	R274H	Deleterious & 0.009	Probably damaging & 1	Deleterious &-5.09	Disease	Probably damaging	Decrease & 8
rs1057521095	G>A	R343C	Deleterious & 0(VEP)	Probably damaging & 1	Deleterious &-6.78	Disease	Probably damaging	Decrease & 5

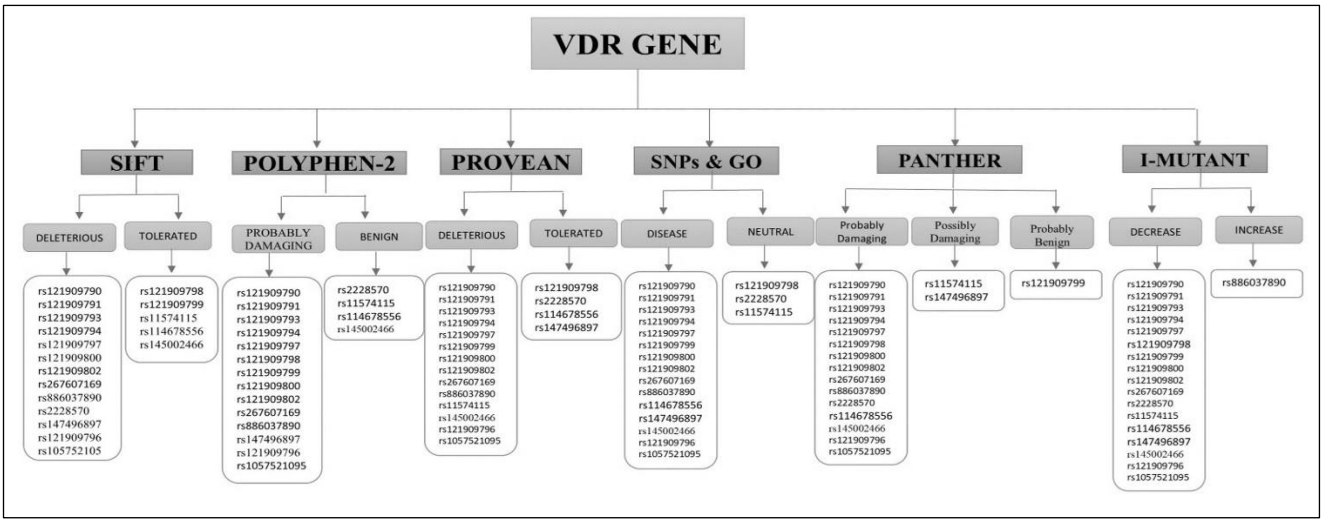

Figure 2: Deleterious or damaging SNPs predicted by online software tools.

Table 2: The position of deleterious and tolerated SNPs in different domains within VDR protein.

Domain	Deleterious (SNP ID)	Tolerated (SNP ID)
Nuclear hormone receptor, ligand-binding domain IPR000536	rs121909800, rs121909802, rs267607169, rs886037890, rs121909796, rs1057521095	rs121909798, rs121909799, rs11574115, rs114678556
Nuclear hormone receptor-like domain superfamily IPR035500	rs121909800, rs121909802, rs267607169, rs886037890, rs121909796, rs1057521095	rs121909798, rs121909799, rs11574115, rs114678556
VDR, DNA-binding domain IPR042153	rs121909793, rs121909794, rs121909797, rs147496897	rs145002466
Zinc finger, nuclear hormone receptor-type IPR001628	rs121909790, rs121909791, rs121909793, rs121909794, rs121909797	rs145002466
Zinc finger, NHR/Gata-Type IPR013088	rs121909790, rs121909791, rs121909793, rs121909794, rs121909797, rs147496897	rs145002466
Vitamin D Receptor IPR000324	rs121909791, rs147496897	NIL
Nuclear hormone receptor IPR001723	rs121909800, rs121909802, rs886037890	NIL

Table 3: Difference between wild- type and mutant-type amino acid properties obtained from Project HOPE software.

SNP ID	Amino acid change	Wild type amino acids			Mutant type amino acids		
		Size	Charge	Hydrophobicity	Size	Charge	Hydrophobicity
rs121909790	G33D	<	neutral	>	>	- charge	<
rs121909791	R73Q	>	+ charge	NIL	<	neutral	NIL
rs121909793	R80Q	>	+ charge	NIL	<	neutral	NIL
rs121909794	R50Q	>	+ charge	NIL	<	neutral	NIL
rs121909796	R274L	>	+ charge	<	<	neutral	>
rs121909797	G46D	<	neutral	>	>	- charge	<
rs121909798	H305Q	>	NIL	NIL	<	NIL	NIL
rs121909799	I314S	>	NIL	>	<	NIL	<
rs121909800	R391C	>	+ charge	<	<	neutral	>
rs121909802	E329K	<	- charge	NIL	NIL	+ charge	NIL
rs267607169	V346M	<	NIL	NIL	>	NIL	NIL
rs886037890	H397P	>	NIL	<	<	NIL	>
rs2228570	M1T	>	NIL	>	<	NIL	<
rs11574115	T362I	<	NIL	<	>	NIL	>

Continued.

SNP ID	Amino acid change	Wild type amino acids			Mutant type amino acids		
		Size	Charge	Hydrophobicity	Size	Charge	Hydrophobicity
rs114678556	R358H	>	+ charge	NIL	>	neutral	NIL
rs147496897	R18W	<	+ charge	<	>	neutral	>
rs145002466	T59I	<	NIL	<	>	NIL	>
rs1057521095	R343C	>	+ charge	<	<	neutral	>

The nuclear hormone receptor, ligand-binding domain IPR000536 and Nuclear Hormone Receptor-Like Domain Superfamily IPR035500 both contain various deleterious SNPs like rs121909800, rs121909802, rs267607169, rs886037890, rs121909796, rs1057521095 and tolerated SNPs rs121909798, rs121909799, rs11574115, rs114678556. The Project HOPE results showed in table 3 refers that the SNP rs121909800 substitute arginine to cysteine (R391C). The charge of the wild-type residue is lost by this mutation. This can cause loss of interactions with other molecules. The mutant residue is smaller than the wild-type residue. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. This will cause a possible loss of external interactions. The hydrophobicity of the wild-type and mutant residue differs. The difference in hydrophobicity will affect hydrogen bond formation. The wild-type residue forms a hydrogen bond with aspartic acid at position 342 and also forms a salt bridge with glutamic acid at position 269 and aspartic acid at position 342. Another substitution of E329K (glutamic acid to lysine) for rs121909802 has a crucial effect. The charge of the buried wild-type residue is reversed by this mutation, this can cause repulsion between residues in the protein core. This wild-type residue was buried in the core of the protein. The mutant residue is bigger and probably will not fit. The wild-type residue forms a hydrogen bond with histidine at position 326 and 371.The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. For rs267607169 V346M (valine to methionine) substitution the mutant residue is bigger than the wild-type residue and the wild-type residue was buried in the core of the protein. The mutant residue probably will not fit. Another substitution of H397P for rs886037890 explicit that the mutant residue is smaller than the wild-type residue and mutation will cause an empty space in the core of the protein. The hydrophobicity of the wild-type and mutant residue differs. This difference in properties between wild-type and mutation can easily cause loss of interactions with the ligand. Because ligand binding is often important for the protein's function, this function might be disturbed by this mutation. The mutation will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding. In case of rs121909796 the R274H substitution there is a difference in charge between the wild-type and mutant amino acid and the charge of the buried wild-type residue is lost by this mutation. The mutant residue is smaller than the wild-type residue and this mutation will cause an empty space in the core of the protein. Another R343C substitution for rs1057521095 shows the wild-type residue forms a hydrogen bond with glutamic acid at position 269 and cysteine at position 337. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. The difference in hydrophobicity will affect hydrogen bond formation. The wild-type residue forms a salt bridge with glutamic acid at position 269 and aspartic acid at position 342. The difference in charge will disturb the ionic interaction made by the original, wild-type residue. The mutant residue is smaller than the wild-type residue and this mutation will cause an empty space in the core of the protein. The mutation will also cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding. Tolerated SNPs also have impact on protein structure and function. SNP of VDR gene rs121909798 make H305Q substitution and the mutant residue is smaller than the wild-type residue. The mutation will cause an empty space in the core of the protein. The difference in properties between wild-type and mutation can easily cause loss of interactions with the ligand of the VDR protein. Because ligand binding is often important for the protein's function, this function might be disturbed by this mutation. The wild-type residue forms a hydrogen bond with Glutamine at position 400. The size difference between wild-type and mutant residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. Another substitution of I314S of rs121909799 is also shows that the mutant residue is smaller than the wild-type residue. This mutation will cause an empty space in the core of the protein. The hydrophobicity of the wild-type and mutant residue differs and this mutation will cause the loss of hydrophobic interactions in the core of the protein. A threonine to isoleucine conversion at the position of 362 (T362I) due to rs11574115 is buried in the core of the domain and the differences between the wild-type and mutant residue might disturb the core structure of this domain. The mutation will cause loss of hydrogen bonds in the core of the protein and as a result disturb correct folding. As wild-type residue is not conserved at this position the other residue type is not similar with mutant residue. Therefore, the mutation is possibly damaging the structure of the protein. Wild-type amino acid arginine at position 358 is converted to histidine (R358H) for the SNP rs114678556 and the mutated residue is located on the surface of a domain with unknown function. The wild-type residue forms a hydrogen bond with proline at position 249 but the size difference between wild-type and mutant

residue makes that the new residue is not in the correct position to make the same hydrogen bond as the original wild-type residue did. The wild-type residue forms a salt bridge with glutamic Acid at position 127 and aspartic acid at position 253. The charge difference will disturb the ionic interaction made by the original, wild-type residue.

The Vdr, Dna-Binding Domain IPR042153 contain deleterious SNPs rs121909793, rs121909794, rs121909797, rs147496897 and tolerated SNP rs145002466. The Vitamin D Receptor IPR000324 only contain deleterious SNPs rs121909791, rs147496897. The Nuclear Hormone Receptor IPR001723 only contain deleterious SNPs rs121909800, rs121909802, rs886037890. The Zinc Finger, Nuclear Hormone Receptor-Type IPR001628 and Zinc Finger, Nhr/Gata-Type IPR013088 both contain deleterious SNPs rs121909790, rs121909791, rs121909793, rs121909794, rs121909797 and tolerated SNP rs145002466 except deleterious SNP rs147496897 which is only situated on Zinc Finger, Nhr/Gata-Type IPR013088.

For the SNP rs121909793 and rs121909794 amino acid substitution is same i.e., Arginine to glutamine in the position of 80 and 50 respectively. For both the amino acids there is a difference in charge between the wild-type and mutant one. The charge of the wild-type residue will be lost, this can cause loss of interactions with other molecules or residues. The wild-type and mutant amino acids differ in size, the mutant residue is smaller, this might lead to loss of interactions. In case of rs121909790 and rs121909797 which is also situated on The Zinc Finger, Nuclear Hormone Receptor-Type IPR001628 both has the same changes in amino acid. Glycine is substituted by aspartate in the 33rd and 46th position. Both the substitution differs in charge between the wild-type and mutant amino acid. The mutation introduces a charge, this can cause repulsion of ligands or other residues with the same charge. The wild-type and mutant amino acids differ in size and the mutant one is bigger; this might lead to bumps. The torsion angles for this residue are unusual. only glycine is flexible enough to make these torsion angles, mutation into another residue will force the local backbone into an incorrect conformation and will disturb the local structure. Another SNP of VDR gene, rs147496897 has the R18W substitution in which there is a difference in charge between the wild-type and mutant amino acid. The charge of the wild-type residue will be lost, this can cause loss of interactions with other molecules or residues. The mutant residue is bigger, this might lead to bumps. The hydrophobicity of the wild-type and mutant residue differs and the mutation introduces a more hydrophobic residue at this position. This can result in loss of hydrogen bonds and/or disturb correct folding. An R73Q substitution of rs121909791 which is also located on The Vitamin D Receptor IPR000324 domain, there is a difference in charge between the wild-type and mutant amino acid which can cause loss of interactions with other molecules or residues. This mutation is probably damaging to the protein. The mutant residue is located near a highly conserved position. The mutant residue is smaller, this might lead to loss of interactions. An T59I substitution for rs145002466, the wild-type and mutant amino acids differ in size. The mutant residue is bigger, this might lead to bumps. The hydrophobicity of the wild-type and mutant residue differs and the mutation introduces a more hydrophobic residue at this position. This can result in loss of hydrogen bonds and/or disturb correct folding. For SNP rs2228570 the amino acid change in first position from methionine to threonine (M1T) this SNP not situated in any domain and the mutation is likely not harmful to the protein as the wild-type amino acid is very conserved. The 3D model of structural effect of the SNPs of VDR predicted by Project HOPE is enlisted in Figure 3.

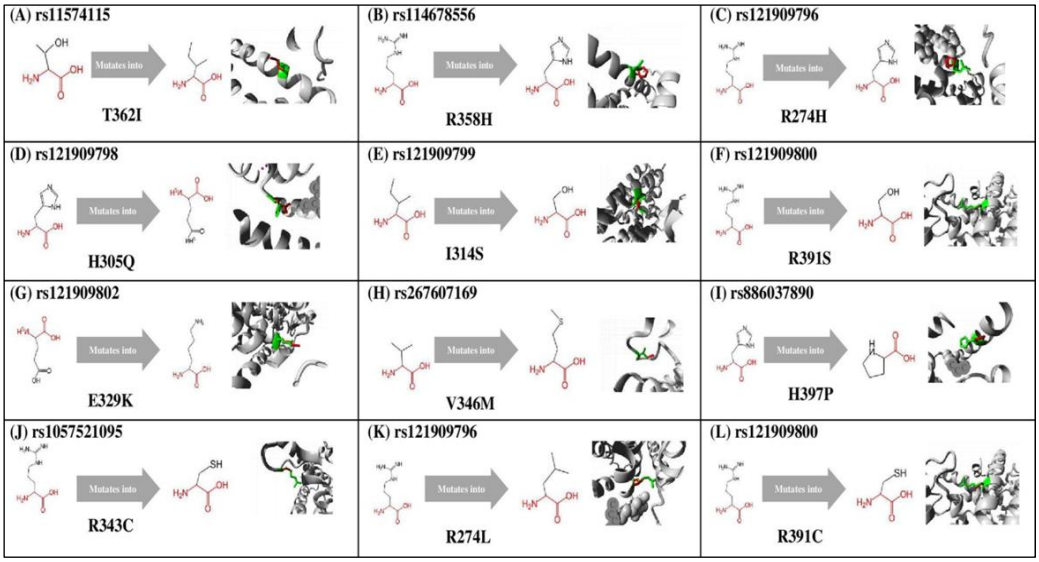

Figure 3: 3D model of structural effect of the SNPs predicted by Project HOPE.

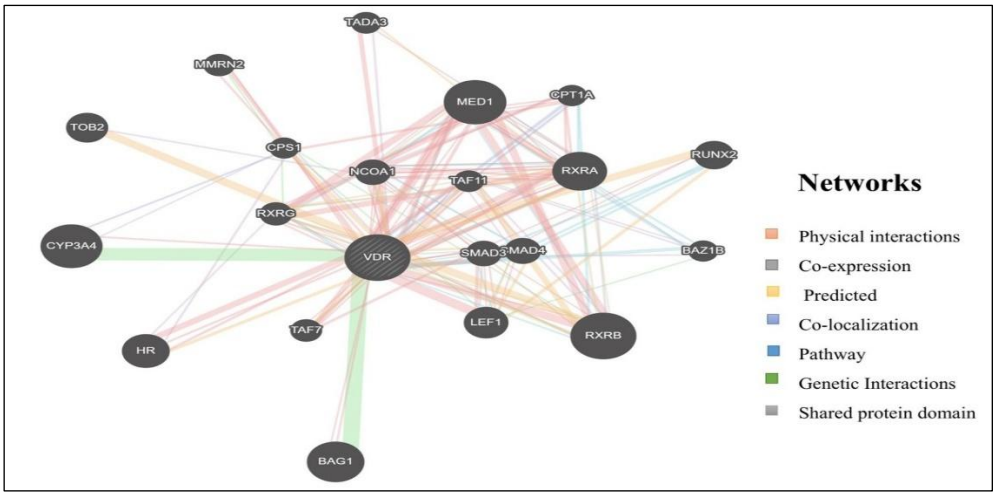

Figure 4: Gene–gene interactions of VDR.

DISCUSSION

This is a comprehensive report of almost all the available information about the structural and functional effects of missense SNPs of VDR gene. Additionally, there is still need to collect more experimental data regarding all possible substitutions of amino acids to fill in the gaps in knowledge. For example, in certain instances, Project HOPE was unable to generate a 3D structure because of a lack of data. Some tolerated SNPs (like rs2228570) were found associated with osteoporosis significantly in some populations of the world.[15,20] Particularly this SNP (rs2228570) has been found to be associated with drug response against osteoporosis. This fact is experimentally proven by another study.[28] Therefore, alternative method should be reconsidered for software driven categorization of the SNPs as deleterious and tolerated. A recent study identified strong physical interaction between VDR and two of its partners RXR and MED1 through a Gene Mania derived proposed genetic network of VDR.[14] In this study, a similar genetic network proposed not only dependent on physical interaction but also co-expression, predicted, co-localization, pathway, genetic interactions and shared protein domain (Figure 4).

Almost all the available data about missense SNPs of VDR were assembled. Besides association studies, functional analyses are also required to investigate the role of these SNPs in disease formation. Hence this article will be an essential pre-document before experimental designing.

This study has few limitations. The analysis of SNPs in the human VDR gene utilizing in silico techniques comes with various limitations. Computational predictions depend on existing databases and algorithms, which might not entirely reflect the intricacies of protein dynamics within a cellular context. Experimental verification is necessary to ascertain the real structural and functional impacts of mutations. Moreover, aspects like protein folding, post-translational modifications, and interactions with other molecules are not always represented accurately in models. The precision of structural forecasts hinges on the quality of the reference protein model, and uncommon or novel SNPs may not have ample data for trustworthy assessment.

Therefore, although in silico methods offer valuable perspectives, they should be supplemented with in vitro and in vivo research for a thorough understanding.

CONCLUSION

The analysis of SNPs in the VDR gene shows that they have a considerable effect on the structure and functionality of the receptor. A total of 18 missense SNPs were examined, with 13 classifieds as detrimental and 5 as benign, impacting essential protein domains such as the Nuclear Hormone Receptor and Zinc Finger domains. The mutations lead to structural alterations that may include changes in charge, variations in size, and interruptions in hydrogen bonding, which can destabilize the VDR protein and hinder its ability to bind with ligands, thereby affecting its biological role. Computational tools such as SIFT, PolyPhen, and I-Mutant 2.0 were employed to forecast these consequences, underscoring the importance of experimental validation to verify the results. Furthermore, an analysis of gene-gene interactions conducted using GeneMANIA revealed RXRB, MED1, CYP3A4, and BAG1 as significant interacting partners of VDR, highlighting its involvement in transcriptional regulation, chromatin remodeling, and bone metabolism. These findings emphasize the significance of VDR SNPs in relation to disease susceptibility and responses to drugs, indicating their potential utility as biomarkers in genetic research. Nevertheless, in silico predictions have their limitations, making it crucial to perform additional in vitro and in vivo studies to confirm the structural and functional impacts of these mutations.

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