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Functional Annotation of Missense Variants in CCR2 Gene: A computational approach to CKD susceptibility

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ABSTRACT

Background: Chronic kidney disease (CKD) affects approximately 9.1% of the global population and is increasingly recognized as a condition influenced by genetic predisposition. Among the genetic factors implicated, missense variants in the *C-C motif chemokine receptor 2 (CCR2)* gene are of particular interest due to CCR2's role in immune regulation. Variants in this gene may disrupt protein structure and function, potentially contributing to CKD pathogenesis. This study employs an *in silico* approach to investigate the structural and functional impact of *CCR2* missense variants and their potential association with CKD susceptibility.

Methods: Missense single nucleotide polymorphisms (SNPs) in the *CCR2* gene were retrieved from the dbSNP database. Ten computational tools—SIFT, PolyPhen-2, PANTHER, SNP&GO, I-Mutant 2.0, MUpro, MutPred2, ConSurf, Phyre2, and STRING—were employed to assess pathogenicity, protein stability, evolutionary conservation, structural alterations, and protein-protein interactions. Variants were classified as deleterious based on consensus predictions from at least six tools.

Results: Of the eight missense variants analysed, six (rs113340633, rs200491743, rs370278890, rs371121141, rs373211972, rs374045702) were consistently predicted to be deleterious. These variants were associated with reduced protein stability and significant structural alterations. Notably, substitutions such as L119P and M249K affected highly conserved residues and were predicted to disrupt chemokine-receptor interactions. Two variants (rs200575131 and rs368219093) yielded inconsistent results across tools, warranting further experimental validation.

Conclusion: This study highlights several *CCR2* missense variants that may impair protein function and contribute to CKD susceptibility through dysregulation of immune responses. These computational findings provide a foundation for future experimental validation and may inform precision medicine strategies in CKD diagnosis and management.

Keywords: *CCR2 gene,Missensevariants,chronic kidney disease,insilicoanalysis,Protein stability, Pathogenicity prediction*

1. INTRODUCTION

Chronic kidney disease (CKD) represents a growing global health burden, affecting approximately 9.1% of the population and contributing to increased morbidity and mortality worldwide [1, 2]. While environmental and lifestyle factors influence CKD progression, genetic predisposition plays a critical role in individual susceptibility. Among the implicated genetic elements, the *C-C motif chemokine receptor 2 (CCR2)* gene has emerged as a candidate of interest due to its regulatory role in immune response pathways, particularly in monocyte recruitment and inflammation—processes closely linked to renal injury and fibrosis [3, 4].

Missense variants in the *CCR2* gene, which result in single amino acid substitutions, have the potential to disrupt protein structure and function, thereby contributing to CKD pathogenesis [5, 6]. However, experimental validation of each variant's functional impact remains time-consuming and resource-intensive. In this context, *in silico* methods provide a rapid, cost-

effective approach to predict the functional and structural consequences of genetic variants. These tools integrate evolutionary conservation, physicochemical properties, and structural modeling to assess variant pathogenicity and their potential roles in disease [7, 8]

This study applies a comprehensive computational framework to evaluate the functional impact of missense variants in the *CCR2* gene associated with CKD susceptibility. A panel of web-based tools—including SIFT, PolyPhen-2, PANTHER, SNP&GO, I-Mutant 2.0, MUpro, MutPred2, ConSurf, Phyre2, and STRING—was employed to assess variant pathogenicity, structural stability, evolutionary conservation, and interaction networks. By integrating these predictive approaches, the study aims to identify potentially deleterious variants that may contribute to CKD and provide a foundation for future experimental validation and personalized therapeutic strategies.

2. MATERIALS AND METHODS

Variant Identification

Missense variants in the *CCR2* gene were identified from the dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), maintained by the National Center for Biotechnology Information (NCBI). Only single nucleotide polymorphisms (SNPs) resulting in amino acid substitutions were selected for downstream analysis. Variants were filtered to retain those with confirmed missense effects on the CCR2 protein sequence.

Functional Impact Prediction

A suite of web-based ***in silico*** tools was used to evaluate the potential functional, structural, and stability-related consequences of each identified missense variant. Each tool was selected for its ability to provide unique insights into the potential pathogenicity, stability, or structural consequences of the variants. The following tools were employed:

1. **SIFT (Sorting Intolerant From Tolerant) (https://sift.bii.a-star.edu.sg/):**SIFT predicts whether an amino acid substitution is tolerated or deleterious based on sequence homology and the physicochemical properties of amino acids. Variants were analyzed using the SIFT 4G algorithm, with scores ≤ 0.05 classified as deleterious and scores > 0.05 considered tolerated [9].

2. **PolyPhen2 (Polymorphism Phenotyping v2) (http://genetics.bwh.harvard.edu/pph2/):** PolyPhen2 assesses the impact of amino acid substitutions on protein structure and function using sequence-based and structural features. Variants were classified as probably damaging (score > 0.909), possibly damaging (0.447 < score ≤ 0.909), or benign (score ≤ 0.446) [10].

3. **PANTHER (Protein Analysis Through Evolutionary Relationships) (http://www.pantherdb.org/):**PANTHER uses evolutionary relationships to predict the functional effects of amino acid substitutions. The pSUB1 score was utilized, with scores < 0.5 indicating a likely deleterious variant [11].

4. **SNP&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/):**This tool employs machine learning to predict the effect of single point mutations on protein function and structure. Predictions were evaluated using the reliability index (RI), with RI > 6 indicating high confidence in the prediction [12].

5. **I-Mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html):**I-Mutant 2.0 predicts changes in protein stability upon mutation. The ΔΔG (DDG) value was calculated, where a negative DDG indicates decreased stability, suggesting a deleterious effect [13].

6**. MUpro (https://www.ics.uci.edu/~baldig/software.html):**MUpro uses support vector machines to predict protein stability changes upon mutation. The confidence score was used to assess the reliability of stability predictions [14].

7. **MutPred2 (http://mutpred2.mutdb.org/):**MutPred2 predicts the molecular mechanisms underlying disease-associated amino acid substitutions. A pathogenicity score was generated, with higher scores indicating a greater likelihood of pathogenicity [15].

8. **Consurf (https://consurf.tau.ac.il/):**Consurf identifies evolutionarily conserved positions in proteins. Conservation scores were used to determine whether variants affect highly conserved residues, which are more likely to be functionally significant [16].

9. **Phyre2 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index):**Phyre2 was used to model the three-dimensional (3D) structure of the CCR2 protein with and without variants. Structural models were visualized to assess changes in secondary structure, binding sites, or overall protein folding [17].

10**. String (https://string-db.org/):**The String database was used to analyze proteinprotein interaction networks. Variants were evaluated to determine if they affectresidues involved in known interactions, potentially disrupting CCR2's role in inflammatory pathways [18].

Data Integration and Variant Classification

To determine the overall pathogenicity of each variant, predictions from all tools were integrated using a consensus-based

approach. A variant was classified as deleterious if at least six out of the ten tools predicted a damaging or destabilizing effect. For variants with inconsistent predictions, further evaluation was conducted using literature review and prioritization based on ConSurf conservation scores. This integrative analysis enabled the identification of high-priority variants for potential experimental validation.

3. RESULTS &DISCUSSION

The computational analysis of missense single nucleotide polymorphisms (SNPs) in the *CCR2* gene provided critical insights into their potential role in chronic kidney disease (CKD) susceptibility. This study utilized a comprehensive bioinformatics pipeline to assess the pathogenicity, protein stability, structural consequences, evolutionary conservation, and protein interaction profiles of eight missense SNPs in *CCR2*.

Pathogenicity Predictions: The results from pathogenicity prediction tools (Table 1) revealed that six SNPs—rs113340633, rs200491743, rs370278890, rs371121141, rs373211972, and rs374045702—were consistently classified as deleterious or probably damaging by multiple algorithms including SIFT, PolyPhen-2, PANTHER, and SNP&GO. These tools incorporate information on sequence homology, amino acid properties, and conservation to infer the functional impact of variants [19, 9, 20].

Table 1:Functional impact of CCR2 missense SNPs predicted by SIFT, PolyPhen-2, PANTHER, and SNP&GO

SNP	Uniprot ID	Amino acid	SIFT	Polyphen 2.0		PANTHER	SNP&GO
				Human div	Human Var		
rs113340633	P41597	L119P	Deleterious	probably damaging	probably damaging	probably benign	Pathogenic
rs200491743	P41597	M249K	Deleterious	probably damaging	Possibily damaging	NA	Pathogenic
rs370278890	P41597	T153M	Deleterious	probably damaging	probably damaging	probably damaging	Pathogenic
rs371121141	P41597	F125L	Deleterious	probably damaging	probably damaging	NA	Pathogenic
rs373211972	P41597	G127V	Deleterious	probably damaging	probably damaging	NA	Pathogenic
rs374045702	P41597	A141V	Deleterious	probably damaging	probably damaging	probably damaging	Pathogenic
rs200575131	P41597	C75R	Deleterious	benign	benign	probably benign	Benign
rs368219093	P41597	G333R	Deleterious	benign	benign	probably benign	Benign

The consensus among these tools for SNPs such as L119P, M249K, T153M, F125L, G127V, and A141V suggests a high likelihood of disrupting *CCR2* function, potentially contributing to CKD susceptibility through altered chemokine receptor activity. However, discrepancies were observed for rs200575131 (C75R) and rs368219093 (G333R), which were predicted as deleterious by SIFT but benign by other tools. These inconsistencies highlight the limitations of individual prediction algorithms and underscore the importance of integrating multiple tools to improve reliability [21]. The variable predictions for L119P in SNPs&GO's Human Var model further emphasize the need for experimental validation to confirm the functional impact of these SNPs.

Table 2:Predicted effects of SNPs on protein stability by I-Mutant2.0 and MUpro

S. No	Amino acid variants	Imutant2.0		Mupro	
		Stability	DDG	Stability	DDG
1	L119P	Decrease	-2.12	Decrease	-1.7738
2	M249K	Decrease	-1.99	Decrease	-2.0126

3	T153M	Increase	0.25	Decrease	-0.5612
4	F125L	Decrease	-2.38	Decrease	-0.5044
5	G127V	Decrease	-1.42	Decrease	-0.6637
6	A141V	Increase	0.83	Decrease	-0.5777

Protein Stability Predictions: Protein stability assessment (Table 2) using I-Mutant2.0 and MUproindicatedthat L119P, M249K, F125L, and G127V consistently reduce *CCR2* protein stability, as indicated by negative DDG values. These findings align with pathogenicity predictions and imply structural destabilization (Table 1) and suggest that these variants may destabilize the protein's tertiary structure, potentially impairing its role as a chemokine receptor [13, 14]. In contrast, T153M and A141V showed conflicting stability predictions between I-Mutant2.0 (increased stability) and MUpro (decreased stability). Such discrepancies may arise from differences in the algorithms' consideration of local versus global structural effects, highlighting the complexity of predicting stability changes [22]. The destabilizing effects of these SNPs could disrupt *CCR2*'s interactions with chemokines, which are critical for immune responses and renal homeostasis [23].

MutPred2 Pathogenic Mechanisms: MutPred2 analysis (Table 3) revealedmechanistic insights into the pathogenicity of the SNPs, with L119P and M249K exhibiting the highest scores (0.76 and 0.783, respectively) and multiple molecular mechanisms, including altered transmembrane protein structure, loss of helix, and changes in ordered interfaces. These mechanisms suggest that these variants may disrupt *CCR2*'s membrane topology and ligand-binding capacity, which are essential for its role in inflammatory responses [24].

Table 3: MutPred2 scores and predicted mechanisms for CCR2 variants

S. No	Amino acid variants	MutPred2 score	Molecular mechanisms	P-value
1	L119P	0.76	Altered Transmembrane protein	1.50E-05
			Loss of Helix	8.10E-04
			Gain of Strand	2.70E-04
			Altered Ordered interface	0.03
			Gain of Catalytic site at K114	0.04
2	M249K	0.783	Altered Ordered interface	0.04
			Altered Disordered interface	0.03
			Altered Metal binding	8.10E-03
			Altered Transmembrane protein	3.20E-03
3	T153M	0.535	Altered Transmembrane protein	8.20E-04
4	F125L	0.432	NA	NA
5	G127V	0.624	Altered Ordered interface	0.04
			Altered Transmembrane protein	0.01
6	A141V	0.484	NA	NA

Lower MutPred2 scores for F125L and A141V, coupled with no identified mechanisms, indicate milder functional effects, consistent with their variable stability predictions. The integration of MutPred2 with other pathogenicity tools enhances the understanding of how these SNPs may contribute to CKD by affecting *CCR2*'s structural and functional integrity [15].

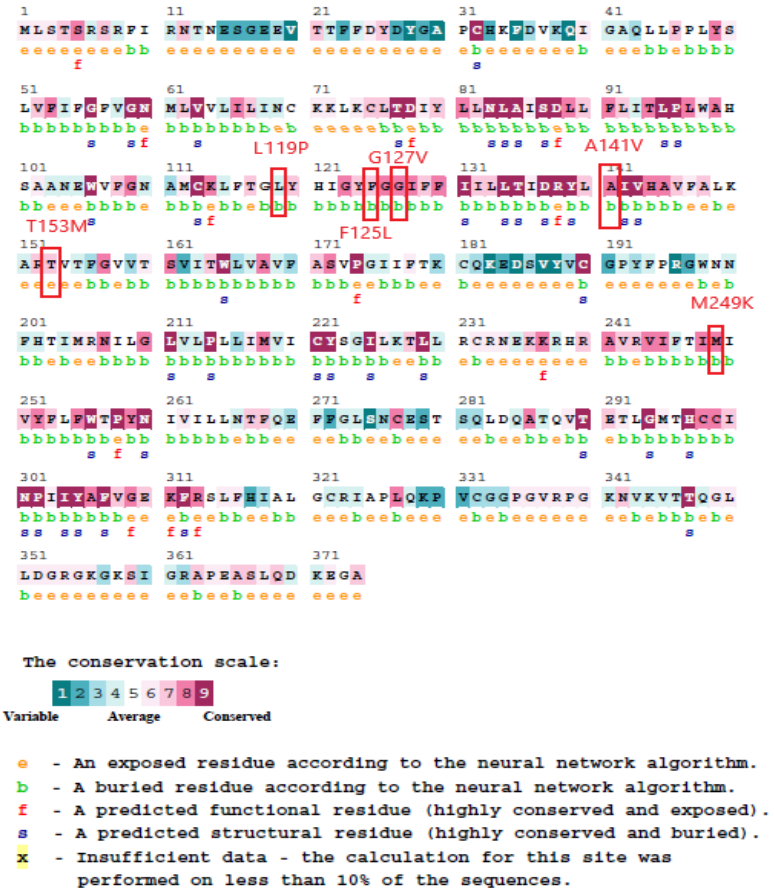

Figure 1.Evolutionary conservation profile of the CCR2 protein generated by ConSurf. Disease causing variants are highlighted.

Evolutionary Conservation: ConSurf analysis (Figure 1) revealed thatmost deleterious SNPs (F125L, G127V, A141V, M249K) occur in highly conserved residues (scores 7–8), which are typically buried and critical for structural stability or function [16]. This conservation underscores their potential to disrupt *CCR2*'s role in chemokine signaling, which is implicated in CKD progression [25]. T153M, located in an exposed residue, may affect surface interactions with chemokines, while L119P's lower conservation score (3) suggests it resides in a variable region. Despite this, its deleterious predictions indicate functional significance, possibly due to local structural disruptions [26].

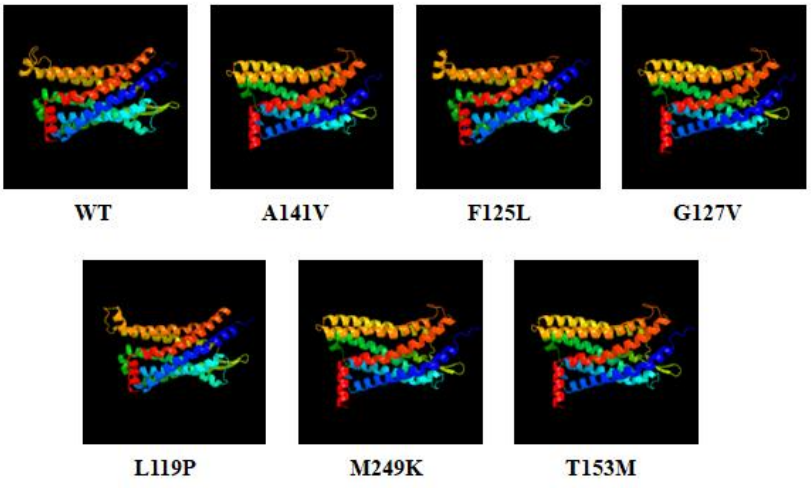

Figure 2.Three-dimensional structural models of wild-type and six mutant CCR2 proteins generated using Phyre2 homology modeling.

Structural Modeling: Phyre2-based structural modeling (Table 4, Figure 2) demonstratedsubtle changes in the mutant *CCR2* models, such as reduced alpha helix content in A141V and increased alpha helix in T153M and G127V. These alterations, though minor, align with the stability reductions observed in Table 2 and may affect *CCR2*'s folding or ligand-binding properties [17]. The high confidence and coverage of the models (76–79%) support their reliability for inferring structural impacts. These findings suggest that even small conformational changes could impair *CCR2*'s function in immune regulation, potentially exacerbating CKD [27].

Table 4:Secondary structure features of WT and mutant CCR2 models (Phyre2)

Models	Alpha helix (%)	Beta strand (%)	Disordered (%)	Confidence (%)	Coverage (%)
WT	61	3	20	100	76
A141V	59	4	20		78
G127V	62	2	20		79
F125L	61	2	20		76
L119P	60	3	20		77
M249K	61	3	20		79
T153M	62	2	20		79

Protein-Protein Interaction Analysis: STRING analysis (Figure 3) identified key CCR2 interactions with chemokines includingCCL11, CCL13, CCL2, CCL3, CCL4, CCL5, CCL7, CCL8, CCR2 (self-interaction), CXCL10, and CXCL8 (Figure 3). This confirmed *CCR2*'s interactions with multiple chemokines (e.g., CCL2, CCL5, CXCL8), reinforcing its role in inflammatory pathways relevant to CKD [18]. SNPs that destabilize *CCR2* or alter its transmembrane structure may disrupt these interactions, leading to dysregulated immune responses and renal injury [28]. The self-interaction of *CCR2* suggests potential dimerization, which could be affected by these variants, further impacting signaling efficiency [29].

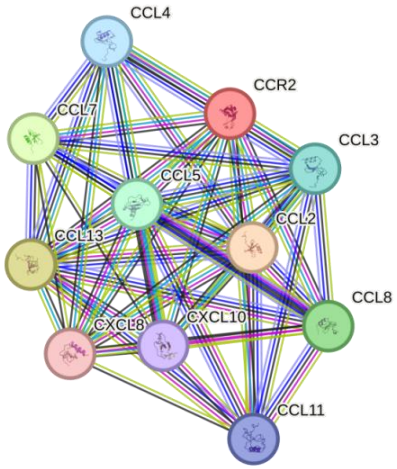

Figure 3: Protein-protein interaction network of CCR2 generated using the STRING database.

4. CONCLUSION

This study presents a comprehensive *in silico* functional annotation of missense single nucleotide polymorphisms (SNPs) in the *CCR2* gene, emphasizing their potential role in chronic kidney disease (CKD) susceptibility. The results indicate that variants such as L119P, M249K, T153M, F125L, G127V, and A141V are likely to impair CCR2 function through a combination of protein destabilization, structural disruptions, and interference with chemokine binding and signaling. The involvement of several variants in evolutionarily conserved regions further supports their functional relevance and potential contribution to immune dysregulation in CKD.

By integrating predictions from a diverse set of computational tools—including SIFT, PolyPhen-2, PANTHER, SNP&GO, I-Mutant 2.0, MUpro, MutPred2, ConSurf, Phyre2, and STRING, this study provides a robust prioritization of functionally significant SNPs for further investigation. However, the observed variability across prediction tools highlights the limitations of computational approaches and underscores the necessity for experimental validation.

Future research should focus on in vitro and in vivo functional assays to confirm the deleterious effects of these variants and elucidate their specific roles in CKD pathogenesis. These findings may contribute to the development of precision medicine approaches, enabling the identification of genetic biomarkers and the design of targeted interventions for CKD management.

5. CONFLICT OF INTEREST

The authors declare that there is no duality of interest associated with authorship, and/or publication of this manuscript.

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