International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No 20s, 2025
https://www.theaspd.com/ijes.php

"In Silico Assessment Of Ccr2 Gene Missense Variants: Implications For Antagonist Binding Affinity"

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ABSTRACT

Background: *The C-C chemokine receptor type 2 (CCR2) plays a pivotal role in modulating immune responses, particularly by guiding monocyte and macrophage chemotaxis in chronic kidney disease (CKD). Missense variants in the CCR2 gene may alter its protein structure and antagonist binding affinity, thereby impacting therapeutic efficacy. This study investigates the effects of six deleterious CCR2 missense variants (rs113340633, rs200491743, rs370278890, rs371121141, rs373211972, rs374045702) on the binding of three CCR2 antagonists—RS102895, RS-504393, and CCX140-B—using comprehensive in silico approaches.*

Methods: *Pharmacokinetic and toxicity profiles of the antagonists were evaluated using the pkCSM web server to predict ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. Homology models of the wild-type and variant CCR2 proteins (L119P, F125L, G127V, A141V, T153M, M249K) were generated using Phyre2. Molecular docking was performed using AutoDock Vina, and protein-ligand interactions were visualized with Discovery Studio.*

Results: *ADMET profiling revealed high intestinal absorption for RS102895 (87.7%) and RS-504393 (89.1%), with CCX140-B showing lower absorption (72.9%). All compounds exhibited predicted hepatotoxicity. Docking analysis showed RS-504393 had the highest binding affinity to wild-type CCR2 (-10.3 kcal/mol) and retained strong binding across all variants. The F125L variant improved RS102895 binding (-9.9 vs. -9.5 kcal/mol WT), whereas L119P and A141V reduced CCX140-B binding affinity (-8.6 vs. -9.6 kcal/mol WT).*

Conclusion: *RS-504393 demonstrates consistent binding across CCR2 variants, indicating broad therapeutic applicability. The F125L variant may enhance RS102895 efficacy, suggesting a potential for genotype-guided treatment strategies in CKD. These findings underscore the clinical importance of integrating genetic information into drug design and warrant further experimental validation.*

Keywords: *CCR2, Missense Variants, Molecular Docking, ADMET Profiling, AutoDock Vina, Chronic Kidney Disease (CKD), Precision Medicine, Pharmacogenomics*

INTRODUCTION

The C-C chemokine receptor type 2 (*CCR2*), a G-protein-coupled receptor (GPCR), plays a critical role in modulating immune responses by directing the chemotaxis of monocytes and macrophages toward sites of inflammation through interaction with its principal ligand, C-C motif chemokine ligand 2 (*CCL2*) (She et al., 2022; Xu et al., 2021). This signaling axis is implicated in the pathogenesis of several inflammatory and fibrotic renal disorders, including chronic kidney disease (CKD), diabetic nephropathy, and glomerulosclerosis, where excessive leukocyte infiltration contributes to tissue injury, interstitial fibrosis, and progressive loss of renal function (Imig& Ryan, 2013; Anders et al., 2003).

Pharmacological blockade of *CCR2* has shown significant therapeutic promise in animal models by attenuating monocyte recruitment, reducing proteinuria, and mitigating renal fibrosis (Awad et al., 2011; Kitagawa et al., 2004). However, genetic variability in the *CCR2* gene—particularly missense variants resulting in amino acid substitutions—may alter protein conformation, receptor stability, or ligand-binding affinity. These alterations have the potential to influence disease susceptibility, patient stratification, and ultimately, the clinical efficacy of CCR2-targeted therapies (Aldeghi et al., 2018).

Our prior in silico study identified six high-risk missense variants in *CCR2* (rs113340633, rs200491743, rs370278890, rs371121141, rs373211972, and rs374045702) based on consensus predictions from multiple bioinformatics tools (Lakkamraju et al., 2025). These variants were predicted to adversely affect structural integrity and receptor function, raising the possibility that they may also influence binding interactions with small-molecule antagonists used to inhibit CCR2 signaling in kidney disease contexts (Kojetin& Burris, 2012; Fantuzzi et al., 2019).

Three CCR2 antagonists—RS102895, RS-504393, and CCX140-B—have shown anti-inflammatory and antifibrotic effects in preclinical and early clinical settings. These compounds have demonstrated efficacy in reducing monocyte infiltration, proteinuria, and glomerular injury in models of diabetic nephropathy and other renal pathologies (He et al., 2023; Sullivan et al., 2013). However, the potential impact of CCR2 missense variants on the binding affinity and pharmacodynamics of these antagonists remains underexplored. This presents a critical knowledge gap, particularly in the context of pharmacogenomics and precision medicine.

Understanding how structural alterations caused by gene variants affect drug-receptor interactions is crucial for optimizing targeted therapies. In silico methodologies, including molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiling, offer robust platforms for predicting such effects with high throughput and low experimental cost (Chang et al., 2022).

This study integrates two computational approaches to investigate the pharmacokinetic properties and binding behaviors of RS102895, RS-504393, and CCX140-B with both wild-type and mutant CCR2 proteins. First, the pkCSM web server is used to evaluate the ADMET profiles of each antagonist (Pires et al., 2015). Second, molecular docking simulations using AutoDock Vina assess how the six CCR2 missense variants alter drug-binding affinities. By exploring these interactions in silico, we aim to provide foundational insights for variant-specific drug responsiveness and the development of personalized therapeutic strategies for CKD patients.

MATERIALS AND METHODS

1. In Silico Analysis of CCR2 Missense Variants

The functional and structural impacts of six missense variants in the *CCR2* gene were assessed using a previously established consensus-based computational pipeline (Lakkamraju et al., 2025). The analysis incorporated ten in silico tools: SIFT,PolyPhen-2, PANTHER, SNP&GO, I-Mutant 2.0, MUpro, MutPred2, ConSurf, Phyre2, and STRING. These tools predicted the pathogenic potential, evolutionary conservation, protein stability changes, and structural consequences of each amino acid substitution. The criteria and thresholds for deleterious variant selection were consistent with those described in the referenced study. Only variants classified as structurally or functionally deleterious were included for downstream pharmacological and structural analysis.

2. ADMET Profiling of CCR2 Antagonists

To evaluate the pharmacokinetic and toxicity properties of the three CCR2 antagonists—RS102895, RS-504393, and CCX140-B—the pkCSM web server (https://biosig.lab.uq.edu.au/pkcsm/) was employed. This platform uses graph-based structural signatures to predict a wide range of ADMET parameters.

Key parameters assessed included:

- **Absorption**: water solubility, Caco-2 permeability, human intestinal absorption, skin permeability, and interaction with P-glycoprotein (substrate/inhibitor status)
- **Distribution**: volume of distribution (VDss), fraction unbound (fu), blood-brain barrier (BBB) permeability, and CNS penetration
- **Metabolism**: interaction with cytochrome P450 (CYP) isoforms, including substrate/inhibitor predictions for CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4
- **Excretion**: total clearance and renal OCT2 substrate prediction
- **Toxicity**: AMES mutagenicity, hepatotoxicity, hERG I and II inhibition, skin sensitization, and maximum tolerated dose in humans

Molecular structures of the antagonists were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SMILES notations were used as input for pkCSM analysis.

3. Homology Modeling of Wild-Type and Variant CCR2 Proteins

3D structures of the wild-type *CCR2* and its six missense variants (L119P, F125L, G127V, A141V, T153M, and M249K) were generated using the Phyre2 web server(http://www.sbg.bio.ic.ac.uk/phyre2/). Each model was built using homology-based threading against known protein structures in the PDB. The top-ranked templates were selected based on alignment confidence, coverage, and sequence identity.

4. Ligand Preparation and Docking Protocol

The 3D conformers of RS102895, RS-504393, and CCX140-B were obtained from PubChem in SDF format and converted to PDB format using PyMOL (Schrödinger &DeLano, 2020). Energy minimization was performed prior to docking.

Molecular docking simulations were carried out using AutoDock Vina (Morris et al., 2009) to evaluate the binding interactions between the ligands and both wild-type and mutant CCR2 proteins. The docking grid was centered on the predicted active site of the CCR2 models, with grid box dimensions adjusted to encapsulate the ligand-binding pocket. A grid spacing of 1.0 Å was used.

Each docking experiment generated 10 poses per ligand-receptor complex. The pose with the lowest binding energy (kcal/mol) was selected as the most favorable conformation. Key protein-ligand interactions, including hydrogen bonds, hydrophobic contacts, and π-π stacking, were analyzed using Discovery Studio Visualizer (BIOVIA, 2021).

RESULTS & DISCUSSION

1. ADMET Analysis of CCR2 Antagonists

The ADMET profiles of RS102895, RS-504393, and CCX140-B were evaluated using the pkCSM web server to assess their pharmacokinetic suitability and toxicity risks (Table 1).

Table 1: Predicted ADMET Profiles of CCR2 Antagonists Using pkCSM

Property	Parameter	RS102895	RS-504393	CCX140-B
Absorption	Water solubility (log mol/L)	-4.895	-3.900	-3.205
	Caco-2 permeability (log Papp)	0.996	0.614	0.455
	Human intestinal absorption (%)	87.75	89.12	72.95
	Skin permeability (log Kp)	-2.887	-2.768	-2.735
	P-gp substrate	Yes	Yes	Yes
	P-gp I inhibitor	Yes	Yes	Yes
	P-gp II inhibitor	Yes	Yes	No
Distribution	Volume of distribution (VDss, log L/kg)	0.923	1.319	0.549
	Fraction unbound in plasma (fu)	0.062	0.071	0.074
	BBB permeability (logBB)	0.455	-0.346	-1.996
	CNS permeability (logPS)	-1.173	-1.736	-3.456
Metabolism	CYP2D6 substrate	Yes	No	No
	CYP3A4 substrate	Yes	Yes	No
	CYP1A2 inhibitor	No	Yes	No
	CYP2C19 inhibitor	No	No	No
	CYP2C9 inhibitor	No	No	Yes
	CYP2D6 inhibitor	Yes	Yes	No
	CYP3A4 inhibitor	Yes	Yes	No
Excretion	Total clearance (log mL/min/kg)	0.855	0.791	0.047
	Renal OCT2 substrate	No	Yes	No
Toxicity	AMES toxicity	No	No	No
	Max tolerated dose (human, log mg/kg/day)	-0.625	-0.393	0.594

	hERG I inhibitor	No	No	No
	hERG II inhibitor	Yes	Yes	Yes
	Oral rat acute toxicity (LD50, mol/kg)	2.674	2.920	2.196
	Oral rat chronic toxicity (LOAEL, log mg/kg_bw/day)	0.92	0.26	2.492
	Hepatotoxicity	Yes	Yes	Yes
	Skin sensitisation	No	No	No
	T. pyriformis toxicity (log μg/L)	1.213	0.308	0.285
	Minnow toxicity (log mM)	-0.795	0.167	4.049

Absorption: All three compounds exhibited favorable water solubility (log S ranging from −4.895 to −3.205), indicating good aqueous compatibility for oral delivery. Human intestinal absorption was highest for RS-504393 (89.1%) and RS102895 (87.7%), while CCX140-B showed slightly reduced absorption (72.9%). Caco-2 permeability followed a similar trend, with RS102895 demonstrating the highest permeability (0.996), suggesting superior intestinal uptake.

Distribution: RS-504393 had the highest predicted volume of distribution (1.319 L/kg), indicating broader tissue distribution, while CCX140-B showed the lowest (0.549 L/kg). RS102895 exhibited the greatest potential for blood-brain barrier penetration (logBB = 0.455), whereas CCX140-B showed poor CNS permeability (logBB = −1.996), making it a more selective option for peripheral (non-CNS) indications such as CKD.

Metabolism: Both RS102895 and RS-504393 were predicted to interact with major CYP450 enzymes (CYP2D6, CYP3A4), suggesting potential drug-drug interaction risks. In contrast, CCX140-B demonstrated minimal metabolic interference, with inhibition reported only for CYP2C9.

Excretion and Toxicity: RS102895 exhibited the highest total clearance (0.855 mL/min/kg), whereas CCX140-B had the lowest (0.047), indicating prolonged retention. All three antagonists were predicted to be hepatotoxic and hERG II inhibitors, raising concerns about potential liver and cardiotoxicity, which require in vivo validation.

Overall, RS102895 and RS-504393 displayed strong pharmacokinetic profiles but may require close monitoring for metabolic and hepatic side effects. CCX140-B, while less permeable and slower to clear, may be safer in metabolically compromised patients but shows weaker absorption.

2. Molecular Docking of Wild-Type and Variant CCR2 Proteins

Docking simulations revealed strong binding affinities for all three antagonists with wild-type (WT) CCR2, with RS-504393 demonstrating the highest binding energy (−10.3 kcal/mol), followed by CCX140-B (−9.6 kcal/mol) and RS102895 (−9.5 kcal/mol). Interaction mapping confirmed stable hydrogen bonding and hydrophobic interactions with key conserved residues (GLU291, TRP98, TYR49, MET295, HIS202) (Table 2).

Table 2: Docking Scores (Binding Affinities) of Wild-Type and Missense Variant CCR2 Proteins with Antagonists

CCR2 Protein	RS102895(kcal/mol)	RS-504393(kcal/mol)	CCX140-B(kcal/mol)
Wild-Type (WT)	-9.5	-10.3	-9.6
L119P	-9.2	-9.7	-8.6
F125L	-9.9	-9.7	-9.4
G127V	-9.0	-10.2	-9.2
A141V	-9.3	-10.2	-8.6
T153M	-9.0	-10.2	-9.0
M249K	-9.1	-10.2	-9.0

2.1. Variant Effects on Ligand Binding

Missense variants in CCR2 altered antagonist binding in distinct ways:

- F125L improved RS102895 binding (−9.9 vs. −9.5 kcal/mol in WT), attributed to an additional hydrogen bond with THR178 and enhanced hydrophobic contacts with TYR49, TRP98, and GLU290 (Figure 1).
- L119P and A141V reduced CCX140-B affinity (−8.6 vs. −9.6 kcal/mol in WT), likely due to disrupted interactions with THR179 and PHE271, respectively (Figure 2).
- RS-504393 maintained consistently strong affinity across all variants (−9.7 to −10.2 kcal/mol), suggesting robustness against genetic variability.

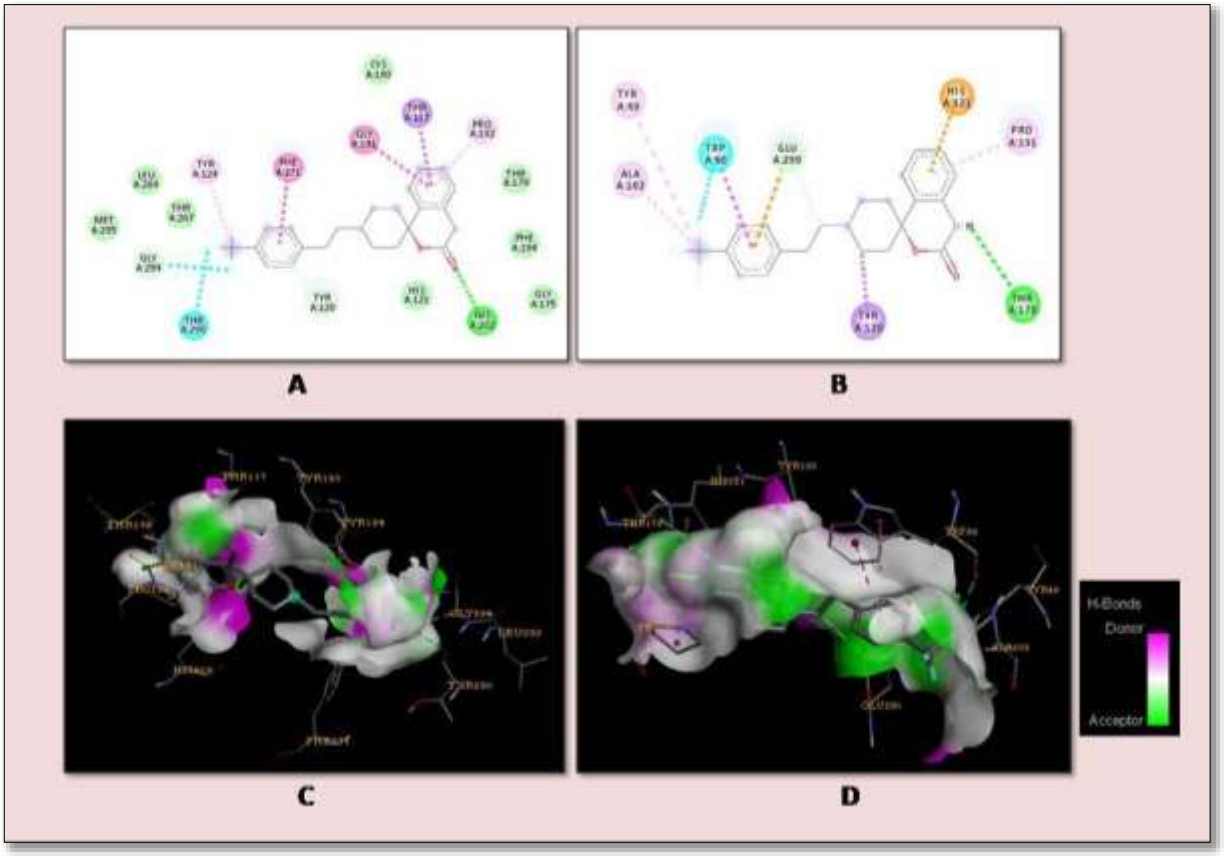

Figure 1: Comparative interaction analysis of RS102895 with wild-type and F125L variant CCR2 proteins.

(A) Two-dimensional (2D) interaction diagram of RS102895 with wild-type CCR2 protein, illustrating key hydrogen bonds with HIS202 and surrounding hydrophobic contacts.
(B) 2D interaction diagram of RS102895 bound to the F125L CCR2 variant, highlighting a newly formed hydrogen bond with THR178 and enhanced hydrophobic interactions with TYR49, TRP98, and GLU290.
(C) Three-dimensional (3D) surface rendering of the wild-type CCR2-RS102895 complex, showing the ligand docked within the binding pocket.
(D) 3D view of the F125L variant complexed with RS102895, depicting the altered binding pocket conformation and favorable ligand accommodation.

This comparison demonstrates that the F125L mutation enhances RS102895 binding by stabilizing interactions at the CCR2 active site.

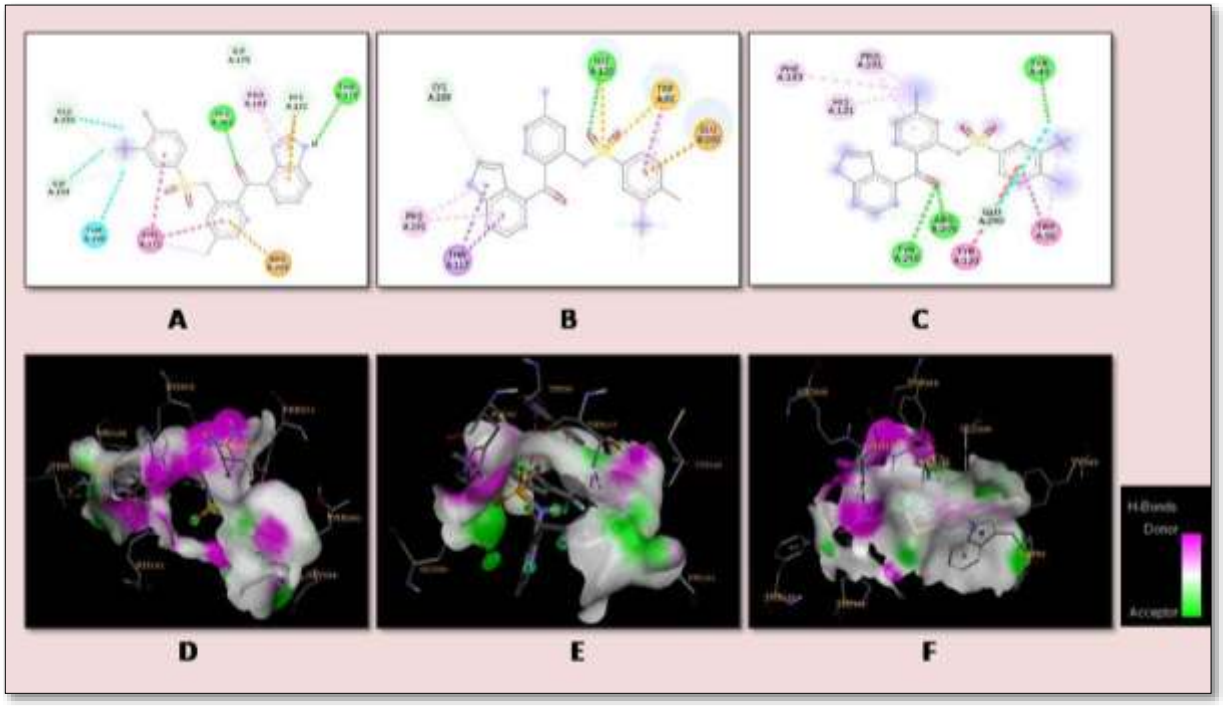

Figure 2: Structural comparison of CCX140-B interactions with wild-type, L119P, and A141V CCR2 variants.

(A) 2D interaction profile of CCX140-B with wild-type CCR2, showing hydrogen bonding with THR179 and HIS202.
(B) 2D diagram of the L119P variant, illustrating the loss of the THR179 interaction and a compensatory hydrogen bond with HIS120.
(C) 2D interaction map for the A141V variant, highlighting disruption of the aromatic contact with PHE271.
(D–F) Corresponding 3D models for wild-type (D), L119P (E), and A141V (F) CCR2 proteins complexed with CCX140-B, showing structural shifts in the binding pocket.

These structural changes suggest that the L119P and A141V mutations reduce CCX140-B binding affinity by altering key polar and hydrophobic contacts critical for stable ligand interaction.
Table 3 summarizes the Δ docking scores and altered interaction residues for the most impactful variants.

Table 3:CCR2 Variants Showing Significant Binding Differences Compared to Wild-Type

Variant	Affected Antagonist(s)	Δ Docking Score vs WT (kcal/mol)	Key Interaction Changes
F125L	RS102895	−9.9 vs. −9.5	Gained H-bond with THR178; enhanced hydrophobic interactions with TYR49, TRP98, and GLU290
L119P	CCX140-B	−8.6 vs. −9.6	Lost H-bond with THR179; gained H-bond with HIS120
A141V	CCX140-B	−8.6 vs. −9.6	Disrupted interaction with PHE271

3. Structural Interpretation of Binding Changes
Variations in docking scores reflect changes in pocket dynamics and residue orientation due to mutations. For instance, L119P introduces a rigid proline, likely causing local structural rearrangement and loss of THR179 interactions, weakening CCX140-B binding. Similarly, A141V disrupts PHE271-mediated contacts, a critical aromatic residue in CCR2's ligand-binding core.Conversely, F125L introduces favorable hydrophobic effects, enhancing RS102895's interaction surface. The minimal impact of other variants (e.g., G127V, M249K, T153M) on binding energy suggests that while structurally destabilizing, they do not significantly affect ligand orientation

or access to key residues.The consistent retention of interactions with residues such as GLU290, TYR49, and TRP98 across most variants underscores their functional conservation and importance in CCR2 antagonist binding, as supported by ConSurf and Phyre2 predictions in our previous study (Lakkamraju et al., 2025; Zacarías et al., 2021).

CONCLUSION

This in silico study highlights the structural and pharmacological impacts of CCR2 missense variants on antagonist binding, offering insights for personalized CKD treatment. Through ADMET profiling and molecular docking, RS102895 and RS-504393 showed favorable pharmacokinetics, though with some toxicity risks, while CCX140-B had a safer metabolic profile but reduced absorption and binding, particularly with L119P and A141V variants. RS-504393 consistently maintained high binding affinity across all variants, suggesting broad therapeutic potential. In contrast, F125L enhanced RS102895 binding, indicating mutation-specific treatment strategies. These findings emphasize the relevance of genetic variation in drug response and support the utility of in silico tools in early drug prioritization. Nonetheless, experimental validation is essential to confirm these results. Future studies integrating molecular dynamics, gene expression assays, and variant-specific pharmacological testing will be key to advancing genotype-guided therapies for CKD, promoting safer, more effective treatments tailored to diverse genetic backgrounds.

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