Original Article :

Molecular Insights into the impact of Deleterious Missense nsSNPs in IL-2 structure and function on Antifungal Immune Responses: An In Silico analysis

Aiah M. Khateb [1,2], Hakeemah H. Al-nakhle [1]

1. Department of Clinical Laboratory Sciences, College of Applied Medical Science, Taibah University, Medina, Saudi Arabia
2. Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

Received on Dec 24, 2024, accepted for publication on Jun 22, 2025, dx.doi.org/10.5455/mjhs.2026.01.005

Corresponding author:
Aiah M. Khateb, Department of Clinical Laboratory Sciences, College of Applied Medical Science, Taibah University, P. O. Box 344, Medina 42353, Saudi Arabia, email: akhateb@taibahu.edu.sa

OPEN ACCESS Distributed under Creative Commons CC BY-NC-ND 4.0

Abstract

Background and Aims:
To evaluate the functional and structural impact of deleterious missense single-nucleotide polymorphisms (nsSNPs) on human Interleukin-2 (IL-2), a key cytokine in antifungal immunity.

Methods:
In silico analysis of nsSNPs using bioinformatics tools (MAPP, PhD-SNP, PolyPhen-1/2, SIFT, SNAP, PredictSNP, I-Mutant 2.0, ConSurf, Project HOPE) to predict their effects on protein function and structure. MusiteDEEP was used for post-translational modification analysis.

Results:
Novel deleterious missense nsSNPs were identified in IL-2 (M1T, F62C, 73P, S107I, G118E, and W141R). Computational analyses predicted significant alterations in IL-2's surface properties, such as altered (signal peptide, transmembrane protein, disordered interface,metal binding, coiled coil, stability,disordered interface), or loss of (n-terminal acetylation, acetylation, strand, loop, transmembrane protein), or gain of (intrinsic disorder, of helix, allosteric site or of relative solvent accessibility), change is hydrophobicity and size were observed. Thus, potentially disrupt interactions with other immune molecules and function. Dramatic changes were obsevered in post-translational modifications for mutations T71I and S107I, resulted in the loss of phosphorylation sites, while the mutations M1T and L92S led to a gain of phosphorylation.

Conclusion:
These findings represents a foundational step in identifying potentially pathogenic IL-2 mutations, suggest the nsSNPs in IL-2 may contribute to impaired antifungal immunity by altering protein function and structure.Further research is warranted to explore the clinical implications of these findings for personalized medicine approaches to fungal infections.

Keywords:
nsSNPs, IL-2, antifungal, immunity, in-silico, protein, mutations, missense, bioinformatics, polymorphism

Introduction:

Approximately 1.5 million people die each year from invasive fungal infections (IFIs), which pose an increasing threat to human health.[1] Research into host-pathogen interactions is crucial for developing diagnostic biomarkers to predict fungal susceptibility and reduce mortality.2 Host mutations, particularly in key immune modulators, can significantly alter the immune response in either direction. Interleukins (ILs) are crucial in the immune response to fungal infections, contributing to B cell maturation and antibody secretion, both vital for combating these pathogens [2]. The 15-kDa cytokine interleukin-2 (IL-2) is primarily produced by activated CD4+ and CD8+ T cells, with natural killer (NK) cells, NK T cells, and dendritic cells (DCs) secreting smaller amounts in response to microbial stimulation.[3] When IL-2 binds to its receptor (IL-2R), it exerts both autocrine and paracrine effects. IL-2 can have both beneficial and detrimental roles; for instance, it inhibits Th17 differentiation but can also promote the expansion of Th17 cells.[4]

In healthy individuals with a balanced cell-mediated immune (CMI) system, IL-2 stimulates the proliferation of activated T-cell populations. IL-2 also plays a key role in regulating CD4+ T-cell differentiation and the effector and memory responses of CD8+ T cells. By supporting the survival and proliferation of regulatory T cells (Tregs), IL-2 is critical for maintaining immunological tolerance, which indicates its essential role in directing the adaptive immune system.[3, 5] Additionally, IL-2 is implicated in the differentiation, expansion, and homeostasis of various effector T-cell subsets, including T helper cells (Th1, Th2, Th17) and memory CD8+ T cells.[3] IL-2 also enhances the cytolytic activity of NK cells, promoting cell death induction and activation.[4] Furthermore, IL-2 plays a strategic role in targeting tumor and fungal cells by activating non-major histocompatibility complex (MHC)-restricted cytotoxic cells, leading to the generation of lymphokine-activated killer cells.[5]

IL-2 regulates the expression of cytokine receptors and transcription factors, either promoting or inhibiting cytokine cascades responsible for T helper (Th) cell differentiation.[6] The common cytokine receptor γ chain (γc), which is mutated in individuals with X-linked severe combined immunodeficiency, is shared by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. As a result, IL-2 has been used therapeutically in regulatory T cells (Tregs) for autoimmune disease treatment and in CD8+ T cells for cancer immunotherapy.[6] Numerous studies have linked the loss of IL-2 or reduced sen-

sitivity to IL-2 with weakened immune responses, as seen in viral infections and tumor progression.[7] IL-2 also activates non-MHC-restricted cytotoxic cells, leading to the production of lymphokine-activated killer cells, which can destroy fungal and tumor targets.[8] Increased vulnerability to fungal infections has been associated with defective cell-mediated immunity (CMI), particularly in mucocutaneous infections caused by Candida albicans. This is especially evident in patients with AIDS, lymphomas, or those undergoing corticosteroid treatment.[8] Research has suggested that IL-2 possesses lectin-like characteristics, showing specific binding to high-mannose glycoproteins such as mannan, ovalbumin, and uromodulin. A distinction appears to exist between the mannose-binding site and the IL-2R binding site.[9] This raises the possibility that IL-2 might bind to Candida albicans, given the high concentration of exposed mannose groups on its cell wall, which could influence the host's response to candidiasis.[9,10] Another study demonstrated that in early-stage murine candidiasis, IL-2 production correlates with the induction of IL-4-producing CD4+ Th2 cells.[10] However, a temporary loss of T cell responsiveness, including IL-2 production, appears necessary for the development of CD4+ Th1 cells.[11] CMI also serves as the primary defense mechanism against Paracoccidioides brasiliensis.[12] Previous studies have shown that individuals with paracoccidioidomycosis (PCM) exhibit an immunological imbalance, indicated by lymphocyte hyporesponsiveness to the main P. brasiliensis antigen, the 43-kDa glycoprotein (gp43).[12] This finding was further supported by comparisons of healthy individuals and those with invasive or chronic PCM. The study revealed poor lymphocyte proliferation and an inverse correlation between antibody levels and IL-2 and IFN-γ production, suggesting that the cytokine imbalance led to the production of non-protective antibodies. [12]

As shown earlier IL-2 is essential for immune system regulation, and even minor structural changes due to missense non-synonymous single-nucleotide polymorphisms (nsSNPs) can have substantial immunological consequences, thus understanding these mutations is vital. This study specifically explored how nsSNPs can functionally alter the human IL-2 protein. The nsSNPs are point mutations that result in a change in the amino acid sequence of a protein which may significantly affect the protein's function and stability, leading to various health implications[13]. For instance, computational analyses have shown that specific nsSNPs in other proteins can destabilize their structure, which

may also apply to IL-2 [13, 14]. Utilizing in silico tools allows researchers to predict the effects of nsSNPs on protein structure and function rapidly, facilitating the identification of potentially harmful mutations to guide the next expermintal extensive laboratory work. These tools allow for the rapid and cost-effective prediction of potentially damaging mutations by simulating their effects on protein structure and stability. While the focus on nsSNPs in IL-2 is critical, it is also important to consider that other genetic factors and environmental influences can contribute to immune system variability, suggesting a multifaceted approach to studying immune responses. Given IL-2's critical role in immune responses, even subtle structural changes induced by nsSNPs could have profound immunological consequences, potentially predisposing individuals to increased susceptibility to infections, including fungal pathogens, or influencing the efficacy of immunotherapies. While the general importance of IL-2 in immunity is well-established, there is a gap in understanding the specific impact of nsSNPs on IL-2's structure and function. To our knowledge, comprehensive studies specifically investigating the effects of nsSNPs on IL-2 protein function and structure have been limited [13]. This highlights a crucial area for investigation to better understand individual variations in immune responses.

IL-2 plays a pivotal role in the immune response to fungal infections. This study explored the potential impact of harmful genetic variations, specifically missense non-synonymous single-nucleotide polymorphisms (nsSNPs), on the function and structural stability of the human IL-2 protein. Using computational analysis, the study seeks to predict how these nsSNPs might alter IL-2's structure and function, potentially compromising its ability to effectively combat fungal pathogens.

Methods:

Study design, data collection, and statistical analysis:

This research is in silico design involving solely use of publicly available data computational methods to analyze and interpret biological data Figure1. The research does not involve any human participants, animals, or sensitive personal information. A no objection letter was obtained for the study protocol from institutional research ethics board. The sample size included 2081 variant extracted from Ensembl genome browser (https://www.ensembl.org) which was then analyzed through different software for variant classification. Each software calculates statistical significance according to best fitted model mentioned in each software below. The nucleotide sequence in FASTA format (accession no.

NP_000577.2) was obtained from NCBI (http://www.ncbi.nlm.nih.gov, accessed on 19 June 2023), along with the IL-2 (P60568) from the UniProtKB database (https://www.uniprot.org/, accessed on 19 June 2023). SNPs of the IL-2 gene were retrieved from the NCBI SNP database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp, accessed on 19 June 2023) and the Ensembl genome browser (https://www.ensembl.org, accessed on 19 June 2023) with accession no. ENSG00000109471. All data were downloaded between 19 -30June 2023.

Identification of deleterious nsSNPs:

To predict the functional effects of nsSNPs collected from the dbSNP and Ensembl databases, the PredictSNP tool (https://loschmidt.chemi.muni.cz/predictsnp/ , accessed on 21 June 2023) was used. This approach provided accurate and stringent results, with SNPs predicted as damaging by all six programs being classified as high-risk. PredictSNP leverages six prediction programs (MAPP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT, and SNAP) to identify disease-related mutations. Additionally, PANTHER was employed to robustly and accurately relate protein sequence relationships to functional relationships. This algorithm classifies proteins based on their evolutionary relationships, molecular functions, and interactions with other proteins, analyzing substitutions using position-specific evolutionary conservation scores derived from alignments of evolutionarily related proteins.

Identification of deleterious nsSNPs on the domains and effect on protein stability of the human IL-2 protein:

The InterPro tool (https://www.ebi.ac.uk/interpro/, accessed on 22 June 2023) was used to identify the locations of missense nsSNPs within the domains of the Interleukin-2 protein, recognizing motifs, active sites, and domains. Protein stability analysis of mutant proteins was conducted using the MuPro and I-Mutant 2.0 tools.13,14 The I-Mutant 2.0 web server (http://gpcr2.biocomp.unibo.it/cgi/predictions/I-Mutant2.0/I-Mutant2.0.cgi, accessed on 22 June 2023) calculates changes in protein structural stability due to single polymorphisms. Using Gibbs free energy measurements, I-Mutant 2.0 differentiates between wild-type and mutant protein stability by categorizing variants as destabilizing mutations ($\Delta\Delta G < -1.0$ kcal/mol), stabilizing mutations ($\Delta\Delta G > 1.0$ kcal/mol), and neutral mutations ($-1.0 \leq \Delta\Delta G \leq 1.0$ kcal/mol). The results are presented with a Reliability Index (RI), ranging from 0 (lowest reliability) to 10 (highest reliability). MuPro (http://mupro.proteomics.ics.uci.edu/, accessed on 22

June 2023) predicts changes in energy based on the plain protein sequence of IL-2, assigning a confidence score between -1 and 1. Scores below 0 suggest a mutation may decrease protein stability, while scores above 0 indicate increased protein stability.

Prediction of the structural effect of ns-SNPs on the human IL-2 protein:

Project HOPE (Have Your Protein Explained; https://www3.cmbi.umcn.nl/hope/, accessed on 22 June 2023) was used to assess the effect of nsSNPs on protein stability. 15,16 The server accepts protein sequences in FASTA format and SNP data as inputs. By integrating and evaluating multiple databases and web servers, such as WHAT IF, UniProt, and the Distributed Annotation System, Project HOPE generates a homology model to provide a better understanding of mutations. The brief report produced by Project HOPE describes how SNPs may influence protein structure and function in both wild-type and mutant forms, along with accompanying notes, images, and animations for further insight.

Potential PTM site prediction:

Post-translational modifications (PTMs) play a crucial role in protein structure, folding, and function. In silico tools were employed to identify potential PTM sites in the IL-2 protein, as well as the gain or loss of PTM sites at all nsSNP positions. GPS-MSP was used to predict methylation sites in the IL-2 protein. For phosphorylation prediction at serine, threonine, and tyrosine sites, NetPhos 3.1 was used, with a threshold set at 0.5. Amino acid residues with values higher than this threshold were predicted to be phosphorylated. NetPhos 3.1 identified phosphorylation sites at serine, threonine, and tyrosine residues in IL-2 protein. NetNGlyc 1.0 and NetOGlyc 4.0 were used to predict potential glycosylation sites in IL-2. For N-glycosylation, residues with a prediction score above 0.6 were predicted to have glycosylation potential. GSP-Uber was used to predict ubiquitylation sites, identifying lysine residues with ubiquitylation potential based on a threshold score of 0.62, with the balanced cut-off selected for the analysis. The STRING database, which currently contains 24,584,628 proteins from 5,090 organisms, was used to predict protein-protein interactions. A FASTA sequence for IL-2 was submitted as input, and the output provided all predicted interactions, each associated with confidence scores (accessed on 22 June 2023).

Identification of mutational impacts on structural and functional properties of proteins:

The MutPred2 program (http://mutpred.mutdb.org, accessed on 24 June 2023) is a machine-learning tool used to

predict pathogenic or non-pathogenic substitutions and their molecular mechanisms.16,17 This method screens for functional and structural variations, including altered stability, catalytic site loss, and the gain of O-linked glycosylation. According to MutPred2, a probability greater than 0.5 indicates a deleterious result, while a probability greater than 0.75 signifies the most deleterious outcomes. The input for this analysis was the FASTA amino acid sequence of the IL-2 protein.

The last, third, tool of data collection was the Obesity Beliefs Scale, which was designed to assess pregnant women's beliefs. Based on the instrument adapted from this research [13], the pregnant women's belief in obesity's impact on breastfeeding duration was evaluated. The scale employed a Likert format with the answers on the scale being 1 disagreeing, 2 being neutral and 3 agreeing. The total belief scores were categorized into two levels: The negative beliefs ranged from 1 and 6 while the positive beliefs ranged from 7 to 12. Like the knowledge tool, the belief scale was evidenced to experts and peers in Saudi Arabia for culture relevancy and relevance to the sample population, with modifications made according to the suggestions

Validity and Reliability:

The content validity, relevance, and clarity of the tools were evaluated by a panel of three experts in community health nursing and maternity care. Adjustments were made accordingly. The reliability of tools (2 and 3) was assessed using Cronbach's alpha test, indicating high dependability (0.78 & 0.77).

Ethical Considerations:

Ethical clearance was obtained from the Local Committee of Bioethics (LCBE) with IRB LOG NUMBER 23-606C and the institutional review board (IRB) at Majmaah University number (MUREC-Des. 18/CONI-2O23 / 35-2). Pregnant women received detailed information about the study's purpose, risks, benefits, and required information, and provided voluntary informed consent. Withdrawal from the study is permitted without penalty. Pregnant women's privacy and confidentiality were ensured, with data stored securely.

Statistical Analysis:

Data was coded, tabulated, and analyzed using a personal computer using the Statistical Package for the Social Sciences (SPSS) program version 25 statistical software. Mean and standard deviation were used for continuous variables. The categorical variable was expressed by frequency and proportions. For categorical variables, .

Results:

SNP annotation and identification of deleterious nsSNPs:

We retrieved SNP information for the

human IL-2 gene using the NCBI Variation Viewer (https://www.ncbi.nlm.nih.gov/variation/view/, accessed on 22 June 2023), which identified 1,440 SNPs in the intronic region, 81 SNPs in the 5' UTR region, 90 SNPs in the 3' UTR region, 57 synonymous variants, and 83 missense variants in the coding region. For this study, we focused only on nsSNPs, as these involve codon changes that result in different amino acids. A schematic diagram (Figure. 1) provides an overview of the entire methodological approach. We used PANTHER and PredictSNP to identify deleterious SNPs that may significantly alter the structure or function of the IL-2 protein. A total of 11 SNPs were predicted to be deleterious by all computational algorithms used (Table 1).

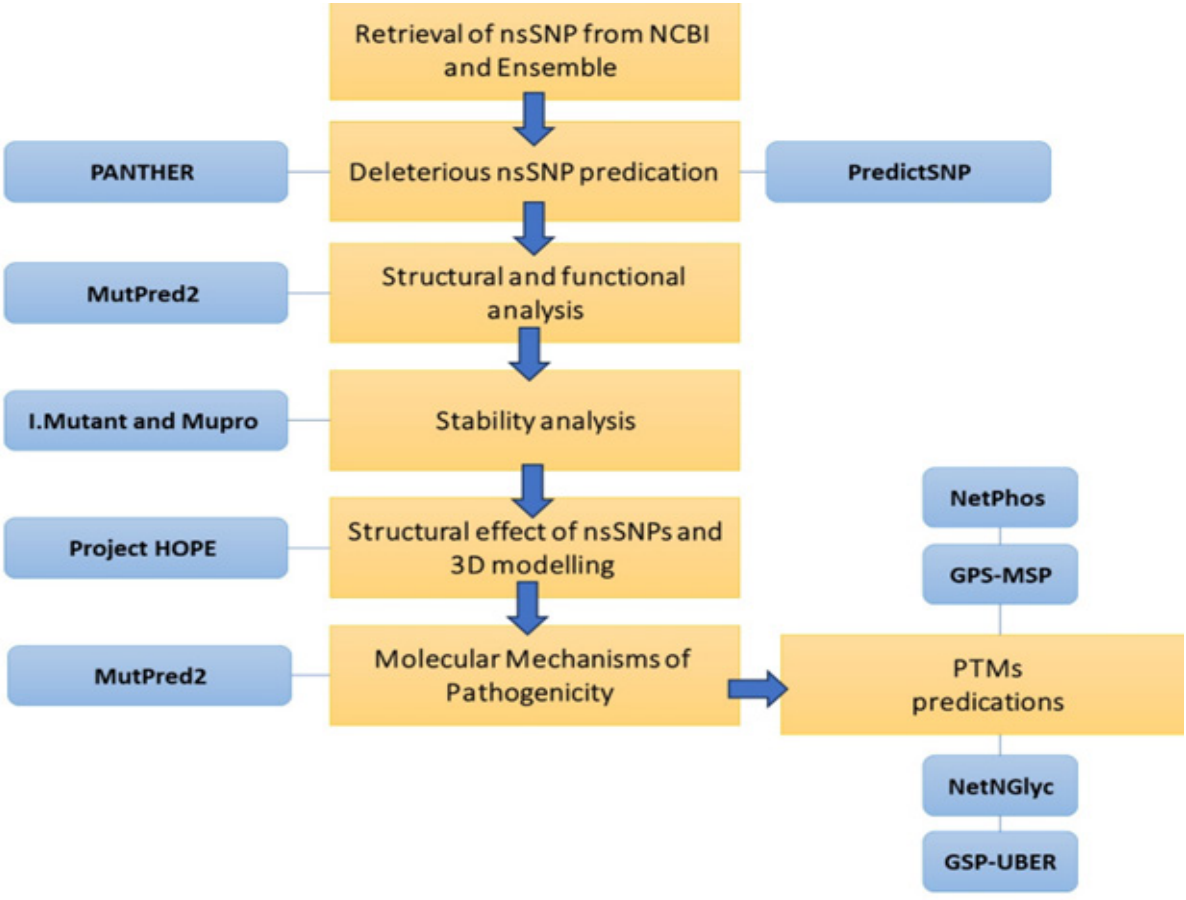


Figure 1: Scheme of the whole methodological approach of the *In silico* analysis of nsSNPs. Using bioinformatics tools (MAPP, PhD-SNP, PolyPhen-1/2, SIFT, SNAP, PredictSNP, I-Mutant 2.0, ConSurf, Project HOPE) to predict their effects on protein function and structure, stability, 3D modleing. MusiteDEEP was used for post-translational modification analysis.

Figer1: Scheme of the whole methodological approach of the In silicon analysis of nsSNPs.

Table 1: The 11 High-risk nsSNPs predicted by predictSNP & PANTHER.

SNP ID	AA change	PredictSNP		MAPP	PhD-SNP	PolyPhen-1		SIFT	SNAP	PANTHER
		Expected Accuracy	Prediction	Expected Accuracy	Expected Accuracy	Expected Accuracy	Expected Accuracy	Expected Accuracy	Expected Accuracy	Expected Accuracy
rs778990655	**M1T**	87%	D	76%	61%	74%	81%	79%	72%	-
rs758928770	**S25I**	87%	D	91%	58%	74%	81%	79%	72%	65%
rs1371545323	**F62C**	87%	D	77%	68%	74%	81%	79%	89%	74%
rs994708825	**P67A**	87%	D	77%	77%	74%	81%	79%	85%	61%
rs774269792	**T71I**	87%	D	88%	73%	74%	50%	79%	62%	61%
rs759849798	**L73P**	87%	D	77%	82%	74%	81%	79%	85%	73%
rs773490003	**L92S**	87%	D	84%	68%	74%	55%	79%	72%	70%
rs1580834442	**S107I**	87%	D	57%	61%	74%	65%	79%	81%	61%
rs1314082605	**G118E**	87%	D	63%	88%	74%	81%	79%	81%	70%
rs377374894	**D129H**	87%	D	86%	68%	74%	65%	79%	81%	68%
rs1162791166	**W141R**	87%	D	86%	88%	74%	81%	79%	89%	72%

D: deleterious

Domain Identification for nsSNPs and Stability of protein:

The InterPro tool identified four domains in the IL-2 protein: the noncytoplasmic domain (residues 26-153), PHOBIUS Signal peptide C region (18-25), PHOBIUS Signal peptide N region (1-5), signal gram-positive region (1-23), and the signal peptide H region (6-17), which is the hydrophobic region of the signal peptide. These domains and regions have specific lengths. The ten nsSNPs predicted to be high-risk pathogens by all in silico tools and their domains were examined. Nine out of 11 nsSNPs were located in the non-cytoplasmic domain of the IL-2 protein, and one was at te N-terminal region (Figures. 2). The protein's function is closely tied to its stability, making it crucial to identify nsSNPs that affect stability. We assessed the impact of damaging nsSNPs on IL-2 protein stability using I-Mutant and MuPro. Each nsSNP was evaluated individually for ΔΔG values. All damaging nsSNPs, except for S25I, were predicted to decrease the stability of the IL-2 protein.

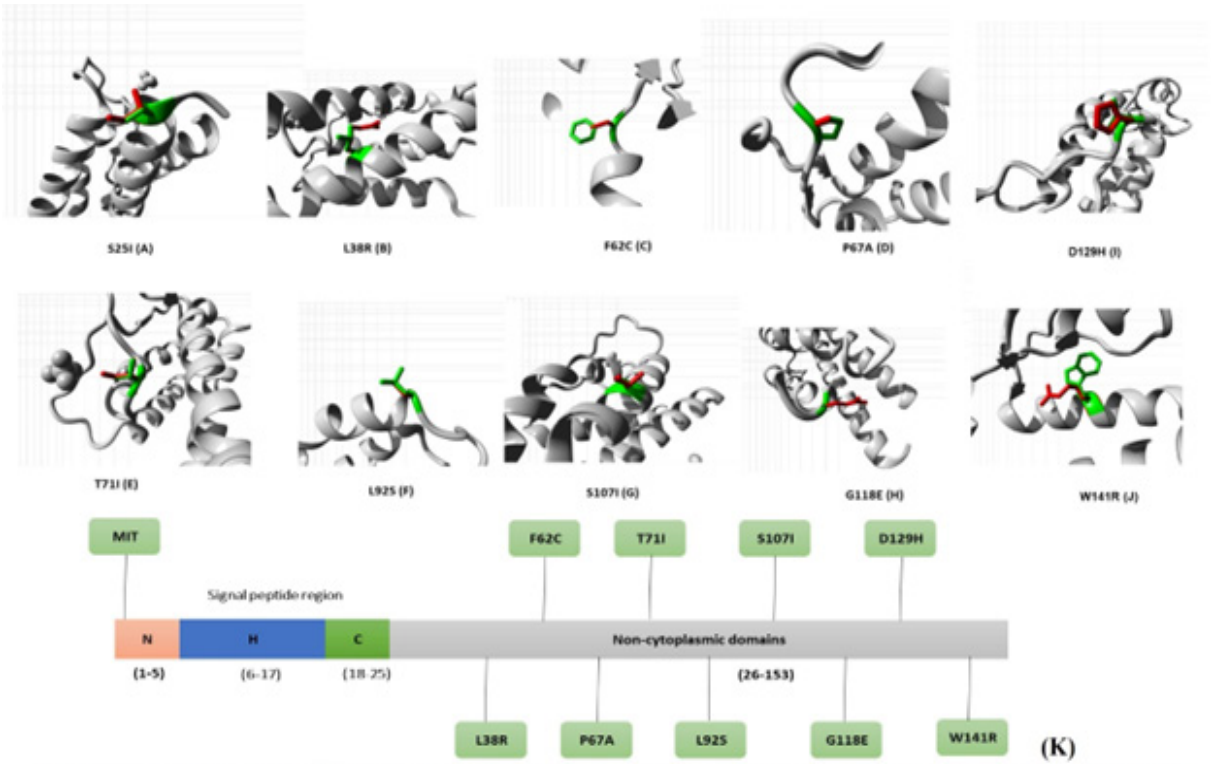


Figure 2. (A–K) The ten nsSNPs which predicted to be high-risk pathogens by all in silico tools and their domains in IL-2 protein. The modified IL-2 protein structure was modeled using Project HOPE. For each SNP, the native residue is indicated in green, and the altered residue is indicated in red. The residue side chain is shown in color, while the whole protein is shown in gray color.

Figer2: (A-K) The ten nsSNPs which predicted to be high-risk pathogens by all in silico tools their domains in IL-2 protein.

PTM predictions:

PTM sites were predicted using various tools. No methylation sites were identified at the nsSNP positions in the IL-2 protein, as predicted by GPS-MSP 3.0 (Table S1). Phosphorylation sites were predicted by NetPhos 3.1, which identified two residues with phosphorylation potential: serine at position 107 and threonine at position 71 (Table S2). The NetPhos 3.1 analysis revealed that the mutations T71I and S107I resulted in the loss of phosphorylation sites, while the mutations M1T and L92S led to a gain of phosphorylation. No glycosylation sites were predicted by N-Glyc and 6 wereidentified by NetOGlyc 4.0 (Table S3 A and B). Additionally, no ubiquitylation sites were found at the deleterious SNP regions using GSP-UBER (Table S4).

Protein structure analysis by HOPE Analysis:

Project HOPE generated 3D models for the ten nsSNPs were predicted to be high-risk pathogens by all in silico tools mutant IL-2 proteins (Figure. 2). In Project HOPE, amino acid substitutions on native proteins are simulated to evaluate their structural effects. For 10 of the 11 nsSNP, the corresponding amino acid in IL-2 was altered in terms of size, charge, and hydrophobicity. The analysis revealed changes in residue size, such as from large to small, and shifts in charge, such as losing or gaining a charge. Hydrophobicity was also observed to increase or decrease, leading to the loss of hydrogen bonds due to nsSNPs. These changes ultimately disrupted protein folding. Table S1 (Supplementary) summarizes the HOPE analysis results.

As a consequence, (1) signal transfer from the binding domain to the activity domain is hindered, affecting the regulation of the protein's activity, and (2) the binding of other molecules is compromised. These effects impair the overall function of the IL-2 protein. Specifically, S107I resulted in the loss of hydrogen bond formation, affecting the protein's structural stability. The wild-type residue forms a salt bridge with lysine at position 68 in D129H, but the charge difference between the wild-type and mutant disrupts the ionic inter-

action. Mutations such as M1T, F62C, P67A, and W141R caused smaller residue sizes and altered hydrophobicity. The remaining nsSNPs resulted in larger residue sizes.

The HOPE analysis illustrated changes caused by each of the ten nsSNPs were predicted to be high-risk pathogens by all in silico tools mutant IL-2 proteins SNPs, Project HOPE used a 3D structure of the IL-2 protein (PDB ID: 7M2G). In one position, wild-type and mutated residues were displayed in different colors within the DNA binding domain (Figure. 2). The nsSNPs were linked to genomic variants using dbNSFP, which provides scores ranging from 0.0 to 1.0. A higher score indicates a greater likelihood of the variant being pathogenic. According to dbNSFP scores, all ten nsSNPs were classified as pathogenic (Table S2, Supplementary). These nsSNPs, located in conserved regions, may affect the structure and function of the IL-2 protein.

Identification of Molecular Mechanisms of Pathogenicity of nsSNPs predicted by MutPred2:

The molecular mechanisms by which 11 nsSNPs contribute to disease were predicted using MutPred2. The reuslats of the structural and functional alterations predicted for 6 out of 11 nsSNPs (M1T, F62C, L73P, S107I, G118E, W141R) include the loss of sulfation, acetylation, and allosteric sites; increased solvent accessibility, intrinsic disorder, loops, B-factor, and catalytic sites; and the gain of solvent accessibility, intrinsic disorder, loops, B-factors, and catalytic sites. MutPred2 provides a general score (g), which represents the average score from all neural networks in the tool (Table2). A g-score greater than 0.50 ($g > 0.50$) indicates that a mutation is likely pathogenic. Mutations with a g-value > 0.5 and p-value < 0.05 are considered actionable hypotheses, while mutations with a g-value > 0.75 and p-value < 0.05 are considered confident hypotheses. According to MutPred2, the substitutions M1T, F62C, L73P, G118E, and W141R had g-values greater than 0.5 and p-values lower than 0.05 (Table 2)

Table 2: Functional, structural modifications and stability change prediction of IL-2 predicted by MutPred2, I-Mutant 2.0, and Mupro were deteceted in 6 out of 11 nsSNPs.

Substitution	MutPred2 score g-value	Molecular mechanisms with P-values <= 0.05	Probability	P-value	Mupro		I. Mutant		
					ΔΔG (kcal/mol)	stability	ΔΔG (kcal/mol)	stability	Reliability Index
M1T	**0.862**	Altered Signal peptide	0.13	1.4e-03	-1.7792	decrease	-0.85	decrease	7
		Altered Transmembrane protein	0.09	0.05					
		Loss of N-terminal acetylation at M1	0.03	4.8e-03					
F62C	**0.604**	Altered Disordered interface	0.31	0.02	-1.6386	decrease	-2.32	decrease	6
		Loss of Acetylation at K63	0.19	0.05					
L73P	**0.772**	Gain of Intrinsic disorder	0.35	0.02	-1.8826	decrease	-1.20	decrease	6
		Altered Metal binding	0.30	0.01					
		Altered Coiled coil	0.29	0.02					
		Altered Stability	0.17	0.02					
S107I	**0.506**	Altered Disordered interface	0.42	3.3e-03	-0.2384	decrease	-0.12	decrease	1
		Gain of Helix	0.27	0.04					
		Altered Transmembrane protein	0.19	6.7e-03	-0.12795	decrease	-0.27	decrease	2
G118E	**0.730**	Loss of Strand	0.34	1.9e-04					
		Altered Transmembrane protein	0.28	5.8e-04					
		Loss of Loop	0.27	0.05					
W141R	**0.893**	Altered Transmembrane protein	0.37	1.5e-05	-1.6600	decrease	-2.02	decrease	9
		Altered Disordered interface	0.32	0.01					
		Altered Ordered interface	0.31	4.2e-03					
		Gain of Allosteric site at W141	0.28	3.3e-03					
		Gain of Relative solvent accessibility	0.24	0.04					

Discussion:

IL-2 plays a critical role in both innate and cellular immunity, and the occurrence of disruptive mutations may significantly impair essential functions, affecting nature and magnitude of immune responses. Increasingly, IL-2 has been used in immunotherapy. For instance, one study demonstrated that IL-2 immunotherapy in patients with systemic lupus erythematosus (SLE) provided a more than threefold protective effect against fungal infections compared to non-IL-2 immunotherapy groups. [7] The potential impact of deleterious missense nsSNPs on the IL-2 protein is a compelling area of research, particularly in understanding susceptibility to fungal infections. This in silico analysis sheds light on how these genetic variations may alter the structure and function of IL-2, offering valuable insights into their role in disrupting the body's antifungal immune response.

By investigating protein folding and intermolecular interactions, this study highlights how specific nsSNPs can destabilize the delicate architecture of IL-2. Such destabilization may impair receptor binding and downstream signaling, ultimately crippling the cascade of immune responses crucial for combating fungal invaders. Further studies are needed to explore the relationship between these mutations and related diseases, providing evidence of their protective or harmful effects in the host's defense against fungal infections. This is particularly important for species that cause a range of illnesses, from mucosal infections such as oral and vaginal thrush to life-threatening invasive infections, such as those caused by Candida species. In active candidiasis, numerous Pattern Recognition Receptors (PRRs), including Dectin-1, Toll-like receptors (TLRs), and nucleotide-binding oligomerization domain-like receptors (NLRs), are bound by Candida species on myeloid cells.[17] This interaction stimulates the production of various cytokines, triggering Th1 and Th17 response.[18] Several studies have found that myeloid dendritic cells (DCs) release IL-2 in response to microbial triggers, due to the cytokine's potency and pleiotropic activities.[19] Exposure of DCs to fungal antigens leads to a Ca^{2+} influx, which activates the cytoplasmic nuclear factor of activated T cells (NFAT). NFAT is dephosphorylated by calcineurin, a Ca^{2+}-calmodulin-dependent phosphatase, resulting in its nuclear translocation and the transcription of IL-2. Another study demonstrated the importance of IL-2 production in a murine model of invasive pulmonary Aspergillus fumigatus infection. Mice with targeted deletion of IL-2 in DCs exhibited increased lung expression of IL-17 and often

yielded to a pathological Th17 response.[20] [21] In the absence of IL-2, DCs released IL-23, which promoted a lethal Th17-mediated immunological response.

In this study, we predicted changes in IL-2's surface properties, which may hinder its interaction with other immune molecules and impair the coordinated effort required for a robust antifungal response. These results highlight the predicted pathogenicity of the identified mutations, thereby guiding future in vitro and in vivo research to experimentally validate their exact functional consequences and clinical significance. Our results section meticulously presents the anticipated effects for each analyzed mutation. To our knowledge, all the protein changes identified in this study, including F62C, S107I, G118E, and W141R, are novel. This study also reported M1T and L73P deleterious nsSNPs in IL-2 structure similar to previously published research by Sadaf & Darakhshan eat al in 2018.[13] Their in silico analysis involved annotating the functional impact of these mutations and evaluating changes in protein instability, protein movement and flexibility at the residual level. Their study also confirmed the highly pathogenic and deleterious mutations, concluding that these specific substitutions may alter the normal structure-function relationship of the IL-2 protein. M1T rs778990655 and L73P rs759849798 making them vital candidates for further investigation in diseases linked to IL-2.[13] Further analyses are required to investigate their associations with diseases like severe combined immunodeficiency (SCID), which leaves individuals vulnerable to a wide range of infections, including fungal threats. SCID, particularly in Omenn syndrome (OS), exemplifies how a compromised immune system, especially in T and B cells, heightens susceptibility to fungal infections. Without this intricate defense network, even common fungal pathogens can cause severe damage. In OS, fungal infections often manifest as firm skin lesions, persistent respiratory issues like pneumonia, and chronic gastrointestinal distress.[22] The unchecked growth of fungal spores in the absence of immune control can lead to life-threatening complications. Early diagnosis, aggressive antifungal treatment, and bone marrow transplantation to address the underlying immune deficiency are crucial for managing these infections and improving long-term outcomes in patients with Omenn syndrome.[23]

Our predicted mutations, if experimentally validated, could contribute to similar clinical scenarios by disrupting the crucial IL-2 pathway. Our findings open the door to further investigations into the clinical implications of IL-2-related nsSNPs.

By identifying specific genetic variations linked to increased vulnerability to fungal infections, this study contributes to the advancement of personalized medicine strategies. Furthermore, a deeper understanding of the molecular mechanisms through which these nsSNPs exert their harmful effects could lead to the development of novel therapeutic approaches aimed at restoring protein structure and function, thereby strengthening the body's defenses against fungal pathogens.

Conclusions:

Several distinct nsSNPs in the IL-2 gene identified in this study may serve as risk factors for fungal infections. Our results highlight their potential pathogenicity, paving the way for future in vitro and in vivo studies to experimentally validate their precise functional consequences and clinical significance. We have ensured that the results section clearly presents the predicted effects for each analyzed mutation. Future epidemiological studies can explore whether these mutations are associated with fungal infections and how frequently they occur in local populations. This research could help identify populations at higher risk for fungal illnesses, such as invasive aspergillosis (IA) or candidiasis, by pinpointing genetic variations that increase susceptibility.

Financial support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest: The authors declare no conflicts of interest.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to Taibah University, Ministry of Education in Saudi Arabia.

References:

1. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J Fungi (Basel). 2017;3(4).
2. Naik B, Ahmed SMQ, Laha S, Das SP. Genetic Susceptibility to Fungal Infections and Links to Human Ancestry. Front Genet. 2021;12:709315.
3. Letourneau S, Krieg C, Pantaleo G, Boyman O. IL-2- and CD25-dependent immunoregulatory mechanisms in the homeostasis of T-cell subsets. J Allergy Clin Immunol. 2009;123(4):758-62.
4. Malek TR. The biology of interleukin-2. Annu Rev Immunol. 2008;26:453-79.
5. Bachmann MF, Wolint P, Walton S, Schwarz K, Oxenius A. Differential role of IL-2R signaling for CD8+ T cell responses in acute and chron-

ic viral infections. Eur J Immunol. 2007;37(6):1502-12.

6. Sharfe N, Dadi HK, Shahar M, Roifman CM. Human immune disorder arising from mutation of the alpha chain of the interleukin-2 receptor. Proc Natl Acad Sci U S A. 1997;94(7):3168-71.
7. Boyman O, Surh CD, Sprent J. Potential use of IL-2/anti-IL-2 antibody immune complexes for the treatment of cancer and autoimmune disease. Expert Opin Biol Ther. 2006;6(12):1323-31.
8. Zhou P, Chen J, He J, Zheng T, Yunis J, Makota V, et al. Low-dose IL-2 therapy invigorates CD8+ T cells for viral control in systemic lupus erythematosus. PLoS Pathog. 2021;17(10):e1009858.
9. Treseler CB, Maziarz RT, Levitz SM. Biological activity of interleukin-2 bound to Candida albicans. Infect Immun. 1992;60(1):183-8.
10. Zheng XF, Hong YX, Feng GJ, Zhang GF, Rogers H, Lewis MA, et al. Lipopolysaccharide-induced M2 to M1 macrophage transformation for IL-12p70 production is blocked by Candida albicans mediated up-regulation of EBI3 expression. PLoS One. 2013;8(5):e63967.
11. Spaccapelo R, Del Sero G, Mosci P, Bistoni F, Romani L. Early T cell unresponsiveness in mice with candidiasis and reversal by IL-2: effect on T helper cell development. J Immunol. 1997;158(5):2294-302.
12. Benard G, Romano CC, Cacere CR, Juvenale M, Mendes-Giannini MJ, Duarte AJ. Imbalance of IL-2, IFN-gamma and IL-10 secretion in the immunosuppression associated with human paracoccidioidomycosis. Cytokine. 2001;13(4):248-52.
13. Tayyaba Sadaf FD. Identification of Deleterious Nssnps of Interleukin-2 (IL-2) Gene and Its Structural Stability Using Computational Methods International Journal of Research 2018;Volume 05 Issue 04
14. Arshad M, Noor N, Iqbal Z, Jaleel H. In silico analysis of missense SNPs in TNFR1a and their possible therapeutic or pathogenic role in immune diseases. Hum Immunol. 2023;84(11):609-17.
15. Ittisoponpisan S, Islam SA, Khanna T, Alhuzimi E, David A, Sternberg MJE. Can Predicted Protein 3D Structures Provide Reliable Insights into whether Missense Variants Are Disease Associated? J Mol Biol. 2019;431(11):2197-212.
16. Pejaver V, Urresti J, Lugo-Martinez J, Pagel KA, Lin GN, Nam HJ, et al. Inferring the molecular and phenotypic impact of amino acid variants with MutPred2. Nat Commun. 2020;11(1):5918.

17. Thompson A, Orr SJ. Emerging IL-12 family cytokines in the fight against fungal infections. Cytokine. 2018;111:398-407.
18. Wells CA, Salvage-Jones JA, Li X, Hitchens K, Butcher S, Murray RZ, et al. The macrophage-inducible C-type lectin, mincle, is an essential component of the innate immune response to Candida albicans. J Immunol. 2008;180(11):7404-13.
19. Granucci F, Vizzardelli C, Pavelka N, Feau S, Persico M, Virzi E, et al. Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. Nat Immunol. 2001;2(9):882-8.
20. Gafa V, Lande R, Gagliardi MC, Severa M, Giacomini E, Remoli ME, et al. Human dendritic cells following Aspergillus fumigatus infection express the CCR7 receptor and a differential pattern of interleukin-12 (IL-12), IL-23, and IL-27 cytokines, which lead to a Th1 response. Infect Immun. 2006;74(3):1480-9.
21. Zelante T, Wong AY, Ping TJ, Chen J, Sumatoh HR, Vigano E, et al. CD103(+) Dendritic Cells Control Th17 Cell Function in the Lung. Cell Rep. 2015;12(11):1789-801.
22. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol. 2008;122(6):1082-6.
23. Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, et al. Omenn syndrome due to ARTEMIS mutations. Blood. 2005;105(11):4179-86.