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Research Article

In silico analysis of IL7RA missense mutations in lung, breast and skin cancers

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Abstract: Interleukin 7 (IL7)-Interleukin 7 Receptor Alpha (IL7RA) signaling is well investigated in hematological cancers, but in solid cancers, its role needs to be investigated further. In a recent study, IL7R was identified as a key gene in leptomeningeal carcinoma. Unfortunately, there is limited patient data on leptomeningeal carcinoma from breast, lung and skin cancers. In this study, IL7RA missense mutations that could have pathologic importance in lung, breast and skin cancers were analyzed *in silico*. Using Genomic Data Commons (GDC) data portal, lung, breast and melanoma data from 3250 patients were filtered to list IL7RA missense mutations. Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), Universal Mutation Database Predictor (UMD-Predictor) and Single Nucleotide Polymorphisms & Gene Ontology (E-SNP&GO) servers were employed to reveal pathogenic variants. Conservation Surface Mapping (ConSurf)was used to analyze conservation scores. Domains were investigated by InterPro tool. Molecular docking of IL7-IL7RA was performed by ClusPro, Mutational Binding free energy change predictor 2 (Mutabind2) and Protein-Ligand Interaction Profiler (PLIP) servers. Stability of the mutations were analyzed by Impact-Mutant 2.0 (I-Mutant2), Mutation Protein Stability Prediction (MUpro) and Impact of Non-synonymous mutations on Protein Stability-Multi Dimension (INPS-MD). Structural changes were determined using Dynamic Mutation predictor 2 (DynaMut2) and Have (y)Our Protein Explained (HOPE) servers. Out of 99 missense mutations identified, 6 (T56P, C57Y, K204I, S207F, G215V and W217C) were defined as pathogenic. All these mutations were primarily found in lung cancer and located in the extracellular domain of IL7RA. Although none were in the interaction interface of IL7, all were located at or next to conserved motifs. This proximity likely destabilizes IL7RA and drastically changes its bonding patterns. The IL7RA missense mutations may have a significant role in lung cancer, as they presumably change the protein's function.

Özet: İnterlökin 7 (IL7)-İnterlökin 7 Reseptör Alfa (IL7RA) sinyali hematolojik kanserlerde iyi araştırılmış, ancak, solid kanserlerdeki rolünün daha fazla araştırılması gerekmektedir. Son yapılan bir çalışmada, IL7R leptomeningeal karsinomda anahtar gen olarak tanımlanmıştır. Meme, akciğer ve deri kanserlerinden kaynaklanan leptomeningeal karsinomda, ne yazık ki sınırlı sayıda hasta verisi bulunmaktadır. Bu çalışmada, akciğer, meme ve deri kanserlerinde patolojik öneme sahip olabilecek IL7RA yanlış anlamlı mutasyonları bilgisayarlı olarak analiz edildi. GDC veri portalı kullanılarak, 3250 hastadan alınan akciğer, meme ve deri kanseri verileri filtrelenerek IL7RA yanlış anlamlı mutasyonları listelendi. Patojenik varyantları ortaya çıkarmak için Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), Universal Mutation Database Predictor (UMD-Predictor)ve Single Nucleotide Polymorphisms & Gene Ontology (E-SNP&GO) sunucuları kullanıldı. Korunma puanları analiz etmek için Conservation Surface Mapping (ConSurf) kullanıldı. Domain adları InterPro aracıyla araştırıldı. IL7-IL7RA'nın moleküler yerleştirilmesi ClusPro, Mutational Binding free energy change predictor 2 (Mutabind2) ve Protein-Ligand Interaction Profiler (PLIP) sunucuları tarafından gerçekleştirildi. Mutasyonların stabilitesi Impact-Mutant 2.0 (I-Mutant2), Mutation Protein Stability

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Prediction (MUpro) ve Impact of Non-synonymous mutations on Protein Stability-Multi Dimension (INPS-MD) ile analiz edildi. Yapısal değişiklikler Dynamic Mutation predictor 2 (DynaMut2) ve Have (y)Our Protein Explained (HOPE) sunucuları kullanılarak belirlendi. Tanımlanan 99 yanlış anlamlı mutasyondan 6'sı (T56P, C57Y, K204I, S207F, G215V ve W217C) patojenik olarak belirlendi. Tüm mutasyonların öncelikli olarak akciğer kanserinde bulunduğu ve IL7RA'nın ekstraselüler alanında yerleştiği tespit edildi. Her ne kadar hiçbiri IL7 etkileşiminin ara yüzünde olmasa da, hepsi korunmuş motiflerin yanında veya yakınında konumlanmışlardı. Bu yakınlık IL7RA'yı istikrarsızlaştırmakta ve bağlanma paternini büyük ölçüde değiştirmektedir. IL7RA yanlış anlamlı mutasyonları muhtemelen proteinin işlevini değiştirdiği için akciğer kanserinde önemli bir role sahip olabilir.

Introduction

Interleukin 7 (IL7) and IL7 Receptor Alpha (IL7RA) signaling is a widely studied pathway, whose role has been extensively defined in B and T cell development and differentiation (Mazzucchelli & Durum 2007, Winer *et al.* 2022). The signaling of IL7 starts upon binding to IL7RA extracellular domain. The receptor heterodimerization occurs after this binding and triggers the phosphorylation of cytoplasmic tyrosine residue on the IL7RA. This phosphorylation activates the Janus kinase/signal transducers and activators of transcription (JAK-STAT) signaling and results in cell survival and proliferation (Barros *et al.* 2021, Winer *et al*. 2022).

The signaling of IL7 is controlled through receptors as IL7 has constitutive expression. One of the control mechanisms is via receptor shedding and converting the membrane bound IL7RA into soluble IL7RA (sIL7RA) (Vranjkovic *et al.* 2007, Lundström *et al.* 2013). Another control mechanism is alternative splicing of the receptor transcript to generate the sIL7RA. The soluble version of the receptor competes with the membrane bound one for IL7 and reduces the amount of ligand that binds to it. However, sIL7RA suppresses the expression of negative controllers of the IL7 signaling and increases the bioactivity via membrane bound IL7RA (Vranjkovic *et al.* 2007, Mazzucchelli *et al.* 2012, Lundström *et al.* 2013, Barros *et al.* 2021, Wang *et al.* 2022).

There are many mutations of IL7RA causing different pathologies, of which those causing immunodeficiency and autoimmune diseases are well defined (Puel *et al.* 1998, Mazzucchelli *et al.* 2012, Campos *et al.* 2019, Yan *et al.* 2023). Alterations causing leukemia were also reported in many studies (Kim *et al.* 2013, Barata *et al.* 2019). In solid cancers, the effect of IL7 - IL7RA signaling was mostly investigated from the perspective of immune cell filtration or tumor microenvironment (Caushi *et al.* 2021, Wang *et al.* 2022,). However, it is reported in the human protein atlas (proteinatlas.org) that tissues like the lung, breast, small intestine, skin and more also express IL7RA and studies reported the expression of the receptor in different cancer cell lines (Cosenza *et al.* 2002). One such study reported that in lung adenocarcinoma cell lines, IL7RA expression was significantly lower when compared with normal lung cell line (Wang *et al.* 2022). Although the expression was documented, there are few studies investigating the effects of IL7RA missense mutations in the context of solid tumors. One such study reported 3 intronic and 2 3'UTR variants in the Chinese-Han population increasing breast cancer susceptibility (Wang *et al.* 2020). Similarly, T244I mutation was reported for susceptibility to breast cancer (Vitiello *et al.* 2018).

IL7R was reported as an important gene in brain metastasis from non-small cell lung cancer (Zu *et al.* 2023). In accordance with this finding, we have recently reported IL7R as an important role player in leptomeningeal carcinoma (Congur *et al.* 2023). This type of cancer is a metastatic form of lung, breast and skin cancer which localizes to the meninges of the brain. The patients after this metastasis unfortunately have a very short lifespan. That is why more investigation is required to understand this type of metastasis for developing better or targeted medications. The main focus of this study is to define and understand the effects of missense mutations of IL7RA in lung, breast and skin cancers by employing computational methods.

Materials and Methods

Data retrieval

The mutations of IL7R were retrieved from the National Institutes of Health Genomics Data Commons (GDC) Data Portal (https://portal.gdc.cancer.gov). The portal's last update was on March 29, 2024 at the time of the last analysis (July 4, 2024). Under the tab Cohort Builder, primary sites lung and bronchus, breast and skin were selected for analysis. Within the selected cohort, mutations of IL7R were searched by using mutation frequency app tool under the analysis tab. Then the mutations listed were filtered to show missense mutations.

Mutation analysis

The pathogenicity of the mutations were analyzed by Sorting Intolerant From Tolerant (SIFT) (https://sift.bii.a-star.edu.sg) (Sim *et al.* 2012), Polymorphism Phenotyping v2 (PolyPhen2) (http://genetics.bwh.harvard.edu/pph2/) (Adzhubei *et al.* 2010), Universal Mutation Database Predictor (UMD-Predictor) (https://umd-predictor.genomnis.com/mutation) (Salgado *et al.* 2016) and Single Nucleotide Polymorphisms & Gene Ontology (E-SNP&GO) (https://esnpsandgo.biocomp.unibo.it/) (Manfredi *et al.* 2022) mutation analysis servers. UMD-Predictor was chosen considering it has high sensitivity (0.95) and accuracy (0.85) when compared with the tools available (Salgado *et al.* 2016). Similarly, E-SNP&GO determines the pathogenicity of the mutation, employing Gene Ontology (GO) functional annotations and has high precision (85.7%) when compared with the other methods (Manfredi *et al*. 2022). FASTA file of the protein sequence used in these tools listed above was obtained from Universal Protein Resource (UniProt) ID: P16871-1 and

the corresponding Ensembl ID ENST00000303115 was used in UMD-Predictor. The conservation analysis was performed by Conservation Surface Mapping (ConSurf) (https://consurf.tau.ac.il/consurf_index.php) (Ashkenazy *et al.* 2016) by using the UniProt ID: P16871. Disease association of the mutations was investigated by Clinical Variation (ClinVar) (https://www.ncbi.nlm.nih.gov/clinvar/).

Domain, Docking and Stability analysis

The domains where the mutations are located were analyzed by InterPro (https://www.ebi.ac.uk/interpro/) (Paysan-Lafosse *et al.* 2023). The stability of the mutated receptor was analyzed by using Impact-Mutant 2.0 (I-Mutant2) (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi) (Capriotti *et al.* 2005), Mutation Protein Stability Prediction (MUpro) (https://mupro.proteomics.ics.uci.edu/) (Cheng *et al.* 2006) and Impact of Non-synonymous mutations on Protein Stability-Multi Dimension (INPS-MD) sequence based (https://inps.biocomp.unibo.it/welcome/default/index) (Fariselli *et al.* 2015) servers. The FASTA sequence of IL7RA protein was retrieved from UniProt ID: P16871.

For the docking of the IL7 to the mutated IL7RA, Cluspro (https://cluspro.org/) (Kozakov *et al.* 2013, Vajda *et al.* 2017, Kozakov *et al.* 2017,Desta *et al.* 2020), Mutational Binding free energy change predictor 2 (Mutabind2) (https://lilab.jysw.suda.edu.cn/research/mutabind2/) (Zhang *et al.* 2020) and Protein-Ligand Interaction Profiler (PLIP) (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) servers (Salentin *et al.* 2015) were used. For the structural comparison analysis of wild type vs mutant receptors Dynamic Mutation predictor 2 (DynaMut2) (https://biosig.lab.uq.edu.au/dynamut2/, Pearson's correlation value 0.72) (Rodrigues *et al.* 2021) and Have (y)Our Protein Explained (HOPE) (https://www3.cmbi.umcn.nl/hope/) (Venselaar *et al.* 2010) servers were employed. The Protein Data Bank (PDB) files used in these analyses were obtained from UniProt available under the codes 7OPB, 3DI2, 3DI3 and 3UP1.

Results

Data Mining and Evaluation of the Pathogenicity Risks

In GDC Data portal the primary sites lung and bronchus, breast and skin revealed 10382 cases. Among these cases 169 IL7RA somatic mutations were reported. Upon filtering for missense only mutations, 102 missense variants were listed, 2 of these mutations were synonymous and 1 was at the splice site. These 3 mutations were excluded and the pathogenicity risk analysis was performed over the remaining 99 mutations. (Supplementary Material 1).

Pathogenicity risks were computed by using SIFT and 40 of the mutations were found to be deleterious. Then these 40 mutations were further analyzed by PolyPhen2 tool which reduced the list to 24. These 24 mutations annotated as "probably damaging" by PolyPhen2 were then run in UMD-Predictor and E-SNP&GO tools. UMD-Predictor scores the mutations for their pathogenicity over 100 where over 75 is categorized as pathogenic. Among these 24 variants 18 were found to be pathogenic and 11 variants were scored 100 for pathogenicity in UMD-Predictor (Supplementary Material 2). The variants that scored 100 were chosen. In E-SNP&GO the mutations were given a pathogenicity probability score over 1 annotating them as pathogenic or benign. Moreover, a reliability index over 9 was determined for the prediction of each variant, indicating 9 as the most reliable. For this purpose, upon the computation of E-SNP&GO tool, the mutations annotated as pathogenic which have a reliability score of 9 were chosen. 8 of the missense mutations were found to be reliably pathogenic according to this tool. As a result, the list of missense mutations was refined to 6 which were annotated as pathogenic in all 4 servers. These mutations were T56P, C57Y, K204I, S207F, G215V and W217C (Table 1).

Positions and Conservation Status of 6 Missense Mutations

IL7RA missense mutations listed in Table 1 were further investigated in ClinVar. Except from G215V mutation, none of the others (T56P, C57Y, K204I, S207F, W217C) were reported in Clinvar. G215V variant was reported as likely pathogenic and was associated with immunodeficiency. The mutations were searched in GDC Data Portal specifically and 5 of the mutations, T56P, C57Y, S207F, G215V and W217C were detected in lung and bronchus but K204I variant was detected at skin as primary site. In this short list, no mutation was associated with breast cancer.

The 6 missense mutations were further analyzed according to the positions of the amino acids by using InterPro server (Supplementary Material 3). Interestingly all these mutations were located at the extracellular domain. T56P, C57Y were located at FN3_7 domain specifically found in IL7RA (IL-7Ralpha, fibronectin type III domain). K204I, S207F, G215V and W217C were found in fibronectin type III (FN3) domain of IL7RA. Moreover, using Conservation Surface Mapping (ConSurf) tool the conservation scores of each amino acid at the IL7RA protein sequence were detected (Supplementary Material 4). The scoring ranges between 1-9,9 being the most conserved. Regarding this tool, all the mutations were at conserved amino acids where for 5 of them (C57, K204, S207, G215 and W217), the score was 9 and T56 had a score of 7 (Table 2).

Comparing the Stability of the IL7RA mutants with the wild type

Since the ConSurf analysis revealed that the mutations were at conserved amino acids, the stability of the mutant proteins was investigated by using different tools (Table 3). When I-Mutant 2 tool was used, T56P, C57Y, K204I, G215V and W217C were found to decrease the stability of the protein while S207F alteration increased the stability.

Table 1. IL7RA mutations analyzed as pathogenic by 4 different tools.

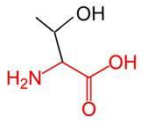
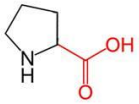
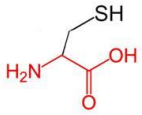
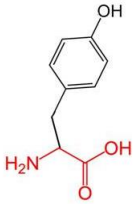
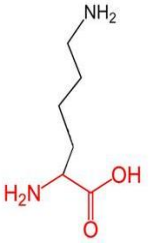
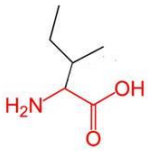
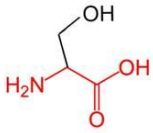
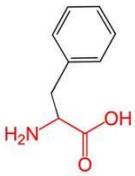
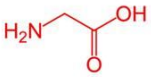
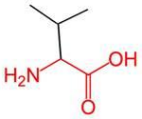
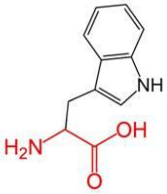
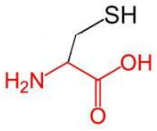
Mutation	Wild type amino acid	Mutant amino acid
T56P (Thr56Pro)		
C57Y (Cys57Tyr)		
K204I (Lys204Ile)		
S207F (Ser207Phe)		
G215V (Gly215Val)		
W217C (Trp217Cys)		

Table 2. ConSurf conservation scores of the wild type IL7RA amino acids at the mutated sites.

AMINO ACID POSITION	T56 (THR56)	C57 (CYS57)	K204 (LYS204)	S207 (SER207)	G215 (GLY215)	W217 (TRP217)
SCORE	7	9	9	9	9	9

Table 3. Stability and ΔΔG of the IL7RA mutants compared to wild type.

	T56P (Thr56Pro)	C57Y (Cys57Tyr)	K204I (Lys204Ile)	S207F (Ser207Phe)	G215V (Gly215Val)	W217C (Trp217Cys)
I-Mutant2 (Stability)	Decrease	Decrease	Decrease	Increase	Decrease	Decrease
MUpro (Stability)	Decrease	Decrease	Decrease	Decrease	Decrease	Decrease
INPS-MD ΔΔG (kcal/mol)	-1.19	-2.06	0.56	-0.32	-1.71	-1.62

However, this latter change had a reliability index of 4 which was the lowest value among all 6 mutations tested. In addition to I-Mutant 2, the stabilities of the mutants were also investigated by MUpro tool. MUpro calculated the stability of all mutants as decreasing. These two algorithms were in consensus about the decreasing stability of all mutants except S207F. Additionally, the ΔΔG (kcal/mol) scores of the mutated proteins were calculated with INPS-MD server by using the sequence as an input. The energy scores were negative for T56P, C57Y, S207F, G215V and W217C but positive for K204I. These algorithms calculate the free energy from folded structure towards unfolded structure (Pancotti *et al.* 2022, Benevenuta *et al.* 2023). In accordance with these results, most of the mutations might have a destabilizing effect on IL7RA.

Structural comparison of the IL7RA mutants with the wild type

After analyzing the stability of the mutations, the docking of IL7 was performed by using ClusPro, Mutabind and PLIP tools, with PDB files obtained from UniProt. 3DI2, 3DI3 and 3UP1 files were analyzed in all 3 tools. 3DI2 and 3DI3 files contained, IL7 and IL7RA structural information, whereas 3UP1 file has the IL7RA homodimer interaction of the extracellular domain. However, although all the mutations were at the extracellular compartment of IL7RA, they were not at the IL7 or IL7RA interaction interface. Therefore, the structural comparison of the wild type versus mutant IL7RA was analyzed for each 6 of the mutations by both HOPE and DynaMut2 servers. These tools after computing the mutation, give graphical outputs of the mutated region of the protein by comparing mutated with the wild type. All 6 variants were analyzed in both tools. HOPE server requests UniProt ID as an input and recognizes the PDB file 7OPB, while DynaMut2 requires the PDB file of choice as an input. 7OPB file was used to standardize the PDB input and the visual figures were presented as the outputs of DynaMut2.

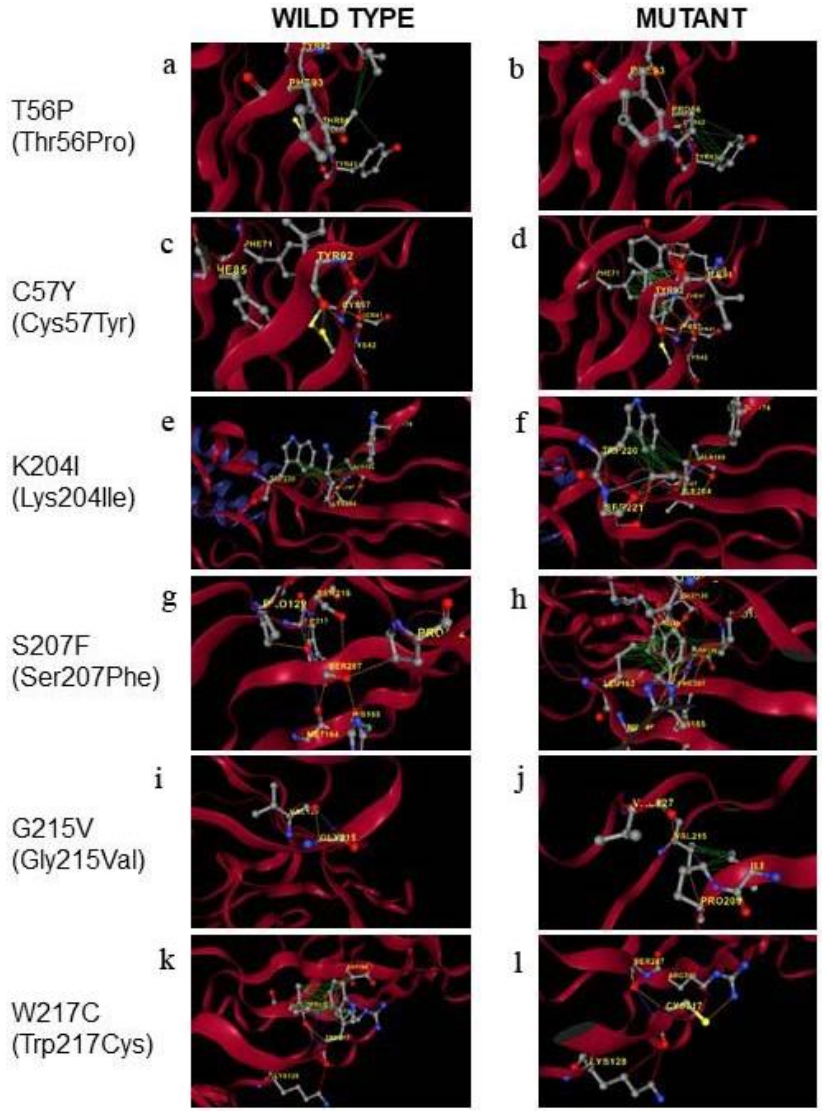


Fig. 1. Wild type versus mutant IL7RA structures executed by the DynaMut2 server. The differences were plotted by choosing single mutation analysis type. The colors of the interactions were annotated by the server as: light blue - Van der Waals attractions, green - hydrophobic bonds, red - hydrogen bonds, yellow - ionic bonds, light green - aromatic interactions, orange - polar bonds.

When each mutation was compared relative to its wild type, all mutations were found to disturb the sequence of FN3 domain, which was already confirmed by InterPro server. C57T mutation replaces cysteine, a small and –S containing amino acid, with a non –S and big amino acid, tyrosine, which also contains a ring. The cysteine at this position is associated with a sulfide bridge occurring between 2 cysteines (Campos *et al.* 2019) and this bridge should be lost at mutant protein, which probably would cause destabilization of IL7RA. Besides, the hydrophobicity of the wild type and mutant residues would be different affecting the hydrophobic interactions of the core protein. Tyrosine is a bigger amino acid by size, and it would not completely fit in the place of cysteine as well. As a result, the cysteine that is buried in wild type protein when altered into a tyrosine was assumed to be in a more exposed position (Fig. 1a-b).

Similar to C57T mutation T56P mutation is just before the cysteine bridge and it is also introducing a ring structure. Proline is known to be present more in beta turns and not compatible with beta strands (Li *et al.* 1996, Mir *et al.* 2023). Consequently, this structure change could disturb both the –S bridge and the beta sheet configurations in the wild type protein. Furthermore, their hydrophobicity differences would change hydrogen bonds in the core IL7RA protein disturbing the correct folding (Fig. 1c-d).

Mutation K204I was found to replace a positively charged amino acid, lysine with a neutral isoleucine. This change would be associated with the disruption of the salt bridge between lysine and glutamic acid at position 202 according to HOPE server. This charge change would affect the internal and external interactions of the receptor (Fig. 1e-f).

Variant S207F also introduced a ring structure to the IL7RA, changing the hydrogen bonds that were established in the wild type receptor. This assumption is also supported by the change in the hydrophobicity of the wild type amino acid. Moreover, serine in this position bears a buried confirmation while the mutant amino acid phenylalanine, would be exposed for being bigger than the wild type (Fig. 1g-h).

Glycine is a flexible amino acid (Senthil *et al.* 2019). G215V mutation for that reason could affect the flexibility of IL7RA protein which could cause functional changes. Additionally, the sizes of glycine in wild type and valine in the mutant differ and would result in an exposed amino acid in the backbone (Fig. 1i-j).

The last mutation W217C is located in a WSXWS motif. This motif is important for the receptor activation of the cytokine receptors (Campos *et al.* 2019). The mutant protein would have a disrupted motif and although the residue is not in the interface of IL7-IL7RA interaction, it could possibly affect the ligand receptor contacts by causing a change in the neighboring interactions (Fig. 1k-l).

Discussion

IL7RA mutations were extensively studied in leukemia, T and B cell development. It is found both as homodimer and heterodimer but the dimerization depends on the type of the ligand bound (Mazzucchelli & Durum 2007, Winer *et al*. 2022). In our recent study, IL7R was proposed as one of the genes that could be important in leptomeningeal carcinoma (Congur *et al.* 2023). However, there are limited number of patient data on leptomeningeal carcinoma which is a deadly metastatic form of mostly breast, lung and skin cancers. Specifically, IL7RA downregulation was reported in lung cancer (Wang *et al.* 2022). Moreover, in non-small cell lung cancer, the knockdown of IL7RA leads to overexpression of multidrug resistance (Ke *et al.* 2019). However, the missense mutations were not very well investigated in these solid cancers. Therefore, a cohort of lung, breast and skin cancer as a primary site was selected to analyze the effects of mutations in IL7RA computationally.

In this study, 99 missense mutations from a cohort of 3250 patients were analyzed by 4 different servers (SIFT, PolyPhen2, UMD-Predictor and E-SNP&GO) to identify the pathogenic variants. SIFT and PolyPhen2 are widely employed servers which detect the pathogenicity score depending on the conservation of the amino acid between different species. UMD-Predictor uses the transcript information instead of the protein sequence as an input, but compares the conservation scores by taking biochemical properties of the proteins and the changes to the transcript into account. As a result of all these combined matrices, the server determines the pathogenicity score of the mutation. Suybeng *et al.* (2020) compared several different variant prediction tools and reported UMD-Predictor as the most accurate one among the tested. However, E-SNP&GO was developed in 2022 as another tool which also determines the pathogenicity by using the gene ontology annotations. As all these tools use different approaches for determining the pathogenicity, the list of missense mutations were analyzed sequentially in all 4 servers. Finally, the intersecting missense variants T56P, C57Y, K204I, S207F, G215V and W217C were selected for further investigation. Although all the tools used involved the conservation value for determining the pathogenicity, the conservation of the wild type residues were also checked by ConSurf tool as well to confirm the conservation of these residues one more time.

It was very interesting to see that all mutations were in the extracellular domain and were mostly very closely located to each other. IL7RA had 2 different fibronectin domains, FN3_7 and FN3. Both domains are very important for the functioning of the IL7RA. These domains contain 2 important structural elements crucial for the normal folding and performance of the protein, disulfide bridge regions and WSXWS motifs (McElroy *et al.* 2009, Campos *et al.* 2019). C57Y, K204I and W217C variants are therefore directly related to the

proper formation of the IL7RA as they reside in these regions. The fibronectin domains as reported before have conserved beta strands in their structures (McElroy *et al.* 2009). C57Y alteration corresponds to one of the disulfide bonds, as a result probably disrupting the correct folding and beta strand configuration of the FN3_7 domain. Similarly, K204I and W217C mutations were located at the WSXWS region of the IL7RA which is known as a conserved sequence for type I cytokine receptors (McElroy *et al.* 2009, Campos *et al.* 2019). Both variants replace the wild type amino acid in such a way that the bonding property of the region drastically changes. Although WSXWS motif is outside of the contact region of the ligand, this sequence was reported to be important for the correct folding of IL7RA which brings the receptor to a structure enabling IL7 binding (McElroy *et al.* 2009). Another mutation, W217X (rs104893893), was previously reported in ClinVar and was associated with immunodeficiency. This mutation was generating a stop codon with a G>A mutation and causing early termination. Whereas W217C mutation is G>T and not reported in ClinVar. A recent study performed with HEK293 cells indicated that cysteine tRNAs when overexpressed could act like a stop codon read-through (Valášek *et al.* 2023). Although this mutation does not replace a stop codon still it raises a possibility that the variant could have other consequences in IL7RA functions or expression that could be enlightened by further research studies.

Besides changing the intra bonding of the amino acids, these 6 mutations have a tendency towards an unfolded state energetically. This tendency presents a destabilized condition (Pancotti *et al.* 2022), that could possibly be interpreted by degradation or aggregation of the unfunctional receptor. Almost all these 6 mutations listed in this study were significantly changing the bonding, hence the folding of the protein. It was also proposed that the IL7RA on tumor cells could be a good prognosis marker for the capacity to regulate immune cell infiltration (Wang *et al.* 2022). Therefore, the structural integrity of IL7RA probably evokes a pivotal role for the survival of the cancer cell.

On the other hand, sequence-wise all 6 mutations are not a part of exon 6 and exon 6 skipping generates the soluble IL7RA (sIL7RA) (Barros *et al.* 2021). The soluble receptor competes with the membrane bound receptor for IL7. The decrease in IL7 concentration however can increase the bioactivity of the cytokine. Moreover, sIL7RA also suppresses the negative regulation of IL7 (Lundström *et al.* 2013, Barros *et al.* 2021). If any of these mutations do increase the contact time of IL7 with IL7R, then it is possible that the proliferative signal through JAK-STAT pathway could be enhanced. Increased proliferation could also contribute to the aggressiveness of the tumor. On the other hand, it was also demonstrated that IL7 binding to IL7RA could trigger membrane shedding and desensitize the cell to IL7 signal (Vranjkovic *et al.* 2007). These mutations could reduce the shedding of the receptor keeping it active with a prolonged signal. Accordingly, the conformational changes that occur due to these missense mutations could have multiple different consequences.

The limitation of this current study is that the sequenced tumor tissue could also have immune cells infiltrated into the tissue. The consequence of tissue sequencing is not discriminating between different cell types in a heterogeneous tumor microenvironment. The second setback is the cohort of 3250 patients whose missense mutation list was obtained. If the number increases more potent mutations could be discovered. Moreover, all these mutations are found in 1 patient indicating that they are not hotspot mutations, which could also be the result of the limited patient number. Accordingly, with all the limitations of this study, the effect of the mutations should be validated before their pathogenicity is attributed to any solid cancer. To understand the nature of these mutations, it is necessary to perform fully controlled *in vitro* and *in vivo* experiments. In these experiments the potent mutations discovered with this research could be introduced into lung adenocarcinoma cell lines and the effects of the mutations could be investigated. Moreover, their tumorigenicity and metastatic capacities could be assessed with *in vivo* experiments. The results of this *in silico* study will help limit the number of mutations to be investigated by reverse genetics strategies.

Conclusion

In this study the missense mutations of IL7RA were investigated and 6 mutations, T56P, C57Y, K204I, S207F, G215V and W217C were evaluated as pathogenic. These mutations were found to affect the structure of IL7RA by drastically changing the confirmation. The confirmation changes occur in important motifs of the protein and they could possibly cause a change in the proper functioning of the IL7RA. Understanding the molecular biology of such mutations with further *in vitro* and *in vivo* studies could elevate our understanding of IL7 signaling in solid cancers.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Data Sharing Statement: All data are available within the study and Supplementary Materials.

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References

1. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S. & Sunyaev, S.R. 2010. A method and server for predicting damaging missense mutations. *Nature Methods*, 7(4): 248-249. https://doi.org/10.1038/nmeth0410-248

2. Ashkenazy, H., Abadi, S., Martz, E., Chay, O., Mayrose, I., Pupko, T. & Ben-Tal, N. 2016. ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. *Nucleic Acids Research*, 44(W1): W344-50. https://doi.org/10.1093/nar/gkw408

3. Barata, J.T., Durum, S.K. & Seddon, B. 2019. Flip the coin: IL-7 and IL-7R in health and disease. *Nature Immunology*, 20(12): 1584-1593. https://doi.org/10.1038/s41590-019-0479-x

4. Barros, P.O., Berthoud, T.K., Aloufi, N. & Angel, J.B. 2021. Soluble IL-7Rα/sCD127 in Health, Disease, and Its Potential Role as a Therapeutic Agent. *Immunotargets and Therapy*, 10: 47-62. https://doi.org/10.2147/ITT.S264149

5. Benevenuta, S., Birolo, G., Sanavia, T., Capriotti, E. & Fariselli, P. 2023. Challenges in predicting stabilizing variations: An exploration. *Frontiers in Molecular Biosciences*, 9: 1075570. https://doi.org/10.3389/fmolb.2022.1075570

6. Campos, L.W., Pissinato, L.G. & Yunes, J.A. 2019. Deleterious and Oncogenic Mutations in the *IL7RA*. *Cancers* (Basel). 11(12): 1952. https://doi.org/10.3390/cancers11121952

7. Capriotti, E., Fariselli, P. & Casadio, R. 2005. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. *Nucleic Acids Research*, 33(Web Server issue): W306-310. https://doi.org/10.1093/nar/gki375

8. Caushi, J.X., Zhang, J., Ji, Z., Vaghasia, A., Zhang, B., Hsiue, E.H., Mog, B.J., Hou, W., Justesen, S., Blosser, R., Tam, A., Anagnostou, V., Cottrell, T.R., Guo, H., Chan, H.Y., Singh, D., Thapa S, Dykema, A.G., Burman, P., Choudhury, B., Aparicio, L., Cheung, L.S., Lanis, M., Belcaid ,Z., El Asmar, M., Illei, P.B., Wang, R., Meyers, J., Schuebel, K., Gupta, A., Skaist A., Wheelan, S., Naidoo, J., Marrone, K.A., Brock, M., Ha J., Bush, E.L., Park, B.J., Bott, M., Jones, D.R., Reuss, J.E., Velculescu, V.E., Chaft, J.E., Kinzler, K.W., Zhou, S., Vogelstein, B., Taube J.M., Hellmann, M.D., Brahmer, J.R., Merghoub, T., Forde, P.M., Yegnasubramanian, S., Ji, H., Pardoll, D.M. & Smith, K.N. 2021. Transcriptional programs of neoantigen-specific TIL in anti-PD-1-treated lung cancers. *Nature*, 596(7870): 126-132. https://doi.org/10.1038/s41586-021-03752-4

9. Cheng, J., Randall, A. & Baldi, P. 2006. Prediction of protein stability changes for single-site mutations using support vector machines. *Proteins*, 62(4): 1125-32. https://doi.org/10.1002/prot.20810

10. Congur, I., Koni, E., Onat, O.E. & Tokcaer Keskin, Z. 2023. Meta-analysis of commonly mutated genes in leptomeningeal carcinomatosis. *PeerJ*. 11: e15250. https://doi.org/10.7717/peerj.15250

11. Cosenza, L., Gorgun, G., Urbano, A. & Foss, F. 2002. Interleukin-7 receptor expression and activation in nonhaematopoietic neoplastic cell lines. *Cell Signal*, 14(4): 317-25. https://doi.org/10.1016/S0898-6568(01)00245-5

12. Desta, I.T., Porter, K.A., Xia, B., Kozakov, D. & Vajda, S. 2020. Performance and its limits in rigid body protein-protein docking. *Structure*, 28(9): 1071-1081.e3. https://doi.org/10.1016/j.str.2020.06.006

13. Fariselli, P., Martelli, P.L., Savojardo, C. & Casadio, R. 2015. INPS: predicting the impact of non-synonymous variations on protein stability from sequence. *Bioinformatics*, 31(17): 2816-2821. https://doi.org/10.1093/bioinformatics/btv291

14. Ke, B., Wei, T., Huang, Y., Gong, Y., Wu, G., Liu, J., Chen, X. & Shi, L. 2019. Interleukin-7 resensitizes non-small-cell lung cancer to cisplatin via inhibition of ABCG2. *Mediators of Inflammation*. 2019: 7241418. https://doi.org/10.1155/2019/7241418

15. Kim, M.S., Chung, N.G., Kim, M.S., Yoo, N.J. & Lee, S.H. 2013. Somatic mutation of IL7R exon 6 in acute leukemias and solid cancers. *Human Pathology*, 44(4): 551-555. https://doi.org/10.1016/j.humpath.2012.06.017

16. Kozakov, D., Beglov, D., Bohnuud, T., Mottarella, S.E., Xia, B., Hall, D.R. & Vajda, S. 2013. How good is automated protein docking? *Proteins*, 81(12): 2159-2166. https://doi.org/10.1002/prot.24403

17. Kozakov, D., Hall, D.R., Xia, B., Porter, K.A., Padhorny, D., Yueh, C., Beglov, D. & Vajda, S. 2017. The ClusPro web server for protein-protein docking. *Nature Protocols*, 12(2): 255-278. https://doi.org/10.1038/nprot.2016.169

18. Li, S.C., Goto, N.K., Williams, K.A. & Deber, C.M. 1996. Alpha-helical, but not beta-sheet, propensity of proline is determined by peptide environment. *Proceedings of the National Academy of Sciences U S A*, 93(13): 6676-81. https://doi.org/10.1073/pnas.93.13.6676

19. Lundström, W., Highfill, S., Walsh, S.T., Beq, S., Morse, E., Kockum, I., Alfredsson, L., Olsson, T, Hillert, J. & Mackall, C.L. 2013. Soluble IL7Rα potentiates IL-7 bioactivity and promotes autoimmunity. *Proceedings of the National Academy of Sciences U S A*, 110(19): E1761-70. https://doi.org/10.1073/pnas.1222303110

20. Manfredi, M., Savojardo, C., Martelli, P.L. & Casadio, R. 2022. E-SNPs&GO: embedding of protein sequence and function improves the annotation of human pathogenic variants. *Bioinformatics*, 38(23): 5168-5174. https://doi.org/10.1093/bioinformatics/btac678

21. Mazzucchelli, R. & Durum, S.K. 2007. Interleukin-7 receptor expression: intelligent design. *Nature Reviews Immunology*, 7(2): 144-154. https://doi.org/10.1038/nri2023

22. Mazzucchelli, R.I., Riva, A. & Durum, S.K. 2012. The human IL-7 receptor gene: deletions, polymorphisms and mutations. *Seminars in Immunology*, 24(3): 225-30. https://doi.org/10.1016/j.smim.2012.02.007

23. McElroy, C.A., Dohm, J.A., Walsh, S.T. 2009. Structural and biophysical studies of the human IL-7/IL-7Ralpha complex. *Structure*, 17(1): 54-65. https://doi.org/10.1016/j.str.2008.10.019

24. Mir, R.A., Shafi, S.M. & Zargar, S.M. 2023. Chapter 1 - Undestanding the OMICS techniques: an introduction to genomics and proteomics. pp 1-28. In: Mir, R.A., Shafi, S.M. & Zargar, S.M. (eds). *Principles of Genomics and Proteomics*. Elsevier, New York. 274 pp.

25. Pancotti, C., Benevenuta, S., Birolo, G., Alberini, V., Repetto, V., Sanavia, T., Capriotti, E. & Fariselli, P. 2022. Predicting protein stability changes upon single-point mutation: a thorough comparison of the available tools on a new dataset. *Briefings in Bioinformatics*, 23(2): 555. https://doi.org/10.1093/bib/bbab555

26. Paysan-Lafosse, T., Blum, M., Chuguransky, S., Grego, T., Pinto, B.L., Salazar, G.A., Bileschi M.L., Bork, P., Bridge, A., Colwell, L., Gough, J., Haft, D.H., Letunić, I., Marchler-Bauer, A., Mi, H., Natale, D.A., Orengo, C.A., Pandurangan, A.P., Rivoire, C., Sigrist, C.J.A., Sillitoe, I., Thanki, N., Thomas, P.D., Tosatto, S.C.E., Wu, C.H. & Bateman, A. 2023. InterPro in 2022. *Nucleic Acids Research*, 51(D1): D418-D427. https://doi.org/10.1093/nar/gkac993.

27. Puel, A., Ziegler, S.F., Buckley, R.H. & Leonard, W.J. 1998. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. *Nature Genetics*, 20(4): 394-397. https://doi.org/10.1038/3877

28. Rodrigues, C.H.M., Pires, D.E.V. & Ascher, D.B. 2021. DynaMut2: Assessing changes in stability and flexibility upon single and multiple point missense mutations. *Protein Science*, 30(1): 60-69. https://doi.org/10.1002/pro.3942

29. Salentin, S., Schreiber, S., Haupt, V.J., Adasme, M.F. & Schroeder, M. 2015. PLIP: fully automated protein-ligand interaction profiler. *Nucleic Acids Research*, 43(W1): W443-W447. https://doi.org/10.1093/nar/gkv315

30. Salgado, D., Desvignes, J.P., Rai, G., Blanchard, A., Miltgen, M., Pinard, A., Lévy, N., Collod-Béroud, G. & Béroud, C. 2016. UMD-Predictor: A high-throughput sequencing compliant system for pathogenicity prediction of any human cDNA Substitution. *Human Mutation*, 37(5): 439-46. https://doi.org/10.1002/humu.22965

31. Senthil, R., Usha, S. & Saravanan, K.M. 2019. Importance of fluctuating amino acid residues in folding and binding of proteins. *Avicenna Journal of Medical Biotechnology*, 11(4): 339-343.

32. Sim, N.L., Kumar, P., Hu, J., Henikoff, S., Schneider, G. & Ng, P.C. 2012. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Research*, 40(Web Server issue): W452-7. https://doi.org/10.1093/nar/gks539

33. Suybeng, V., Koeppel, F., Harlé, A. & Rouleau, E. 2020. Comparison of pathogenicity prediction tools on somatic variants. *The Journal of Molecular Diagnostics*, 22(12): 1383-1392. https://doi.org/10.1016/j.jmoldx.2020.08.007

34. Vajda, S., Yueh, C., Beglov, D., Bohnuud, T., Mottarella, S.E., Xia, B., Hall, D.R. & Kozakov, D. 2017. New additions to the ClusPro server motivated by CAPRI. *Proteins*, 85(3): 435-444. https://doi.org/10.1002/prot.25219

35. Valášek, L.S., Kučerová, M., Zeman, J. & Beznosková, P. 2023. Cysteine tRNA acts as a stop codon readthrough-inducing tRNA in the human HEK293T cell line. *RNA*, 29(9): 1379-1387. https://doi.org/10.1261/rna.079688.123

36. Venselaar, H., Te Beek, T.A., Kuipers, R.K., Hekkelman, M.L. & Vriend, G. 2010. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. *BMC Bioinformatics*, 11: 548. https://doi.org/10.1186/1471-2105-11-548

37. Vitiello, G.A.F., Losi Guembarovski, R., Amarante, M.K., Ceribelli, J.R., Carmelo, E.C.B. & Watanabe, M.A.E. 2018. Interleukin 7 receptor alpha Thr244Ile genetic polymorphism is associated with susceptibility and prognostic markers in breast cancer subgroups. *Cytokine*, 103: 121-126. https://doi.org/10.1016/j.cyto.2017.09.019

38. Vranjkovic, A., Crawley, A.M., Gee, K., Kumar, A. & Angel, J.B. 2007. IL-7 decreases IL-7 receptor alpha (CD127) expression and induces the shedding of CD127 by human CD8+ T cells. *International Immunology*, 19(12): 1329-1339. https://doi.org/10.1093/intimm/dxm102

39. Wang, C., Kong, L., Kim, S., Lee, S., Oh, S., Jo, S., Jang, I. & Kim, T.D. 2022. The role of IL-7 and IL-7R in cancer pathophysiology and immunotherapy. *International Journal of Molecular Sciences*, 23(18): 10412. https://doi.org/10.3390/ijms231810412

40. Wang, X., Chang, S., Wang, T., Wu, R., Huang, Z., Sun, J., Liu, J., Yu, Y., Mao, Y. 2022. IL7R is correlated with immune cell infiltration in the tumor microenvironment of lung adenocarcinoma. *Frontiers in Pharmacology*, 13: 857289. https://doi.org/10.3389/fphar.2022.857289

41. Wang, Z., Wang, X., Gao, Y., Wang, Y., Xu, M., Han, Q. & Zhao, X. 2020. IL-7R gene variants are associated with breast cancer susceptibility in Chinese Han women. *International Immunopharmacology*, 86: 106756. https://doi.org/10.1016/j.intimp.2020.106756

42. Winer, H., Rodrigues, G.O.L., Hixon, J.A., Aiello, F.B., Hsu, T.C., Wachter, B.T., Li, W. & Durum, S.K. 2022. IL-7: Comprehensive review. *Cytokine*, 160: 156049. https://doi.org/10.1016/j.cyto.2022.156049

43. Yan, L., He, Y., Zhang, Y., Liu, Y., Xu, L., Han, C., Zhao, Y. & Li, H. 2023. A novel 268 kb deletion combined with a splicing variant in IL7R causes of severe combined immunodeficiency in a Chinese family: a case report. *BMC Medical Genomics*, 16(1): 323. https://doi.org/10.1186/s12920-023-01765-8

44. Zhang, N., Chen, Y., Lu, H., Zhao, F., Alvarez, R.V., Goncearenco, A., Panchenko, A.R. & Li M. 2020. MutaBind2: Predicting the impacts of single and multiple mutations on protein-protein interactions. *iScience*, 23(3): 100939. https://doi.org/10.1016/j.isci.2020.100939

45. Zu, L., He, J., Zhou, N., Tang, Q., Liang, M. & Xu, S. 2023. Identification of multiple organ metastasis-associated hub mRNA/miRNA signatures in non-small cell lung cancer. *Cell Death and Disease*, 14(12): 798. https://doi.org/10.1038/s41419-023-06286-x