Unraveling TRIM21 Mutations and Their Role in Systemic Lupus Erythematosus Using Bioinformatic Modeling

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Abstract. Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by immune system imbalance, particularly involving the overactivation of type I interferon (IFN-1) and NF-κB pathways. TRIM21, an E3 ubiquitin ligase, plays a crucial role in downregulating these pathways by targeting specific regulatory proteins for degradation via the proteasome. This study utilized an in-silico approach to assess the effects of three-point mutations—C16A, C31A, and H33A—within the essential RING finger domain of TRIM21. Protein stability analysis using I-Mutant 2.0 revealed that the C16A and C31A mutations reduced protein stability (negative ΔG) and increased binding affinity (low Kd), while the H33A mutation showed a slight increase in stability (positive ΔG) but decreased binding affinity (higher Kd). Structural visualization demonstrated that these mutations disrupted the integrity of the RING domain. These changes are predicted to impair TRIM21's ability to regulate immune signalling, potentially resulting in uncontrolled IFN-1 and NF-κB activation—key features in SLE development. The study highlights TRIM21's vital function in immune regulation and suggests its potential as a therapeutic target in autoimmune diseases.

1 Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by immune system dysfunction, leading to the immune system attacking the body's own tissues and organs. This dysregulation results in inflammation that can affect various organ systems, including the skin, joints, kidneys, brain, heart, lungs, and blood vessels, ultimately causing organ damage [1].

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SLE exhibits varying degrees of severity, ranging from mild to life-threatening conditions. The disease onset typically occurs between the ages of 20 and 40. However, SLE can also manifest in children aged 0-18 years, with paediatric cases accounting for approximately 10% of total SLE cases [2,3].

The global prevalence of paediatric SLE varies widely and is influenced by demographic factors, including female gender, adolescence, non-Caucasian race, and urban residency, which are associated with a higher risk. The prevalence of paediatric SLE is reported to range from 1.9 to 25.7 per 100,000 children, with an incidence of 0.3 to 0.9 per 100,000 per year. The disease is rare in children under five years old but increases significantly during the second decade of life [4].

In the pathogenesis of SLE, type I interferon (IFN-1) and nuclear factor-κB (NF-κB) are closely interrelated and play a critical role in exacerbating inflammation and organ damage. IFN-I is a potent cytokine that acts as a primary driver of autoimmunity and inflammation in SLE. This pathway undergoes chronic activation, often referred to as an "IFN signature," triggered by the recognition of nucleic acids from damaged cells or immune complexes by intracellular innate receptors. This excessive IFN-I activation leads to the over-maturation of dendritic cells, robust B cell stimulation resulting in massive autoantibody production, and the activation of T cells. This entire process creates a sustained pro-inflammatory environment and triggers a self-damaging immune response, significantly contributing to the widespread inflammation and organ damage observed in SLE patients [5,6].

On the other hand, NF-κB pathway is another central regulator of inflammation and immune responses that is highly active in SLE. NF-κB is a family of proteins that function as transcription factors responsible for regulating the expression of genes, including those coding for proinflammatory cytokines such as TNF-α, IL-6, and IL-1β, as well as other immune modulators involved in cellular and molecular processes in the pathogenesis of SLE. This uncontrolled NF-κB activation not only drives increased production of pro-inflammatory cytokines, chemokines that recruit immune cells, and adhesion molecules facilitating their migration into inflamed tissues, but also leads to the activation of autoreactive T cells, subsequently triggering B cell activation, massive autoantibody production, and the formation of autoimmune complexes [7,8]. Crucially, these two pathways—IFN-I and NF-κB—mutually reinforce each other in a synergistic cycle that accelerates chronic inflammation and tissue damage. This intricate interplay establishes a pervasive state of systemic inflammation, a hallmark of SLE pathogenesis.

The exact cause of SLE remains unknown; however, it is believed to involve a combination of genetic, environmental, and hormonal factors that interact to produce diverse clinical manifestations. Over the past decades, numerous studies have been conducted to elucidate the molecular mechanisms underlying SLE, focusing on immune dysregulation, genetic predisposition, and inflammatory pathways. Despite this progress, the genetic factors contributing to the development and progression of SLE remain incompletely understood [9]. One gene that has recently garnered attention for its role in the immune system is TRIM21 (tripartite motif-containing 21), also known as Ro52 [10]. TRIM21 is a 52 kDa E3 ubiquitin ligase protein located on the short arm of chromosome 11 at region 11p15.5. This gene plays a crucial role in both physiological immune responses and pathological autoimmune processes [11]. The most common autoimmune disease associated with TRIM21 is SLE [12]. A study by Cifuentes et al., using an in-silico approach to identify genes contributing to autoimmune processes, revealed that TRIM21 plays a significant role and contributes substantially to the SLE disease cluster [13].

Physiologically, TRIM21 is vital in regulating the immune system by suppressing the production of IFN-1 through the ubiquitination of interferon regulatory factors (IRFs). In SLE, an increased expression pattern of the TRIM21 gene is observed via the interferon pathway. This elevated gene expression disrupts IRF degradation through the protease

pathway, resulting in increased Type I IFN production. Elevated IFN levels enhance B cell activation to produce autoantibodies such as anti-dsDNA and anti-SSA/Ro, which contribute to immune complex formation and subsequent tissue damage. Additionally, Type I IFN promotes the proliferation of T helper 1 (Th1) and 17 (Th17) cells while inhibiting regulatory T cell (Treg) proliferation, thereby exacerbating inflammation and loss of immune control [10,11].

Moreover, TRIM21 influences NF-κB activation by modifying inhibitory kappa B kinase beta (IKKβ) through monoubiquitination. This process is essential for maintaining the balance of NF-κB activation, ensuring a well-regulated immune response. When TRIM21 function is disrupted, its stability or activity is compromised, leading to impaired regulation of IKKβ. Consequently, NF-κB activation becomes dysregulated, resulting in excessive release of proinflammatory cytokines such as TNF-α, IL-6, and IL-1β [14]. These cytokines contribute to chronic inflammation and exacerbate tissue damage in autoimmune conditions such as SLE. However, the specific molecular mechanisms governing TRIM21 expression in SLE pathogenesis remain poorly understood.

This study aimed to investigate mutations in the TRIM21 gene related to SLE pathogenesis using an in-silico approach. The analysis focuses on the impact of mutations on TRIM21 protein stability, utilizing Gibbs free energy (ΔG) predictions to evaluate how these mutations affect protein function. The findings of this study are expected to provide new insights into the molecular mechanisms of SLE involving TRIM21 and open avenues for the development of more targeted therapeutic strategies.

2 Methods

2.1 Data and information sources

TRIM21 protein sequence data was obtained from the UniProt database with access code P19474 (RO52_HUMAN). Information related to mutations and natural variants was analyzed to determine the position of specific mutations and amino acid changes that occurred.

2.2 Evaluation of protein structure stability

Evaluation of changes in protein structure stability due to mutations was carried out using the I-Mutant 2.0 application available at https://folding.biofold.org/i-mutant/i-mutant2.0.html. The analysis parameters were TRIM21 protein sequence data and specific mutation information as input, with pH = 7.4 and temperature 37°C, and the Gibbs free energy (ΔG) with the interpretation that $\Delta G<0$ show there is a decrease in protein structure stability and $\Delta G>0$ show there is an increase in protein structure stability. Place the figure as close as possible after the point where it is first referenced in the text. If there is a large number of figures and tables, it might be necessary to place some before their text citation.

2.3 Protein mutation visualization

Visualization of the three-dimensional (3D) structure of TRIM21 protein with mutations was performed using the PolyPhen-2 application available at http://genetics.bwh.harvard.edu/pph2/. The modified protein structure was visualized to understand the spatial effects of mutations on protein stability and function.

2.4 Data analysis

Gibbs free energy data, dissociation constant (Kd), and 3D visualization were analyzed to evaluate the impact of mutations on the stability and possible functional effects of TRIM21 protein.

3 Results

This study investigated the impact of specific mutations in TRIM21 on its key biophysical parameters: Gibbs free energy (ΔG) and dissociation constant (Kd), under physiological conditions (pH 7.4, 37°C). There were 3 mutations in TRIM21, which are the C→A mutation at positions 16 and 31 causing changes in cysteine residues to alanine and the H→A mutation at position 33 causing changes in histidine residues to alanine. This can be seen in Table 1.

Table 1. Gibbs free energy(ΔG) and in TRIM21 mutase at pH 7.4 and 37°C.

Position		pH	T (°C)	ΔG (kcal/mol)	Kd
16	C→A	7.4	37	-1.18	1.36×10^{-1} M
31	C→A	7.4	37	-1.58	6.93×10^{-2} M
33	H→A	7.4	37	0.12	1.22

Evaluation of protein structure stability using the I-Mutant 2.0 application indicate that cysteine to alanine substitutions at position 16 (C16A) and 31 (C31A) yielded negative ΔG values (-1.18 and -1.58 kcal/mol, respectively) and relatively low Kd values (0.136 M and 0.0693 M). These negative ΔG value indicating a decreased in protein structure stability and the measured interaction or process (e.g., ligand binding or mutase activity) proceeds spontaneously at these conditions. Low Kd value indicates high binding affinity (strong binding), or this means the ligand and receptor "prefer" to remain bound.

While the H33A mutation produced a higher ΔG and Kd value (0.12 and 1.22 respectively) than at position 16 and 31. It indicating an increase in protein structure stability and an endergonic reaction is non-spontaneous, meaning it requires an input of energy to proceed, and weak binding affinity.

Visualization using the PolyPhen-2 application can be seen in Fig. 1. The C16A, C31A and H33A mutations changed the local configuration of the RING-finger domain.

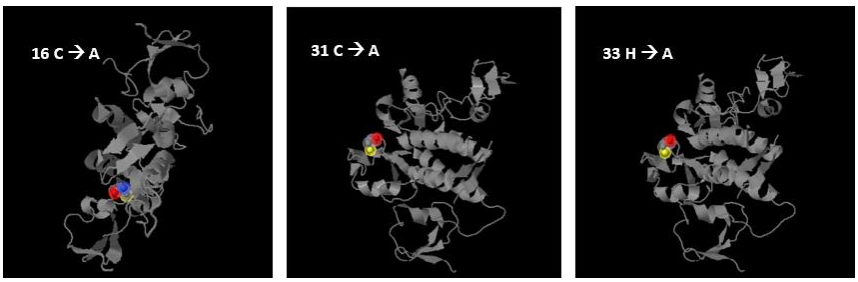

Fig. 1. Visualization of the 3D structure of the mutation in the TRIM21 protein.

4 Disscussion

Systemic lupus erythematosus is a chronic systemic autoimmune disease characterized by excessive immune system activation that targets the body's own cells [1]. The exact cause of SLE remains unclear, but it is believed to be multifactorial, involving interactions between genetic, environmental, and hormonal factors. One key factor is genetic mutations that lead to immune system dysregulation. Among the genetic mutations often associated with SLE is the mutation in the TRIM21 gene, which plays a crucial role in immune regulation and contributes to the pathogenesis of this disease [9,10].

TRIM21 suppress IFN-1 production by regulating interferon regulatory factors (IRFs) through its role as an E3 ubiquitin ligase. The primary mechanism involves the ubiquitination of IRF3 and IRF7, two key transcription factors responsible for inducing IFN-1. TRIM21 catalyses the attachment of K48-linked ubiquitin chains to both IRF3 and IRF7. This K48-linked ubiquitination serves as a proteasomal degradation signal, meaning the ubiquitinated IRF proteins are targeted and destroyed by the cellular proteasome. Consequently, TRIM21 reduces the amount of active IRF3 and IRF7 within the cell, thereby limiting the cell's ability to trigger IFN-1 expression, ultimately suppressing the production of IFN-β and IFN-α. This negative feedback mechanism is crucial for preventing excessive and damaging IFN-1 responses, which can contribute to the pathogenesis of autoimmune diseases such as SLE [14,15].

Additionally, TRIM21 suppress the NF-κB pathway by regulating the IκB kinase (IKK) complex. One proposed mechanism is that TRIM21 can mediate the K48-linked ubiquitination of IKK components themselves, such as IKKβ, which then targets them for proteasomal degradation. The degradation of IKKβ would inhibit its ability to phosphorylate IκB (the NF-κB inhibitor), a protein that typically binds to NF-κB in the cytoplasm and prevents its nuclear translocation. Without IκB phosphorylation by IKKβ, IκB remains undegraded, and NF-κB stays trapped in the cytoplasm, thereby preventing the transcriptional activation of pro-inflammatory and immunogenic NF-κB target genes. Thus, by reducing the availability or activity of IKKβ and indirectly suppressing NF-κB activation, TRIM21 can act as a negative regulator, which in the context of SLE pathogenesis, can reduce chronic inflammation and autoimmunity by inhibiting the expression of pro-inflammatory genes, cytokines, chemokines, and adhesion molecules that contribute to tissue damage and excessive immune cell activation [16].

This study aimed to explore the specific molecular mechanisms associated with mutations in the TRIM21 gene, known for its role in SLE pathogenesis, using an in-silico approach. This method investigates mutations that potentially affect TRIM21's activity as an E3 ubiquitin ligase, evaluates conformational changes in the protein caused by these mutations, and assesses disruptions in critical domains, such as the RING finger domain.

The evaluation of TRIM21 gene mutations through in silico analysis revealed the occurrence of mutagenesis at specific amino acid residues: cysteine (C) at positions 16 and 31, and histidine (H) at position 33, all of which are substituted with alanine (A) within the TRIM21 protein structure. The cysteine-to-alanine substitutions disrupt the RING finger domain of TRIM21, which is essential for its activity as an E3 ubiquitin ligase responsible for ubiquitination. Cysteine residues within this domain are critical for zinc ion (Zn^{2+}) coordination, necessary to maintain the "cross-braced" structure of the RING domain [17,18]. Similarly, histidine residues also play a role in coordinating zinc ions alongside cysteine residues. The histidine-to-alanine substitution further contributes to structural

instability within this vital domain [19]. These mutations lead to domain instability and the loss of ubiquitin ligase activity, impairing the regulation of type I IFN and NF-κB, both of which are pivotal in SLE pathogenesis [12]. This finding aligns with a study by Wada et al., which highlighted that TRIM21 ubiquitination activity depends on the RING finger domain, with mutations like C16A disrupting its ability to regulate type I IFN and NF-κB [18].

Mutations in proteins can significantly influence the spontaneity of biological processes (ΔG) and have broad implications for cellular function. These alterations frequently modify protein structure, stability, and interaction capabilities, consequently affecting the bioenergetics of reactions involving the mutated protein. Key impacts include changes in protein stability, where mutations can render proteins more susceptible to denaturation or misfolding, often leading to a loss of inherent function. Furthermore, mutations can alter binding affinity (Kd) for ligands or substrates; while a decrease in Kd (indicating stronger affinity) can lead to uncontrolled signalling pathway activation or abnormal binding, an increase in Kd (indicating weaker affinity) disrupts essential interactions. Mutations can also affect enzymatic catalytic activity by altering the activation energy, even if the reaction remains thermodynamically spontaneous. Lastly, mutations may modify protein localization or regulation, indirectly impacting the spontaneity of downstream processes. Overall, disruptions in protein bioenergetics due as a result of mutations can disturb cellular homeostasis, cause the accumulation of harmful products, inhibit essential functions, or trigger abnormal responses, all of which can manifest as various diseases [20,21].

The results indicated that the C16A, C31A, and H33A mutations exhibit highly detrimental bioenergetic characteristics. The C16A and C31A mutations yield negative ΔG values, sugesting the spontaneity of certain molecular processes, along with low Kd values that reflect a strong binding affinity. In addition, the H33A mutation shows a positive ΔG value, suggesting that the measured interaction is non-spontaneous, and a Kd that reflects a weak binding affinity. These data collectively indicate that all three mutations, particularly the C31A mutation which has the lowest ΔG value (indicating a most spontaneous initial process) but the highest Kd (reflecting the strongest binding affinity), drastically impair TRIM21's ability to form stable and functional complexes with its targets.

The functional impact of this severely impaired binding affinity has significant implications for TRIM21's regulatory role in the immune system. As an E3 ubiquitin ligase and immune sensor, TRIM21 is essential in suppressing signalling pathways such as NF-κB and type I interferon (IFN type I) production through the ubiquitination of key proteins like IRFs and IKK components. With binding affinity strengthen due to these mutations, the mutated TRIM21 is expected to be incapable of efficient ubiquitination, potentially leading to uncontrolled signalling pathway activation This dysregulation, characterized by increased NF-κB activity and persistent type I IFN production, is a hallmark of the pathogenesis of autoimmune diseases like SLE. Therefore, mutations at these critical sites can directly contribute to the molecular mechanisms behind uncontrolled autoimmune responses [15,22].

Supporting this, studies by Kamiyama et al. revealed impaired proteasomal degradation of IRF3 and IRF5 in SLE peripheral blood mononuclear cells, which contributes to increased IFN-1 synthesis [10]. In SLE patients positive for anti-TRIM21 antibodies, there was a positive correlation between TRIM21 mRNA expression and IFN-1, suggesting that anti-TRIM21 antibodies may interfere with TRIM21-dependent IRF ubiquitination [10]. Study by Santer et al directly demonstrates that TRIM21 also plays a role in the negative regulation of NF-κB. They found that in fibroblasts lacking TRIM21, TLR-mediated NF-κB activation and pro-inflammatory cytokine production (such as IL-1β, IL-6, TNFα) were higher compared to wild-type cells. This indicates that TRIM21 physiologically works to suppress NF-κB activity. Therefore, mutations causing TRIM21 dysfunction or loss of function can lead to uncontrolled NF-κB activation, which is consistent with the pathogenic mechanism in autoimmune diseases like SLE [22].

Several factors are hypothesized to contribute to TRIM21 mutations, including ultraviolet or ionizing radiation, mutagenic agents such as nitrosamines or acridines, viral or pathogenic infections that integrate DNA into the host genome, increased reactive oxygen species causing DNA damage and mutations, hereditary predisposition as part of genetic susceptibility to SLE, or spontaneous mutagenesis [23].

5 **Conclusion**

In conclusion, this studies in silico analysis of TRIM21 gene mutations, specifically within its critical RING finger domain, provides compelling evidence that these genetic alterations significantly impair TRIM21's crucial ubiquitin ligase activity. The observed shifts in Gibbs free energy (ΔG) and dissociation constant (Kd) for the C16A, C31A, and H33A mutations collectively indicate a compromised ability of TRIM21 to perform its physiological functions—namely, the suppression of type I interferon and NF-κB pathways. Such functional disruption leads to severe immune dysregulation, characterized by uncontrolled pro-inflammatory responses and sustained autoantibody production, which are central to the pathogenesis of Systemic Lupus Erythematosus. Understanding these precise molecular mechanisms of TRIM21 dysfunction offers invaluable insights, paving the way for the development of more targeted therapeutic strategies aimed at restoring immune balance in SLE patients.

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