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Immunological and Bioinformatics Meta-Analysis Linking the TLR4-rs4986790 SNP and Bacterial Infections in Patients with Cystitis and Polynephritis

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Abstract:

Toll-like receptor genes, especially TLR-4, produce transmembrane receptors that, depending on their expression, induce a pro- or anti-inflammatory response. TLRs are the first line of the body's defense against pathogens, including infectious ones. The most prevalent kind of mutation occurs in the human genome: single nucleotide polymorphisms (SNPs), which can contribute to functional changes in genes. The main goal of the current investigation is to examine how the TLR4 SNP rs4986790 is associated with infectious diseases, either susceptibility or protection. A meta-analysis of the studied SNP's dominant genotypic model for both analyses of the general population and its subgroup of the kind of infectious agent among random effects.

A total of 30 controls and 30 cases were included in the meta-analysis. It has been shown that this polymorphism is linked to a significant number of infectious disorders, either in terms of severity or susceptibility to them. Additionally, the receptor created is crucial for the development of cell immunologic signaling pathways and the immune response against pathogens. According to the current meta-analysis, infections with a greater risk are significantly associated with the TLR4-rs4986790 SNP.

Keywords: Toll-like receptor genes TLR-4, single nucleotide polymorphisms (SNPs), immunologic signaling.

Introduction:

One of the risk factors for TLRs is genetic variation, including SNPs that can alter the balance between the production of both pro- and anti-inflammatory cytokines, modulating the risk of chronic inflammation and other infections [1].

Single nucleotide polymorphisms have been recognized in the TLR gene based on databases from NCBI SNP, in which the change allele is related to reduced transcription activity of *the TLR gene* and may be susceptible to diseases associated with the *TLR* gene compared to the T allele [2].

Toll-like receptors are receptors of innate immunity that have an essential role in response to pathogenic and non-pathogenic microorganisms and immune surveillance, due to their ability to recognize pattern recognition molecules such as DAMPs and PAMPs that enable them to send signals when exposed to internal or external danger so they act as innate immune sensors, to drive acquired immunity through these signals, which in turn activates NF-κB, IRF3 and induces genes that produce inflammatory cytokines, furthermore during the past years, various studies have shown that polymorphisms of TLRs work to alter the immune response susceptibility are linked to sensitivity to cancer in addition to inflammatory and infectious diseases [3].

The family of TLRs is an important group of host proteins that helps induce an innate immune response against microbes. To date, 11 types of TLRs have been studied in humans, all of which share structural properties such as the intracellular domain that

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mediates signaling cascades that include a complex series of intermediates leading to activation of NF-κB; this domain consists of Toll/Interleukin_1 receptor domain (IL_1), while the extracellular domain contains leucine-rich repeats that are responsible for interaction between TLRs and ligands [4].

The direct involvement of TLRs signaling has been shown by some studies to have a beneficial role in the tumor cells of some types of cancer such as (TLR4, TLR5)involve in T reg activation, (TLR4) as chemoresistance, TLR3 and TLR4 demonstrated in the induction of proliferation, and (TLR2, TLR9) as proangiogenic, Other studies showed that tumor cells show their ability to induce the expression of TLRs compared to normal cells, as the risk factor for some types of cancer may be genetic variations known as polymorphisms of TLRs due to the role of these genetic changes in modulating the risk of infection, pro and anti-inflammatory cytokine, cancer and chronic inflammation [5].

Innate immunity is believed to function as an immune system sentinel, activating rapidly in immune response to a multitude of microbial pathogens. Pathogen signature molecules are recognized by a number of pattern-recognition receptors in innate immune cells. Pathogen-associated molecular patterns (PAMPs), which include many groups of PRRs such as Toll-like receptors (TLRs), are considered important components for the pathogen's survival [6].

The TLRs recognize pathogen-associated molecular patterns originating from different pathogens and have a significant role in the innate immune response. Over the past ten years, a variety of techniques, such as molecular, metabolic, structural, cell biology, and bioinformatics research, have clarified the precise processes underpinning TLR signaling in all ways. In several facets of the pathogen's innate immune response, TLR activation is often diverse and vigorous [7].

The TLRs were the first to be discovered, and they remain the most well-studied. The TLR family has ten members in humans (TLR1–10) and twelve in mice (TLR1–9, TLR11–13). TLRs can be found in internal compartments such as the ER, endosome, lysosome, or endolysosome, or on the exterior of the cell. They identify different or similar PAMPs in all different forms [8].

Depending on where they are found, TLRs are categorized as either intracellular or cell surface TLRs. Many classes of them (TLR1, 2, 4, 5, 6, and 10) are cell-surface types, while TLR3, 7, 8, and 9 are intracellular TLRs exclusive to endosomes. TLRs were expressed by both non-immune cells, including fibroblasts and epithelial cells, and innate immune cells like dendritic cells (DCs) and macrophages [9].

TLRs recognize several microbial components: TLR2 recognizes phospholipomannans and B-glucans, while CD14 and TLR4 recognize glucuronoxylomannans. The most studied family of pattern recognition receptors (PRRs) is the Toll-like receptors (TLRs). TLRs serve a crucial role in the first line of body defense against infections that frequently promote and induce adaptive immune responses by binding to these patterns and triggering a range of pro-inflammatory and antimicrobial reactions. Changes in susceptibility to infection are linked to single nucleotide polymorphisms (SNPs) in the different human TLR proteins [10].

By integrating and analyzing multi-omics data, bioinformatics holds great promise for understanding complex biological systems in the twenty-first century, recognized as a science in the scientific field. In order to facilitate the analysis, connecting, and manipulation of various biological data types, it employs information technology (IT) data types in order to comprehend novel biological insights, and researchers are always trying to understand how the biological system works [11].

The amount of data generated from each experiment indicates that efforts are at a point of unparalleled development and expansion. These huge datasets from the tests are transformed into useful knowledge using bioinformatics [12].

To put it another way, bioinformatics is a method of managing and manipulating data for the scientific and practical domains of applied molecular biology, biochemistry, agriculture, environmental science, and the medical field. It addresses drug discovery, system modeling, structural and functional annotations of genes and proteins, and data mining and processing [13].

Recently, protein sequences are used to build phylogenetic trees for the study of evolutionary links and to predict the structure and function of newly studied proteins. To close the gap that exists between the whole system, organism, biochemical, gene, and protein levels, studies are conducted at several levels. All of these fields have contributed to the accumulation of vast amounts of biological knowledge because of previously unheard-of research endeavors to support experimental research. Combining biology and computing technology, bioinformatics aims to develop novel techniques for in-depth biological system research [14].

Materials and methods:

Immunologic measures involved five milliliters of venous blood drawn from a patient with gram-negative bacteria with *TLR-4 (rs4986790)* Polymorphism in uropathogenic *E. coli*, patients with Cystitis and Polynephritis Infections, and placed in a gel tube. After clotting, the serum was separated and stored at -20 degrees Celsius until immunological analyzes were completed. In accordance with the manufacturer's instructions, TLR4 and radial immunodiffusion plate (LTA-Italy) values in the serum of both patient and control groups.

The ELISA method, which is based on Hoffman's double antibody sandwich method, was used to detect TLR4, IgE, and serum soluble CD14 concentration, which was measured as a marker for cell-mediated immunity in accordance with the manufacturer's instructions (Elabscience Biotechnology Co., Ltd.) The results were expressed as international units per milliliter (iu/ml).

The In Silico Study. In this investigation, numerous web servers were used for sequence retrieval; DNA nucleotide translation; protein sequence analysis, including the computation of physicochemical attributes; and protein structure prediction [8]. The National Center for Biotechnology Information (NCBI) website uses a tool for in silico inspection. TLRS Gene to Amino Acids via a Translation Tool: DNA nucleotide translation to amino acids using Augustus server version 4.0 [15].

Determine the kind of amino acids in the protein structure using the Physicochemical Properties Prediction Protein Structure Prediction Server program [16]. To determine the physicochemical properties of the TLR protein for the human sequence obtained from NCBI, use the online tool ProtParam (protein parameters). ProtParam calculates the following parameters: M.W., theoretical pI, atomic composition, amino acid composition, instability index, aliphatic index, and grand average of hydropathicity (GRAVY) [17].

The secondary structure was predicted using SOPMA (version 3.0) for Prediction of Protein Structure, a self-optimized prediction approach with alignment [18], and the hydropathy plot for TLR4 was calculated using the TMpred program. [19]. The protein sequence's structural features, including B-sheets, helices, and coils, are presented when the sequence is given in FASTA format. Finding an approximate structure that shows homology with the target, selecting the best template with the highest degree of identity with the target sequence once it has been aligned with the target, and modeling the structure utilizing these four basic steps comprise the modeling process. A service called SWISS-MODEL models the structural homology of proteins entirely autonomously. The Swiss-Model automated system uses homology modeling techniques to model a protein's quaternary structure based on its amino acid sequence [20].

Interaction between Proteins: Protein-protein interactions and function have been predicted using the STRING (Search Tool for the Retrieval of

Interacting Genes/Proteins) software version 12.0 when the amino acid sequence was supplied in the FASTA format [21,22].

This reaction, simulation, docking, and analysis of next-generation sequencing (NGS) data are examples of bioinformatics techniques that can be used to study the function of these genes or proteins at the system level and to examine or alter the sequence to better fit essential genes for a given function. This particular genetic, genomic, and proteomic data might then be used to find medications and other uses [23].

The SNPs can help determine an individual's reaction to certain substances like environmental pollutants, customizing a therapeutic response profile and supporting patient care [24].

Result:

Members of the family that contain Toll-like receptors (TLRs) are proteins encoded by these genes, which are essential for pathogen identification and innate immune activation. Human TLRs are structurally and functionally identical, and they are highly conserved. They mediate the triggering of cytokines required for the establishment of efficient immunity and identify pathogen-associated molecular patterns displayed on infectious pathogens. Different expression patterns are displayed by the different TLRs. Signal transduction events triggered by lipopolysaccharide, which is present in the majority of Gram-negative bacteria, have also been linked to a particularly strong binding of surface TLR4 to the receptor, according to in silico studies. Mutations that happen in this gene have been linked to vulnerability to age-related macular degeneration and variations in LPS response. For this gene, many transcript variants encoding distinct isoforms have been discovered in a patient with gram-negative bacteria with *TLR-4 (rs4986790)* Polymorphism in uropathogenic *E. coli* Patients with Cystitis and Polynephritis Infections

Secondary structure prediction of TLR4 protein with and without SNP:

The secondary structure of the protein was predicted computationally using the SOPMA program. The protein primary sequence for the SOPMA analysis was obtained using the software. The coils, helices, and strands of bone morphogenetic protein-15 were described in the prior program. The secondary structure of the proteins depicted in Figure 1 was predicted using SOPMA analysis

Table 1: Serological Estimation of TLR4 *(rs4986790)* concentration in patients associated with cystitis and polynephritis in gram-negative bacterial infection and control.

Groups N= 30	TLR4 concentration Mean ±SD	P value
Cystitis with poly nephritis in gram-negative bacterial infection	0.9509±0.5145	0.00538*
Cystitis with gram-negative bacterial infection	1.1303±0.6711	0.00005*
Healthy Control	0.5223±0.2055	

* The result is highly significant at $p < .05$.

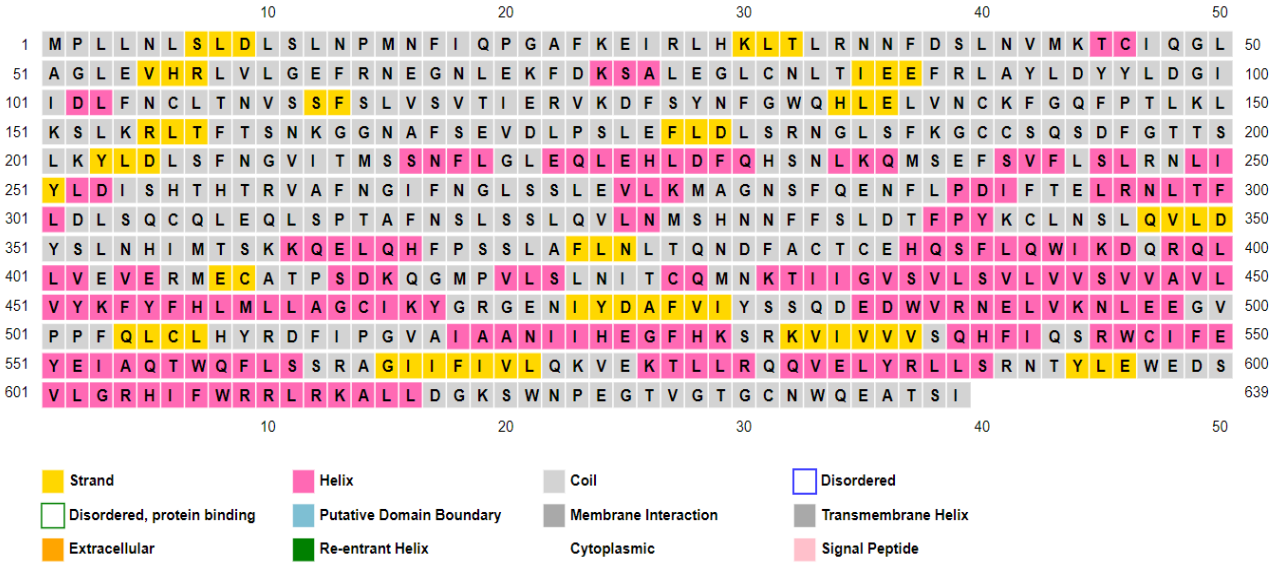

Figure (1): The secondary structure features (alpha helixs, extended strands, and random coils) of TLR 4 protein with SNP: rs 4986790, via PSIPRED.

Table (2): The parameters of the secondary structure of TLR4 protein.

Parameters	Without: rs 4986790	With: rs 4986790
Alpha helix(%)	26.9	31.6
Coil(%)	50.6	49.5
Beta strand(%)	22.5	18.9

Table (3): The composition of amino acids of TLR4 protein.

Parameters (%)	Without: rs 4986790	With: rs 4986790
Polar amino acids	34.1%	33.9%
Non-polar amino acids	24.1%	24.3%
Aromatic plus cysteine amino acids	13.6%	13.6 %
Hydrophobic amino acids	28.2%	28.2%

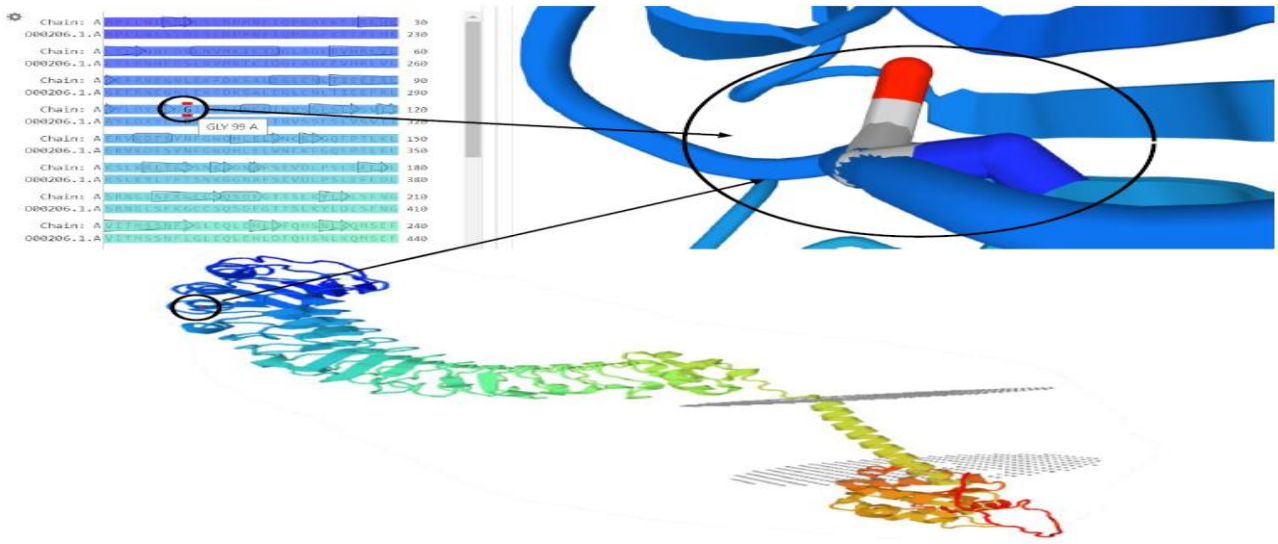

Figure (2): The three-dimensional structure prediction for the quaternary structure of the TLR4 protein, without SNP rs4986790, and the position of the Asp (D) amino acid at position 99 of the TLR4 protein, by the SWISS-MODEL tool.

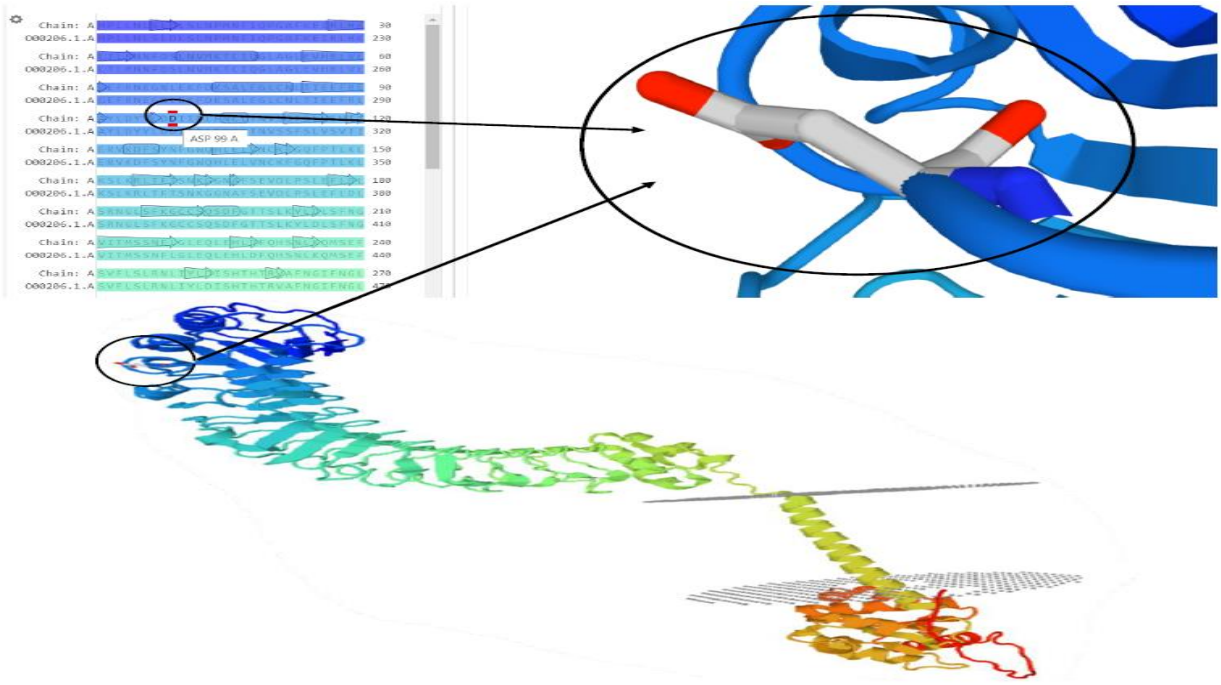

Figure 3: The three-dimensional structure prediction for the quarterly structure of the TLR4 protein with SNP rs4986790, and the position of the Gly (G) amino acid at position 99 of the TLR4 Protein, by the SWISS-MODEL tool.

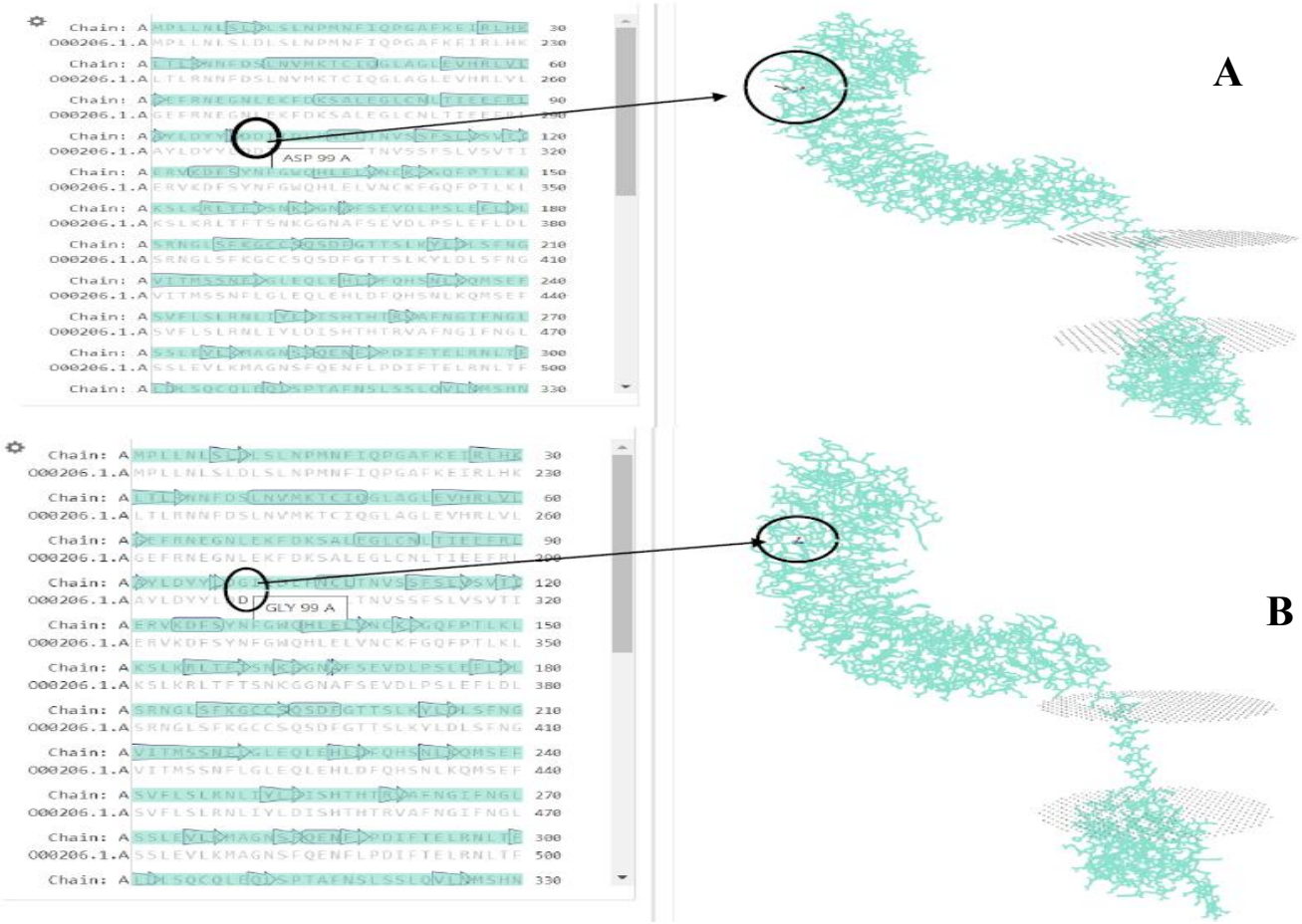

Figure 4: The linear structure prediction of the quarterly structure of the TLR4 protein **(A)**without SNP: rs4986790, determines the Asp(D) amino acid at 99 position and (B) withSNP: rs 4986790 determine the position of Gly (G) instead of Asp(D) amino acid at 99 position of TLR4 Protein, by SWISS model tool.

The helical structure spanned 81 residues, or 20.6% of the protein, according to the secondary structure study. However, the random coil structure spanned 250 residues, or 63.78% of the protein. In contrast, 61 residues (15.5%) of the protein were added to the strand structure. According to this study, random coils have the highest structure in bone morphological protein-15, helical structure comes second, and strand structure has the lowest.

Physicochemical Properties of TLR4:

The results of the current study using the amino acid sequence to predict physicochemical properties with ProtParam are shown in Table 4.

Table (4): Computed physicochemical properties of TLR4 protein with and without rs4986790.

Parameter	Result of TLR4 protein without rs 4986790	Result of TLR4 Protein with rs 4986790
Number of amino acids	639	639
Molecular weight	73301.36	73243.32
Theoretical pI	6.04	6.12
Total number of negatively charged residues (Asp + Glu)	65	64
Total number of positively charged residues (Arg + Lys)	56	56
Total number of atoms	10316	10310
Extinction coefficients	75830	74830
half-life	30 hours	30 hours
instability index (II)	41.56	41.43
Aliphatic index	100.63	100.63
Grand average of hydropathicity (GRAVY):	0.009	0.014

By using the ProtParam tool to analyze physico-chemical factors, protein structural stability can be predicted. The strength of the protein structure was evaluated using an instability index value. A protein is considered stable if the index value is less than 40 and unstable if the index value is greater than 40. The current results demonstrate that bone morphogenetic protein-15 has a MW of 45055.01 daltons and a 392-sequence length. An extinction value of 55390/280 nm indicates that the TLR4 protein is capable of absorbing light at that WL. The extinction coefficient was used to calculate a protein's concentration in the solution. The pH at which a protein surface is charged but its net charge remains zero is known as the isoelectric point (PI). The BMP15 protein demonstrated a basic PI.

Discussion:

The primary correlation between the major TLR4 ligand, which is represented by LPS, and the main link found in the present study in a multi-gene model for susceptibility to SNPs and infectious diseases demonstrates the impact of the allele(G) in numerous higher investigations for Gram-negative bacteria. The primary ligand and accurate expression of TLR4 are highlighted, demonstrating its ability to identify the molecular patterns associated with pathogens (PAMPs) of Gram-negative bacteria [25]. There are several steps involved in how LPS activates TLR4. The first stage concentrates on the binding of protein to LPS, which extracts it from bacterial membranes and vesicles containing it and then transports it to the cluster of TLR4 (CD-14). CD-14 comes in two forms: soluble and membrane-bound. Both of them

can interact with LPS-loaded LBP. After CD-14 breaks down LPS aggregates, monomer LPS is transferred to a hydrophobic pocket in (MD-II), a part of the MD-II/Toll-R4 complex. TLR4 activation results in the production and secretion of pro-inflammatory soluble mediators, which recruit intracellular adaptor proteins, mainly MyD88 and/or (IL1R)-containing interferon-beta [26]. A particular cell receptor for microorganisms (bacteria, fungi, viruses, and microbes) is called TLR4. It can promote endosomes and the cell surface to identify the linker, initiate the generation of cytokines in the natural immune response, and support the specific immunological responṣe [27]. It is a crucial mediator in the detection of mannuronic acid polymers and LPS, both of which are present in Gram-negative bacteria. The systemic inflammatory response that can result in sepsis, septic shock, and organ failure is largely triggered by LPS in patients with Gram-negative bacteria with *TLR-4 (rs4986790)* Polymorphism in Patients with Cystitis and Polynephritis Infections [28].

Additionally, lipoteichoic acid (LTA) from Gram-positive bacteria, mannans/fungal pathogens, and mycobacteria like Mycobacterium tuberculosis are recognized by TLR-type 4 [29]. The location and physico-chemical characteristics of LTA in Gram-positive bacteria are comparable to those of LPS in Gram-negative bacteria. Oxidized phospholipids have the ability to activate TLR4 as an immune response. As a result, it triggers the innate immune response and can recognize specific molecular DAMPs produced by lysed or dead cells following viral infection or host tissue damage [30]. The TLR4 human gene, which has two introns and three exons, is located on chromosome 9q33.1 (Vaure and Liu, 2014). SNP/rs-4986790, commonly known also as 896A-G, is mutation type non-synonymous in this gene that causes a guanine (G) to replace an adenine (A) at position 896A>G in the TLR4 gene's third exon. This substitution changes the conserved aspartic acid residue to a gly. in amino acid 299 of the protein sequence (Asp299Gly) in, on, or at the TLR4 extracellular structure domain. It is the cause of the encoded protein's altered or reduced function, or missense [31]. The G allele frequency of this SNP varies between 0 - 20% among groups [33], and it has already been linked to protection against and susceptibility to a number of disorders yields from infectious and non as well as the severity degree of some of them [32]. The position of SNP rs4986790 in the TLR4 gene and protein in a didactic model is shown in Fig. 1. In bacterial infections, SNP rs4986790 in the TLR4 gene. The primary correlation between the major TLR4 ligand (LPS) and the many offer and probability for genetic susceptibility to SNP and infectious diseases discovered in this research shows the impact of the G (Fig. 4). Begg's funnel plot for the meta-analysis of the 4986790 SNP of the TLR4 gene. Genetic Frontiers frontiersin.org 12 Silvaetal [34]. 10.3389/fgene.2022.1045725 allele in different functional investigations for Gram-negative bacteria.

The ability of TLR4 to identify Gram-negative bacterial PAMPs is highlighted by the accurate expression of bio- TLR4 and its primary ligand (LPS) in a patient with Gram-negative bacterial infection with *TLR-4 (rs4986790)* Polymorphism in Patients with Cystitis and Polynephritis Infections [35].

TLR4 activation by LPS is a multi-phase, complex process. The first phase focuses on TLR4 differentiation coreceptors-14 (CD-14), vesicles carrying LPS, and its binding protein (LBP). CD-14 comes in two varieties/forms: membrane-bound and soluble. Both of them can react with LPS-loaded LBP. CD-14 breaks down LPS aggregates and transfers LPS (monomer) to a hydrophobic pocket in MD-II as a component of the MD-II/TLR4 complex. Intracellular proteins (adaptors), mainly myeloid differentiation response 88 and/or (TIR-1)-containing interferon-beta (TRIF), are activated by TLR4 and recruited, which results in the synthesis and release of pro-inflammatory mediators [36].

Because the basic buffers have a soluble isoelectric point of 9.28, they can be utilized in buffer systems for protein purification [19]. The relative volume that a protein's aliphatic side chains—such as leucine, isoleucine, valine, and alanine—occupy is known as its aliphatic index. It was found to be 88.80, which could indicate thermostability. The hydrophobic nature of the protein was shown by the Grand Average Hydropathy (GRAVY) values. The TLR4's GRAVY score of -0.345 represented an insoluble characteristic [20]. Protein sequences with negative values could indicate that the protein is stable. Amino acids that are hydrophobic may be involved in the recognition and binding of ligands.

For genotypic comparison, odds ratios (ORs) and matching confidence intervals (95% CIs) were computed. Funnel plots were examined for signs of publication bias, and X^2 statistics were computed to evaluate the existence of heterogeneity between studies. Bacterial infections (29.6%), viral infections (22.2%), and urinary tract infections (UTIs) (7.4%) were the disease categories most closely linked to the SNP under investigation. A total of 5871 controls and 5599 cases were included in this meta-analysis (Sekar PKC, 2024) [37].

Conclusion:

The TLR4 rs4986790 polymorphism and susceptibility to bacterial infection were noted to be strongly correlated in this meta-analysis. To confirm and reinforce these results, more research including a variety of demographics is required. TLR4 serves as a particular cell receptor for bacterial infection, whereas it breaks down LPS aggregates and transmits monomer LPS in a patient with gram-negative bacteria with *TLR-4 (rs4986790)* Polymorphism in Patients with Cystitis and Polynephritis Infections.

Conflict of interest: NIL

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