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# Frequency of Meningococcal Meningitis Susceptibility Associated TLR4 +896 A/G (rs4986790) Allele in the Saudi Population

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## Abstract

**Meningococcal meningitis (MM) is a severe central nervous system (CNS) infection that occurs primarily in children. MM can damage brain areas associated with hearing, learning, reasoning, focus, and memory. Genetic changes, including single nucleotide polymorphisms (SNPs), which compromise pathogen recognition increase the risk and severity of MM. There is little data on how the variation in the frequency of the rs4986790 polymorphism in the Toll-like receptor 4 (TLR4) gene may affect the population of Saudi Arabia. This study sought to determine the allelic frequency and distribution of the TLR4 rs4986790 A/G polymorphism in the Saudi population and compare the data to other global populations. Data from epidemiological studies conducted in various ethnic groups were extracted using PUBMED (Medline) and similar web databases. An estimated 5.88% of the Saudi population harbors the TLR4 rs4986790 G variant allele. This differed significantly from the frequencies in populations in China (p=0.0002), Japan (p=0.0001), Korea (p=0.0001), and Mexico (p=0.01). The TLR4 rs4986790 polymorphism variant allele has a unique pattern in the Saudi population, which may be the result of racial differences. These findings could assist in the risk assessment of people harboring the TLR4 +896 GG genotype susceptible to MM in the Saudi population.**

**Keywords:** Meningococcal Meningitis, Toll-like Receptor 4, rs4986790 Allele, Saudi Population, Single Nucleotide Polymorphism (SNP)

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## INTRODUCTION

Genetic epidemiological studies have shown that genetic variations in human groups influence susceptibility to infections. There are several obstacles to overcome to identify the relevant genes and translate these results into biological mechanistic explanations.[1,2] Meningococcal Meningitis (MM), a severe infection of the central nervous system (CNS) that affects hearing and learning capacities, frequently occurs in childhood.[3-5] The main objective of the immune response is to neutralize the pathogen by recognizing microbial ligands and then induce the release of certain cytokines. However, these cytokine reactions may also incidentally harm healthy brain tissue, which would be detrimental. [6,7]

Mutations in pathogen recognizing receptors (PRRs) including Toll-like receptors (TLRs) and nucleotide oligomerization domain like receptors (NLRs) in macrophages and epithelial cells critically modulate the inflammatory response.[8] These receptors are also expressed by neuro-epithelial cells, resident macrophages in the CNS, and microglia. Thus, any mutation of these receptors significantly increases risk and severity of MM.

Early reports showed that single nucleotide polymorphisms (SNPs) located in genes responsible for the development of innate immunity increase meningococcal, pneumococcal, and meningitis susceptibility.[9-11] A severity analysis linked SNPs located in TLR2, TLR4, and TLR9 with deafness in MM patients.[12] MM usually begins with Neisseria meningitidis and Streptococcus pneumoniae growth in the nasopharynx and epithelium, progressing to bacteremia in the blood circulation. Bacteria may eventually cross the blood–brain barrier and proliferate in the subarachnoid area.[13]

Microglia, astrocytes, and non-neuronal structures near the cerebrospinal fluid (CSF), including dendritic cells and macrophages, detect the presence of bacteria in the CNS and activate the immune response. PRR activation causes the production of inflammatory cytokines and chemokines, which are also present in the CNS.[8] Brain edema, infarction, increased intracranial pressure, and neuronal damage result from the local inflammatory response within the brain, which is exacerbated by cytokine-induced increased blood–brain barrier permeability and entry of inflammatory cells into the CNS 13. To clear these microbes, the host must be able to recognize microbial CNS invasion in order to clear the infection. However, the ensuing inflammatory response produces few cytotoxic mediators that affect healthy bystander neurons, ultimately resulting in poor prognosis. [13,14]

Immune cells recognize gram-positive and gram-negative bacteria with the participation of TLR2 and TLR4 surface receptors. Animal studies have established that a lack of TLR2 and TLR4 reduces the ability of the CNS to remove germs after an infection with S. pneumoniae.[15]

Although the rs4986790 SNP is located in a critical genomic region for MM susceptibility, its prevalence and impact in Saudi Arabia populations is unclear. The present study sought to determine the frequency of genetic variation in TLR4 +896 A/G (rs4986790) that is associated with an increased risk of MM. The frequency distribution of the TLR4 rs4986790 polymorphism among healthy Saudi Arabians was compared with data from multiple epidemiological studies conducted worldwide.

## MATERIALS AND METHODS

### Search criteria of gene variants

The PUBMED (Medline), Web of Science, and EGEMS databases were searched using the keywords "TLR4," "rs4986790," and "polymorphism". Studies on human subjects written in any language were included in the search. Studies reporting genotype frequencies for the control population were included. Studies that reported only allele frequencies and no genotype frequencies were excluded. For every study that met the requirements, the first author's name, year of publication, subjects' country, number of controls, research type, inclusion/exclusion criteria, and subjects' allele and genotype frequencies were all abstracted. The most recent publication data were used for the Saudi population. The prevalence of the TLR4 rs4986790 polymorphism was extracted from 48 studies and included in the current analysis and compared to the Saudi population (Table 1). [16]

**Table 1.** Studies included in the TLR4 +896 A/G (rs4986790) gene variant analysis in different populations

| No. | Study | Year | Ethnicity | Reference |
|---|---|---|---|---|
| 1 | Semlali | 2019 | Arab | 16 |
| 2 | Martinez-Rios | 2013 | Mexican | 17 |
| 3 | Ameziane | 2003 | Caucasian | 18 |
| 4 | O'Halloran | 2006 | Caucasian | 19 |
| 5 | Edfeldt | 2004 | Caucasian | 20 |
| 6 | Zee | 2005 | Caucasian | 21 |
| 7 | Koch | 2006 | Caucasian | 22 |
| 8 | Dzumhur | 2012 | Caucasian | 23 |
| 9 | Nebel | 2007 | Caucasian | 24 |
| 10 | Balistreri | 2004 | Caucasian | 25 |
| 11 | Morange | 2004 | Caucasian | 26 |
| 12 | Golovkin | 2014 | Caucasian | 27 |
| 13 | Guven | 2015 | Turks | 28 |
| 14 | Van well | 2013 | Caucasian | 29 |
| 15 | Sargın | 2017 | European | 30 |
| 16 | Machado | 2016 | Mixed | 31 |
| 17 | Qin | 2009 | Asian (China) | 32 |
| 18 | Na | 2008 | Asian (Korea) | 33 |
| 19 | Burton | 2007 | European | 34 |
| 20 | Snelgrove | 2007 | European | 35 |
| 21 | Adam | 2006 | European | 36 |
| 22 | Gergely | 2006 | European | 37 |
| 23 | van der | 2005 | European | 38 |
| 24 | van Well | 2013 | European | 29 |
| 25 | Ahmad-Nejad | 2011 | Caucasian | 39 |
| 26 | Nakada | 2005 | Asian (Japan) | 40 |
| 27 | Agnese | 2002 | Multi-ethnic | 41 |
| 28 | Bronkhorst | 2013 | Caucasian | 42 |
| 29 | Carregaro | 2010 | Multi-ethnic | 43 |
| 30 | Elkilany Atia | 2015 | Caucasian | 44 |
| 31 | Everett | 2007 | Undefined | 45 |
| 32 | Feterowski | 2003 | Caucasian | 46 |
| 33 | Guarner-Argente | 2010 | Undefined | 47 |
| 34 | Henckaerts | 2009 | Caucasian | 48 |
| 35 | Horcajada | 2009 | Caucasian | 49 |
| 36 | Kompoti | 2015 | Caucasian | 50 |
| 37 | Kumpf | 2010 | Caucasian | 51 |
| 38 | Lorenz | 2002 | Caucasian | 52 |
| 39 | Mensah | 2009 | Multi-ethnic | 53 |
| 40 | Ozgur | 2009 | Undefined | 54 |
| 41 | Rodriguez-Osorio | 2013 | Mexican-Mestizo | 55 |
| 42 | Read | 2001 | Caucasian | 56 |
| 43 | Sampath | 2013 | Multi-ethnic | 57 |
| 44 | Schnetzke | 2015 | Caucasian | 58 |
| 45 | Shalhub | 2009 | Caucasian | 59 |
| 46 | Tellería-Orriols | 2014 | Caucasian | 60 |
| 47 | Van der Graaf | 2006 | Undefined | 61 |
| 48 | Yoon | 2006 | Asian (Korea) | 62 |
| 49 | Yuan | 2008 | Caucasian | 63 |

**Table 2.** Observed and expected genotypic frequencies of TLR4 +896 A/G (rs4986790) polymorphism in the control group

| Study | Genotype observed (n) | | | Genotype Expected (n) | | | MAF | p-value (HWE) |
|---|---|---|---|---|---|---|---|---|
| | A/A | A/G | G/G | A/A | A/G | G/G | | |
| Semlali et al.[16] | 166 | 20 | 1 | 166 | 21 | 1 | 0.059 | 0.83 |

**Statistical analysis**

SPSS version 21 software was used for the Pearson's χ2 test to match the genotype and allelic frequencies of various populations. The Hardy-Weinberg equilibrium (HWE) was investigated using Court-Lab. A p-value <0.05 denoted statistical significance.

## RESULTS

The minor allele frequency (MAF) of the TLR4 rs4986790 polymorphism in the Saudi population was 5.88%, according to the genotype distribution. The value was in accordance with HWE (Table 2). Different minor allele frequencies were found in the genotypic (A/A, A/G, and G/G) and allelic frequency distributions of the studied polymorphisms in various populations (Table 3). When the frequency in Saudi Arabia was compared to that of other populations, a substantially different MAF was observed for the ethnicities of populations of China (p=0.0002), Japan (p <0.0001), Korea (p <0.0001), and Mexico (p=0.01).

## DISCUSSION

Many human diseases, including multiple sclerosis, diabetes, asthma, cancer, and birth abnormalities exhibit multifactorial inheritance patterns. A complex interplay between genetic factors, including copy number variation, epistatic interactions, and modifier effects, as well as numerous environmental factors, results in disease onset and progression. It is difficult to predict whether a disease will develop in situations where there is discontinuous trait variation due to the number of factors that may or may not exceed the liability threshold. Common alleles that contribute to the hereditary component of widespread multifactorial disorders can be identified using genome-wide association studies (GWAS). The alleles discovered using this method typically have small impact sizes and cannot fully explain the disease susceptibility.

This gap might emerge as a result of the difficulty in utilizing GWAS to find rare variants with low to medium penetrance. The percentage of people in a group that has a specific allele and displays an associated phenotype signifies penetration. Mendelian diseases, in contrast to multifactorial illnesses, have strong penetrance and a very low allele frequency.

Several techniques have been developed to study complicated illnesses. GWAS have identified the common genetic variables underlying the most severe complex illnesses. However, much remains to be discovered regarding the origins and characteristics of many multifactorial illnesses.

The majority of diseases are multifactorial, and the consequences of an intricate web of hereditary and environmental factors affect how the disease develops over the course of a person's lifetime. A growing body of research suggests that genetic variation makes people more susceptible to conditions such as diabetes, cardiovascular disease, and cancer.[64-66] Therefore, a primary priority in understanding the pathophysiological mechanisms underlying common human illnesses is the detection of genetic variations associated with common complicated diseases. The possible impact of common functional germline polymorphisms on disease risk, development, and prognosis has attracted increasing attention.

Genetic variety refers to the genomic variation present within a population or species.[67] Given the richness of the human genome, genetic variation is recognized as a factor that affects a person's phenotype.[68] Individual gene variation is referred to as genetic diversity and serves as

**Table 3.** TLR4 +896 A/G (rs4986790) gene variant genotype and allele frequency distribution in different populations and p-values in contrast to Saudi Arabian population

| | Study | Year | Ethnicity | Total No. of subjects | Genotype distribution of TLR4 +896 A/G | | | Allele A | Allele G | Total Alleles | G allele frequency | A Allele frequency | p-value | MAF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | AA | AG | GG | | | | | | | |
| 1 | Semlali | 2019 | Arab | 187 | 166 | 20 | 1 | 352 | 22 | 374 | 0.059 | 0.941176 | Ref | 5.88 |
| 2 | Martinez-Rios | 2013 | Mexican | 283 | 267 | 16 | 0 | 550 | 16 | 566 | 0.028 | 0.971731 | 0.01* | 2.83 |
| 3 | Ameziane | 2003 | Caucasian | 216 | 187 | 28 | 1 | 402 | 30 | 432 | 0.069 | 0.930556 | 0.54 | 6.94 |
| 4 | O'Halloran | 2006 | Caucasian | 386 | 343 | 42 | 1 | 728 | 44 | 772 | 0.057 | 0.943005 | 0.88 | 5.70 |
| 5 | Edfeldt | 2004 | Caucasian | 1508 | 1,374 | 133 | 1 | 2881 | 135 | 3016 | 0.045 | 0.955239 | 0.22 | 4.48 |
| 6 | Zee | 2005 | Caucasian | 695 | 605 | 87 | 3 | 1297 | 93 | 1390 | 0.067 | 0.933094 | 0.57 | 6.69 |
| 7 | Koch | 2006 | Caucasian | 1211 | 1,069 | 138 | 4 | 2276 | 146 | 2422 | 0.060 | 0.939719 | 0.92 | 6.03 |
| 8 | Dzumhur | 2012 | Caucasian | 120 | 98 | 22 | 0 | 218 | 22 | 240 | 0.092 | 0.908333 | 0.12 | 9.17 |
| 9 | Nebel | 2007 | Caucasian | 323 | 293 | 30 | 0 | 616 | 30 | 646 | 0.046 | 0.95356 | 0.38 | 4.64 |
| 10 | Balistreri | 2004 | Caucasian | 182 | 155 | 23 | 4 | 333 | 31 | 364 | 0.085 | 0.914835 | 0.16 | 8.52 |
| 11 | Morange | 2004 | Caucasian | 490 | 439 | 50 | 1 | 928 | 52 | 980 | 0.053 | 0.946939 | 0.68 | 5.31 |
| 12 | Golovkin | 2014 | Caucasian | 300 | 253 | 46 | 1 | 552 | 48 | 600 | 0.080 | 0.92 | 0.21 | 8.00 |
| 13 | Guven | 2015 | Turks | 150 | 134 | 14 | 2 | 282 | 18 | 300 | 0.060 | 0.94 | 1 | 6.00 |
| 14 | Van well | 2013 | Caucasian | 1141 | 1001 | 136 | 4 | 2138 | 144 | 2282 | 0.063 | 0.936897 | 0.75 | 6.31 |
| 15 | Sargın | 2017 | European | 41 | 41 | 0 | 0 | 82 | 0 | 82 | 0.000 | 1 | not calculated | 0.00 |
| 16 | Machado | 2016 | Mixed | 200 | 178 | 22 | 0 | 378 | 22 | 400 | 0.055 | 0.945 | 0.82 | 5.50 |
| 17 | Qin | 2009 | Asian (China) | 112 | 112 | 0 | 0 | 224 | 0 | 224 | 0.000 | 1 | 0.0002* | 0.00 |
| 18 | Na | 2008 | Asian (Korea) | 197 | 197 | 0 | 0 | 394 | 0 | 394 | 0.000 | 1 | <.0001* | 0.00 |
| 19 | Burton | 2007 | European | 1465 | 1,335 | 123 | 7 | 2793 | 137 | 2930 | 0.047 | 0.953242 | 0.30 | 4.68 |
| 20 | Snelgrove | 2007 | European | 98 | 93 | 5 | 0 | 191 | 5 | 196 | 0.026 | 0.97449 | 0.07 | 2.55 |
| 21 | Adam | 2006 | European | 125 | 107 | 17 | 1 | 231 | 19 | 250 | 0.076 | 0.924 | 0.39 | 7.60 |
| 22 | Gergely | 2006 | European | 140 | 127 | 12 | 1 | 266 | 14 | 280 | 0.050 | 0.95 | 0.62 | 5.00 |
| 23 | van der | 2005 | European | 170 | 153 | 16 | 1 | 322 | 18 | 340 | 0.053 | 0.947059 | 0.72 | 5.29 |
| 24 | van Well | 2013 | European | 1141 | 1001 | 136 | 4 | 2138 | 144 | 2282 | 0.063 | 0.936897 | 0.75 | 6.31 |
| 25 | Ahmad-Nejad | 2011 | Caucasian | 112 | 99 | 12 | 1 | 210 | 14 | 224 | 0.063 | 0.9375 | 0.86 | 6.25 |
| 26 | Nakada | 2005 | Asian (Japan) | 214 | 214 | 0 | 0 | 428 | 0 | 428 | 0.000 | 1 | <.0001* | 0.00 |
| 27 | Agnese | 2002 | Multi-ethnic | 39 | 34 | 5 | 0 | 73 | 5 | 78 | 0.064 | 0.935897 | not calculated | 6.41 |
| 28 | Bronkhorst | 2013 | Caucasian | 139 | 118 | 20 | 1 | 256 | 22 | 278 | 0.079 | 0.920863 | 0.30 | 7.91 |
| 29 | Carregaro | 2010 | Multi-ethnic | 205 | 178 | 26 | 1 | 382 | 28 | 410 | 0.068 | 0.931707 | 0.59 | 6.83 |
| 30 | Elkilany Atia | 2015 | Caucasian | 21 | 19 | 2 | 0 | 40 | 2 | 42 | 0.048 | 0.952381 | not calculated | 4.76 |
| 31 | Everett | 2007 | Undefined | 167 | 145 | 22 | 0 | 312 | 22 | 334 | 0.066 | 0.934132 | 0.69 | 6.59 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 32 | Feterowski | 2003 | Caucasian | 154 | 135 | 19 | 0 | 289 | 19 | 308 | 0.062 | 0.938312 | 0.88 | 6.17 |
| 33 | Guarner-Argente | 2010 | Undefined | 105 | 97 | 8 | 0 | 202 | 8 | 210 | 0.038 | 0.961905 | 0.27 | 3.81 |
| 34 | Henckaerts- | 2009 | Caucasian | 293 | 264 | 27 | 2 | 555 | 31 | 586 | 0.053 | 0.947099 | 0.69 | 5.29 |
| 35 | Horcajada | 2009 | Caucasian | 114 | 100 | 14 | 0 | 214 | 14 | 228 | 0.061 | 0.938596 | 0.88 | 6.14 |
| 36 | Kompoti- | 2015 | Caucasian | 245 | 213 | 30 | 2 | 456 | 34 | 490 | 0.069 | 0.930612 | 0.53 | 6.94 |
| 37 | Kumpf | 2010 | Caucasian | 176 | 150 | 24 | 2 | 324 | 28 | 352 | 0.080 | 0.920455 | 0.27 | 7.95 |
| 38 | Lorenz | 2002 | Caucasian | 73 | 65 | 8 | 0 | 138 | 8 | 146 | 0.055 | 0.945205 | 0.86 | 5.48 |
| 39 | Mensah | 2009 | Multi-ethnic | 48 | 42 | 6 | 0 | 90 | 6 | 96 | 0.063 | 0.9375 | 0.88 | 6.25 |
| 40 | Ozgur | 2009 | Undefined | 70 | 66 | 4 | 0 | 136 | 4 | 140 | 0.029 | 0.971429 | 0.16 | 2.86 |
| 41 | Rodriguez-Osorio | 2013 | Mexican-Mestizo | 126 | 122 | 4 | 0 | 248 | 4 | 252 | 0.016 | 0.984127 | 0.008* | 1.59 |
| 42 | Read | 2001 | Caucasian | 879 | 787 | 81 | 11 | 1655 | 103 | 1758 | 0.059 | 0.941411 | 1 | 5.86 |
| 43 | Sampath | 2013 | Multi-ethnic | 318 | 287 | 31 | 0 | 605 | 31 | 636 | 0.049 | 0.951258 | 0.48 | 4.87 |
| 44 | Schnetzke | 2015 | Caucasian | 81 | 76 | 5 | 0 | 157 | 5 | 162 | 0.031 | 0.969136 | 0.17 | 3.09 |
| 45 | Shalhub | 2009 | Caucasian | 451 | 400 | 50 | 1 | 850 | 52 | 902 | 0.058 | 0.94235 | 0.92 | 5.76 |
| 46 | Tellería-Orriols | 2014 | Caucasian | 66 | 60 | 4 | 2 | 124 | 8 | 132 | 0.061 | 0.939394 | 0.92 | 6.06 |
| 47 | Van der Graaf | 2006 | Undefined | 166 | 148 | 17 | 1 | 313 | 19 | 332 | 0.057 | 0.942771 | 0.92 | 5.72 |
| 48 | Yoon | 2006 | Asian (Korea) | 179 | 179 | 0 | 0 | 358 | 0 | 358 | 0.000 | 1 | <.0001* | 0.00 |
| 49 | Yuan | 2008 | Caucasian | 409 | 364 | 44 | 1 | 772 | 46 | 818 | 0.056 | 0.943765 | 0.86 | 5.62 |

a mechanism for population survival by enabling adaptation to a dynamic environment. The key to understanding the biology of human diseases has long been thought to be genetic heterogeneity within and between populations.[69-71]

TLRs are central to the activation of the innate immune system and its response to CNS infections. [72] Early studies have linked SNPs located in TLR4 with meningitis, tuberculosis, malaria, and lupus risk.[73] TLR2 and TLR4 activation leads to variable gene expression through nuclear factor-kappa B (NF-κB) regulated transcription.[74] Toll/interleukin 1-domain-containing adapter inducing interferon-beta (TRIF) also contributes to TLR signaling. When TLR4 is activated, MyD88 and TRIF are recruited. When TLR2 is activated, only MyD88 is recruited. Due to variations in the timing of NF-κB activation, MyD88 and TRIF are believed to coordinate distinct intracellular pathways.[74] TLR2 and TLR4 activation also leads to the production of pro-inflammatory TNF-α in murine macrophages.[75,76] Previous genetic studies have shown a strong association between TLR4 and Crohn's disease in the pediatric population.[77]

Experimental studies have shown that TLR4+896 SNP is associated with a reduced response to lipopolysaccharide (LPS) in mice and humans.[78,79] Compared to healthy volunteers, adult surgical intensive care unit patients have a higher risk of developing gram-negative infections owing to the same TLR4 SNP 41. TLR4 +896 has also been associated with mortality, greater need for respiratory assistance, use of inotropic agents, skin grafting, and limb loss in a pediatric population with meningococcal infections.[80] Decreased pro-inflammatory intracellular signaling and impaired TLR4-mediated LPS responses are probable mechanisms.

Identifying genetic variations that predispose individuals to the development of MM is important because it helps to clarify the specifics of MM pathogenesis. Additionally, this knowledge makes it possible to forecast a person's risk of developing MM and may help in identifying people at the highest risk of developing serious complications from their condition and needing specialized care. Furthermore, the outcome can be useful in the identification and immunization of individuals with the highest MM risk. Another

possibility is to supplement existing prediction models for difficulties in hearing, memory, or behavior after MM with genetic risk factors.[81-83]

Global human genome variation is a product of numerous evolutionary processes, including population separation, mixing, migration, selective pressure, and genetic drift.[84-86] Footprints conserved throughout the genomes of multiple groups provide evidence to support our understanding of health and disease.[87,88] The Human Genome Diversity Project has recently made significant contributions to the development of a single nucleotide alteration database by identifying genetic differences between and within individuals of various ethnic groups worldwide.[89-91] The likely heterogeneous genetic diversity of the Saudi population could be investigated to help develop early preventative and intervention techniques. This study compared the frequency distribution of the TLR4 +896 A/G polymorphism variant in the Saudi population with that of other populations worldwide.

TLR4 detects bacterial LPS on the surface of gram-negative bacteria. Previous research has revealed a connection between TLR4 and bacterial-related phenotypes such as Crohn's disease, ascites, scrub typhus, and tuberculosis.[92,93] Similarly, the rs4986790 SNP located in TLR4 has been used to assess variable manifestations of disease.[94,95] These results suggest that the rs4986790 SNP of the TLR4 gene modulates the antibacterial actions of TLR4 because genetic changes result in functional alterations.[96,97]

The present study involving the Saudi population revealed a 5.88% frequency of variant allele (G) of rs4986790. This frequency is substantially different from China, Japan, Korea, and Mexico. Differences in allele frequencies among separate datasets can affect the ultimate SNP effect because most SNPs are less penetrant, and diseases are polygenic in nature. A change in MAF of 0.02 will result in significant statistical changes in genetic association studies. Any change, even as small as <0.1, in a particular allelic prevalence will significantly influence the individual effect of one SNP in the case of interaction between two SNPs.[98]

Variations in allelic frequencies in genetic association studies can be attributed to racial variance, demographic heterogeneity, and varying sample sizes. The TLR4 gene exhibits a wide range of patterns compared to other people worldwide.[99] The varying incidence of these SNPs in various populations shows that different groups are differently affected by susceptibility factors. It is important to note that the genotype and allele frequencies examined in this analysis may not accurately represent all possible variants at a location. However, such investigations can inform the subsequent creation of epidemiological and clinical databases. Large data repositories have been created over the past ten years as a result of GWAS and genetic association studies.[100] Multiple genetic association tests are required to identify important genes and/or their SNPs involved in the development of early disease prevention programs and treatments. However, before novel genetic biomarkers for application in gene-disease-association research can be identified, a number of bottlenecks must be solved. These include statistical and computational trials as well as the repeatability factor.[101]

## CONCLUSION

The TLR4 rs4986790 polymorphism variant allele in the Saudi population differs significantly from that of many other populations worldwide. These findings may help with population screening and evaluation of the relevance and propensity of MM. The evaluation of diseases may be aided by variations in the frequency distribution of important MM-related genes in healthy Saudi populations and other racial groups. Better management of the affected pediatric cohort in the Saudi population may result from the identification of susceptibility factors linked to individual susceptibility and predisposition to increased frequencies of support for artificial breathing, use of inotropic agents, skin grafting, and limb loss. To utilize this polymorphism as a biomarker, future large-scale research investigating gene-gene and gene-environment interactions is necessary.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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