PHARMACOGENETIC AND MOLECULAR DOCKING ANALYSIS OF HLA-A, -B, AND -DRB1 MARKERS IN ANTIEPILEPTIC DRUG-INDUCED SEVERE CUTANEOUS ADVERSE REACTIONS IN THE IRAQI POPULATION

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by

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LIST OF ABBREVIATIONS

ADRs	Adverse Drug Reactions
AEDs	Antiepileptic Drugs
AGEP	Acute Generalised Exanthematous Pustulosis
CBZ	Carbamazepine
DRESS	Drug Reaction with Eosinophilia and Systemic Symptoms
HLA	Human Leukocyte Antigens
LTG	Lamotrigine
MPE	Maculopapular exanthema
PHT	Phenytoin
SCARs	Severe cutaneous adverse Reactions
SJS	Stevens-Johnson syndrome
TEN	Toxic Epidermal Necrolysis
USM	Universiti Sains Malaysia
GBFDE	Generalised Bullous Fixed Drug Eruptions
SNPs	Single-Nucleotide Polymorphisms
CLA	Cutaneous Lymphocyte Antigen
SFasL	soluble FasL
CTL	cytotoxic T lymphocytes
HHV	Human Herpes Virus
EBV	Epstein-Barr virus
HIV	Human Immunodeficiency Virus
PBMC	Peripheral blood mononuclear cell
PDB	Protein Data Bank
LTT	Lymphocyte Transformation Test
MHC	Major Histocompatibility Complex
β2m	β2-microglobulin

ER	Endoplasmic Reticulum
NK	Natural Killer
PCR	Polymerase Chain Reaction
PCR-SSO	Polymerase Chain Reaction with Sequence-Specific Oligonucleotide
EDTA	Ethylenediaminetetraacetic acid
OR	Odds Ratios
gDNA	genomic DNA
F	frequency
CI	Confidence Interval
NGS	Next-Generation Sequencing
CPIC	Clinical Pharmacogenetics Implementation Consortium
n	Number
RT	Room temperature
SD	Standard deviation
UV	Ultraviolet device
WHO	World health organisation
ALA	Alanine
ARG	Arginine
GLN	Glutamine
GLU	Glutamic acid
GLY	Glycine
ILE	Isoleucine
LEU	Leucine
LYS	Lysine
MET	Methionine

PRO	Proline
SER	Serine
THR	Threonine
TRP	Tryptophan
TYR	Tyrosine

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ANALISIS FARMAKOGENETIK DAN PENGEDOKAN MOLEKUL UNTUK PENANDA HLA-A, -B, DAN DRB1 BAGI REAKSI ADVERS KULIT TERUK TERARUH UBAT ANTIEPILEPTIK DALAM POPULASI IRAQ

ABSTRAK

Reaksi Kulit Teruk (Severe Cutaneous Adverse Reactions, SCARs) yang diaruh ubat antiepileptik (AED) adalah keadaan mengancam nyawa yang dimediasi oleh sistem imun dan menimbulkan cabaran klinikal yang ketara. Farmakogenetik, khususnya kajian tentang antigen leukosit manusia (*HLA*), memainkan peranan penting dalam meramalkan kerentanan terhadap SCARs dan membimbing strategi rawatan jitu. Kajian ini memberi tumpuan kepada pengenalpastian alel HLA yang dikaitkan dengan SCARs aruhan AED dalam populasi Iraq, khususnya yang berkaitan dengan carbamazepine (CBZ), phenytoin (PHT), dan lamotrigine (LTG). Kajian ini bertujuan untuk menentukan frekuensi alel bagi variasi gen *HLA-A, HLA-B,* dan *HLA-DRB1* dalam kalangan individu sihat, meneliti ciri klinikal dan jenis SCARs, menilai hubungan antara genotip *HLA* dan kerentanan terhadap SCARs dengan membandingkan kes yang terkesan dengan pesakit yang terjejas dengan kumpulan kawalan, , serta meneroka asas mekanisme tindak balas ini melalui pendokaan molekul alel *HLA* yang terlibat. Kajian ini melibatkan 300 dewasa sihat dan 50 pesakit yang didiagnosis dengan SCARs, bersama 90 individu kawalan yang toleran. Peserta diambil dari Hospital Dr. Saad Al-Wattari untuk Sains Neurologi dan Hospital Baghdad - Medical City. Pengenotipan *HLA-A, -B,* dan *-DRB1* dijalankan menggunakan kaedah reaksi rantai polimerase oligonukleotida khusus jujukan (PCR-

SSO). Analisis frekuensi alel HLA dalam kalangan sukarelawan sihat menunjukkan penemuan utama terhadap kepelbagaian genetik dalam populasi Iraq. Antara alel kelas I *HLA, HLA-A*02:01* adalah yang paling lazim (25%), diikuti oleh *HLA-A*03:01* (12.83%) dan *HLA-A*01:01* (9.5%). Bagi *HLA-B, B*35:01* diperhatikan dalam 10.16%, *B*50:01* dalam 8.15% dan B*50:02 dalam 6% dari populasi kajian. Untuk alel *HLA* kelas II, *HLA-DRB*103:01* mempunyai frekuensi alel sebanyak 17.16%, *HLA-DRB*107:01* sebanyak 16.5%, dan *HLA-DRB1*13:01* sebanyak 12.33%. Keputusan ini menonjolkan keutamaan alel ini dalam susunan genetik populasi Iraq. Dalam kajian ini, *HLA-B*15:02* dikenal pasti sebagai faktor risiko genetik yang signifikan untuk kedua-dua sindrom Stevens-Johnson (SJS) dan nekrolisis epidermal toksik (TEN). Selain itu, *HLA-DRB1*03:01* didapati mempunyai kaitan yang kuat secara khusus dengan TEN. Bagi SJS, *HLA-A*24:02* dan *HLA-DRB1*13:01* muncul sebagai alel berisiko yang utama. Dalam kes DRESS, *HLA-B*40:02* menunjukkan risiko tertinggi dalam kalangan alel yang dianalisis. Analisis menunjukkan bahawa SJS adalah jenis SCAR yang paling lazim dilihat dalam kumpulan kes (54%) ,dengan CBZ sebagai ejen pencetus paling biasa. PHT menyebabkan 34% daripada kes SJS, manakala LTG dikaitkan dengan 12%. Kehadiran alel *HLA* tertentu seperti *HLA-A*01:02, HLA-A*02:01, HLA-A*30:02, HLA-B*15:02, HLA-B*51:02, HLA-DRB1*15:01, HLA-DRB1*03:01*, dan *HLA-DRB1*13:01* menekankan kepentingan genotipgenotaip *HLA* dalam meramalkan kerentanan terhadap SCARs dan menyesuaikan rawatan antiepileptik untuk mengurangkan risiko reaksi teruk yang mengancam nyawa ini. Hasil pendokaan molekul menunjukkan afiniti pengikatan yang berbeza antara pelbagai AED dan alel *HLA* tertentu yang dikaitkan dengan SCARs. Interaksi ini mencadangkan satu mekanisme iaitu pengikatan ubat dalam alur penyampaian antigen molekul *HLA*

tertentu boleh mengubah persembahan peptida, yang berpotensi mencetuskan tindak balas sel T yang abnormal dan mengakibatkan reaksi kulit teruk. *HLA-B*15:02* menunjukkan pengikatan paling kuat dengan PHT (-8.8 kcal/mol), manakala *HLA-A*01:02* menunjukkan afiniti yang lebih rendah terhadap ketiga-tiga ubat (CBZ, PHT, dan LTG) dengan nilai antara -6.9 hingga -8 kcal/mol. *HLA-DRB1*03:01 dan HLA-DRB1*13:01* menunjukkan afiniti pengikatan terkuat dengan CBZ pada -9.1 kcal/mol. Kajian ini menekankan kepentingan pengenotipan *HLA* dalam memahami faktor genetik yang menyumbang kepada SCARs akibat AED dalam populasi Iraq. Pengenalpastian alel *HLA* tertentu yang berkaitan dengan SCARs memberikan pandangan berharga terhadap penanda genetik dalam populasi ini, seterusnya memudahkan terapi jitu dan memperbaiki strategi terapeutik sambil mengurangkan risiko kesan advers yang teruk.

PHARMACOGENETIC AND MOLECULAR DOCKING ANALYSIS OF *HLA-A, -B,* AND *-DRB1* MARKERS IN ANTIEPILEPTIC DRUG-INDUCED SEVERE CUTANEOUS ADVERSE REACTIONS IN THE IRAQI POPULATION

ABSTRACT

Severe Cutaneous Adverse Reactions (SCARs) induced by antiepileptic drugs (AEDs) are life-threatening immune-mediated conditions that present significant clinical challenges. Pharmacogenetics, particularly the study of human leukocyte antigens (*HLA*), plays a crucial role in predicting susceptibility to SCARs and guiding precision treatment strategies. This study focuses on identifying *HLA* alleles associated with AED-induced SCARs in the Iraqi population, particularly those linked to carbamazepine (CBZ), phenytoin (PHT), and lamotrigine (LTG). This study aims to determine the allele frequencies of *HLA-A, HLA-B,* and *HLA-DRB1* gene variations in healthy individuals, examine the clinical characteristics and types of SCARs, evaluate the correlation between *HLA* genotypes and susceptibility to SCARs by comparing affected cases and control group, and explore the mechanistic basis of these reactions through molecular docking of the implicated *HLA* alleles. The study comprised 300 healthy adults and 50 patients diagnosed with SCARs, alongside 90 tolerant controls. Participants were recruited from Dr. Saad Al-Wattari Hospital for Neurological Sciences and Baghdad Hospital - Medical City. *HLA-A, -B, and -DRB1* genotyping was performed using the polymerase chain reaction-

sequence-specific oligonucleotide (PCR-SSO) method. Analysis of *HLA* allele frequencies among healthy volunteers revealed key insights on the genetic diversity of the Iraqi population. Among *HLA* class I alleles, *HLA-A*02:01* was the most prevalent (25%), followed by *HLA-A*03:01* (12.83%), and *HLA-A*01:01* (9.5%), For HLA-B, *B*35:01* was observed in 10.16%, *B*50:01* at 8.15% and *B*50:02* at 6% in the study population. For *HLA* class II alleles, *HLA-DRB1*03:01* had allele frequency of 17.16%, *HLA-DRB1*07:01* at 16.5%, and *HLA-DRB1*13:01* at 12.33%. These results underscore the prominence of these alleles in the genetic makeup of the Iraqi population. In this study, *HLA-B*15:02* was identified as a significant genetic risk factor for both Stevens Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN). Additionally, *HLA-DRB1*03:01* was found to be strongly associated with TEN. For SJS, *HLA-A*24:02* and *HLA-DRB1*13:01* emerged as key risk alleles. In the case of DRESS, *HLA-B*40:02* demonstrated the highest risk among the alleles analysed. The analysis revealed that SJS was the most prevalent form of SCAR observed in the case group (54%), with CBZ being the most common inducing agent. PHT was responsible for 34% of SJS cases, while LTG was associated with 12%. The presence of specific *HLA* alleles, including *HLA-A*01:02, HLA-A*02:01, HLA-A*30:02, HLA-B*15:02, HLA-B*51:02, HLA-DRB1*15:01, HLA-DRB1*03:01,* and *HLA-DRB1*13:01*, highlights the importance of *HLA* genotyping in predicting susceptibility to SCARs and tailoring antiepileptic therapy to reduce the risk of these life-threatening reactions. The Molecular docking poses revealed that these drugs occupy the antigen-binding groove of the *HLA* molecules, overlapping with the peptide-binding region. Such positioning may competitively alter the repertoire of self- and non-self-peptides presented to T cells, potentially triggering aberrant immune activation. This mechanism aligns with the p-

i concept (pharmacological interaction with immune receptors), in which direct, non-covalent binding of a drug to *HLA* can initiate T-cell–mediated hypersensitivity without prior haptenation. Therefore, these structural and energetic findings provide a mechanistic explanation for how specific *HLA*–drug interactions may contribute to the pathogenesis of SCARs in patients. While these findings are most consistent with the p-i concept, they may also align with the altered peptide repertoire hypothesis, which posits that drug binding modifies the spectrum of presented peptides. Other mechanisms, such as altered T-cell receptor recognition or hapten formation, cannot be excluded but were not directly assessed in this study. In this study, *HLA-B*15:02* showed the strongest binding with PHT (–8.8 kcal/mol), which could enhance the likelihood of altered peptide presentation and immune activation. In contrast, *HLA-A*01:02* exhibited relatively lower affinities for all three drugs CBZ, PHT, and LTG, with values ranging from –6.9 to –8.0 kcal/mol, suggesting a weaker interaction potential. Notably, *HLA-DRB1*03:01* and *HLA-DRB1*13:01* displayed the strongest binding affinity for CBZ at –9.1 kcal/mol, further supporting the hypothesis that high-affinity drug–*HLA* interactions may contribute to the pathogenesis of SCARs.This study underscores the importance of *HLA* genotyping in understanding the genetic factors contributing to AED-induced SCARs in the Iraqi population. The identification of specific *HLA* alleles associated with SCARs offers valuable insights into the genetic markers within this population, further facilitating precision therapy and improving therapeutic strategies while reducing the risk of severe ADRs.

CHAPTER 1
INTRODUCTION

1.1 Research Background

Severe cutaneous adverse reactions (SCARs) to medications are a unique and unpredictable a form of delayed hypersensitivity reaction that is independent of the drug dosage (Tempark, John, Rerknimitr, Satapornpong, & Sukasem, 2022). Serious and chronic adverse reactions (CARs) make for approximately 15 to 20% of all negative responses to medication (Thong & Tan, 2011). Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) conditions resulting from the death of keratinocytes leading to the blistering and erosion of the skin and mucous membranes. SJS and TEN, however infrequent, are associated with death rates of 1-10% and 30%, respectively (M. K. Kim, Yoon, Yoon, & Seo, 2021). Drug reaction with eosinophilia and systemic symptoms (DRESS) is another example of a delayed hypersensitivity reaction, affecting various organs throughout the body (Chen et al., 2023). Maculopapular exanthema (MPE) is a comparatively benign cutaneous reaction to medications, characterized by a rash that generally resolves spontaneously upon the cessation of the offending treatment. (M. Mehta, Shah, Khakhkhar, Shah, & Hemavathi, 2014). In contrast, SCARs like SJS and TEN are characterized by significant morbidity and mortality in patients (Calle, Aguirre, Ardila, & Villa, 2023).

The drugs include commonly used antiepileptic drugs (AEDs) like Carbamazepine (CBZ), phenytoin (PHT), and lamotrigine (LTG) are well documented to result in SJS/TEN. CBZ, which is the first-line antiepileptic drug, is most prominently associated with these severe reactions (Rashid et al., 2022). In a population study, the RegiSCAR (Registry of Severe Cutaneous Adverse Reactions) group identified that the frequency of SJS or TEN varies among new users of

antiepileptic drugs, with CBZ presenting a risk of 1.4 cases per 10,000 users, LTG presents a risk of 2.5 cases per 10,000 users, while PHT poses a higher risk, with 8.3 cases Out of every 10,000 users. (Mockenhaupt, Messenheimer, Tennis, & Schlingmann, 2005).

The other recent development of this area is identifying the relation of several Human Leukocyte Antigen *(HLA)* alleles to SCAR syndromes. By this discovery, the relevant genomic susceptibilities predisposing to SCARs have been identified, and prevention can be achieved once they are detected prior to a SCAR. It aligns well with the new concept of precision medicine stating that treatment should also be based on the variations in the genes of the patient. The research deems it crucial to differentiate the specific type of SCARs while attempting to identify the *HLA* alleles associated with this hypersensitive condition (Yang et al., 2021).

CBZ-induced cases of SJS/TEN showed a significant correlation with *HLA-B*15:02* allele in Taiwan (Hsu, Yeh, Lee, & Liu, 2021). This correlation was later evidenced in other Asian populations such as the Thai population. (Tiwattanon et al., 2022), Vietnamese (Nguyen, Sukasem, Nguyen, & Pham, 2023), Han Chinese from Hong Kong (Kwan, Ng, & Lo, 2014), Mainland China (He et al., 2013), and Malays in Malaysia (C. Chang et al., 2017). However, the strength of this correlation was not constant with these populations. Moreover, the association between *HLA-B*15:02* and SCARs has been expanded beyond CBZ to include cases triggered by other AEDs such as PHT and LTG. This expanded association suggests a broader role of *HLA-B*15:02* in mediating susceptibility to these life-threatening reactions. The association between *HLA-B*15:02* and CBZ is particularly strong, with an odds ratio (OR) of 113.4 (95% CI = 51.2–251). This means that individuals carrying the *HLA-B*15:02* allele have over 113 times higher odds of developing CBZ-induced SCARs

compared to individuals without this allele. Such an exceptionally high OR indicates a very strong genetic risk factor, highlighting the importance of *HLA-B*15:02* screening before initiating CBZ therapy in populations where the allele is prevalent. (X. Li et al., 2015; V. Yip, Marson, Jorgensen, Pirmohamed, & Alfirevic, 2012). In contrast, PHT and LTG show weaker associations, with ORs of 5.65 (95% CI: 2.76–11.57) and 4.51 (95% CI: 1.57–12.98), respectively, according to a meta-analysis study. This means that individuals carrying the *HLA-B*15:02* allele have approximately 5.6 times higher odds of developing PHT-induced SCARs and about 4.5 times higher odds for LTG-induced SCARs compared to non-carriers. Although these associations are weaker than that observed with CBZ, they still indicate a clinically relevant genetic risk, supporting the need for consideration of genetic screening before initiating these drugs in at-risk populations. (X. Li et al., 2015). Han Chinese and Thai individuals have shown associations between *HLA-B*15:02* and SJS/TEN concerning PHT and LTG. However, there is insufficient evidence to conclusively establish that these linkages hold for all other ethnic groups (Tham, Yek, & Liu, 2024).

The link between *HLA-B*15:02* and CBZ-induced SJS/TEN is influenced by clinical phenotype, ethnicity, and drug specificity. Clinical phenotype refers to the observable traits of SCARs, like rash severity, organ involvement, and symptom patterns, which help distinguish between conditions like SJS, TEN, and DRESS. For example, *HLA-B*15:02* is strongly associated with CBZ-induced SJS/TEN, but not necessarily with other phenotypes or drugs, and this association is most pronounced in certain Asian populations. This association was not observed in Japanese, Korean, or European populations. In both Japan and Korea, the *HLA-B*15:11* allele was found to be associated with CBZ-induced SJS/TEN. (Kaniwa et al., 2010; Kim et al.,

2011; Ozeki et al., 2011). *HLA-B*15:11* and *HLA-B*15:02* are classified within the B75 serotype category, this fact may indicate a possibility of genetic or structural homology that would explain their similar immunological reaction. This could offer valuable insight into why specific *HLA* alleles are linked to hypersensitivity reactions such as SCARs, emphasizing the significance of understanding serotype subfamilies in predicting adverse drug reactions (Phillips et al., 2018). Recent research has identified a link between the *HLA-A*31:01* allele and various carbamazepine-induced hypersensitivity reactions, including SJS/TEN, DRESS, and MPE, in both Japanese and European populations. (McCormack et al., 2011; Ozeki et al., 2011). However, a later meta-analysis study presented conflicting results, showing that *HLA-A*31:01* exhibited a stronger link with CBZ-DRESS rather than with CBZ-SJS/TEN in Han Chinese and European populations (Genin et al., 2014). Two additional case-control studies have validated the link between *HLA-A*31:01* and hypersensitivity to CBZ in the Japanese (Kashiwagi et al., 2008; Niihara et al., 2012).

Risk variations relevant to our community must be investigated due to many risk alleles in different ethnicities. Although a report has confirmed an association between antiepileptic drugs used and SJS/TEN in Iraqi ethnic groups, no other research has examined the correlation between antiepileptic medications and *HLA* alleles with SJS/TEN (Almaroof & Wahaab, 2021).

Despite extensive research on *HLA* associations with SCARs in various ethnic groups, there remains a critical gap regarding the Iraqi population. The *HLA-B*15:02* allele is highly prevalent in East Asian populations such as Japanese and Chinese and strongly linked to AED-induced SCARs. Other alleles, such as *HLA-B*13:01* among Thai and Han Chinese, *HLA-A*31:01* in Japanese and *HLA-*

*B*15:11* in Koreans, have also been implicated in SCAR susceptibility. In Middle Eastern populations, including Iranians, alleles like *HLA-B*51:01* have been reported in association with SCARs. Although alleles such as *HLA-A*03:01, B35*, and *HLA-DRB1*11* are frequently observed in the Iraqi population from genetic studies, their association with SCARs has not yet been investigated. This gap highlights the need for population-specific research to identify which *HLA* alleles contribute to SCAR susceptibility in Iraqis. Therefore, this study aims to investigate the association of *HLA* alleles with AED-induced SCARs within the Iraqi population to uncover relevant genetic risk factors and enhance patient safety. While specific *HLA* alleles are associated with SCARs, the molecular processes through which these genetic variants elicit immune-mediated responses in AED-induced SCARs are poorly comprehended. The immunological pathways involved and the influence of individual *HLA* alleles on drug-induced immune activation remain ambiguous. To address these gaps, this study employed an Insilco technique to determine the AEDs –*HLA* allele interactions. Therefore, the aim is to understand how these genetic factors modulate immune reactions and participate in the formation of SCARs to improve prognoses and prevention of these critical adverse drug effects. Understanding these associations may inform improved risk evaluation and mitigation in clinical practice for this group and had the potential to support safer drug practices and improved patient outcomes.

1.2 Adverse drug reactions

An adverse drug reaction (ADR) refers to an unwanted and unexpected effect resulting from a pharmaceutical agent given in its recommended proportions for prophylactic, diagnostic, therapeutic or physiological function modification

(Organisation 2004) (Hanafi, Torkamandi et al. 2012). Ensuring drug safety through post-marketing surveillance is crucial for identifying adverse effects that may not appear during clinical trials (Ji, Ying et al. 2011).

ADRs are a major global public health concern and contribute significantly to mortality. Studies report that fatal ADRs account for up to 10% of hospital admissions, with median ADR-related death rates around 1.7–1.8% in both developing and developed countries (Kamtane and Jayawardhani 2012, Al-Worafi 2020). (Chen, Fan et al. 2012). (Angamo, Chalmers et al. 2016).

ADRs are broadly classified into two types: Type A reactions, which are predictable and dose-dependent, and Type B reactions, which are unpredictable, not dose-related, and often immune-mediated (Kaufman 2016). SCARs fall under Type B reactions and are characterized by their idiosyncratic nature and high morbidity.

1.3 Severe cutaneous adverse reactions (SCARs)

SCARs are classified as Type B drug hypersensitivity reactions. These reactions are idiosyncratic, unpredictable, and driven primarily by genetic and immunological factors, in contrast to Type A reactions., which are characterized by their dose dependency and predictability. Type B drug hypersensitivity typically begins as a minor rash, characterised by small raised bumps that spread across the body, but can escalate into more severe and life-threatening conditions like SJS or TEN This rash tends to go away by itself after the drugs causing the reaction are stopped. Pharmacological hypersensitivity may sometimes manifest as a strong pharmacological reaction. These SCARs, also known as severe reactions, provide a considerable threat to life.

SCARs include multiple disease presentations, including SJS/TEN, DRESS, acute generalized exanthematous pustulosis (AGEP), and generalized bullous fixed drug eruptions (GBFDE) (Paulmann and Mockenhaupt 2015). Their rates of morbidity and mortality are markedly increased. Each SCAR, however, demonstrates distinct pathomechanisms, dermatological manifestations, etiological factors, clinical trajectories, and potential treatment modalities. Consequently, it is essential to understand the importance of SCARs and administer appropriate treatment to properly manage these conditions and mitigate their detrimental effects.

1.3.1 Phenotyping and Causality of SCARs

The clinical phenotype of SCARs refers to the observable signs and symptoms, including rash severity, organ involvement, and laboratory abnormalities, which vary between individuals and have important diagnostic implications (Kardaun, Sidoroff et al. 2007, Han, Agusti et al. 2010).

The European Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) has developed standardized clinical criteria to improve the diagnosis, phenotyping, and classification of SCARs in both research and clinical settings (Sassolas, Haddad et al. 2010) .RegiSCAR also collects clinical and biological data, including photographs, skin biopsies, and associated medical history, to support accurate phenotyping of SCARs. In diagnosing conditions such as DRESS, key clinical factors like rash morphology, organ involvement, and the need for hospitalization are considered. Non-genetic factors such as drug exposure, re-exposure, disease etiology, and prior case reports are essential in assessing drug causality (Kardaun, Sidoroff et al. 2007 ; Sassolas, Haddad et al. 2010).

Several tools have been developed to assess drug causality in adverse drug reactions, including the Naranjo algorithm and RUCAM. However, for SCARs particularly SJS/TEN the ALDEN algorithm, developed by the RegiSCAR group, provides a specialized, validated method for evaluating drug causality and is now widely used in clinical and research settings (Bégaud, Evreux et al. 1985, Sassolas, Haddad et al. 2010). Timely diagnosis of SCARs, particularly SJS/TEN, within 7 days of symptom onset is critical for improving survival. Clinical assessment should include rash morphology, symptom onset timing, systemic involvement, and histological confirmation via skin biopsy (Duong, Valeyrie-Allanore et al. 2017).

Research on SCARs has focused on assessing drug causality (e.g., using the ALDEN algorithm), patient outcomes, and epidemiological patterns. Ongoing studies also aim to define SCAR phenotypes more precisely, explore lymphocyte antigen specificity, and identify genetic susceptibility markers such as single-nucleotide polymorphisms (SNPs) (Palmieri, Greenhalgh et al. 2002).Not all individuals exposed to high-risk medications develop SCARs, suggesting a role for genetic predisposition. Advances in pharmacogenetics have facilitated the identification of genetic markers that predict hypersensitivity reactions (Alfirevic and Pirmohamed 2017). Key susceptibility genes include *HLA* alleles, drug transporter genes (e.g., ABCB1, SLCO1B1), drug-metabolizing enzymes (e.g., cytochrome P450), glucose-6-phosphate dehydrogenase (G6PD), and NUDT15 (Hazell and Shakir 2006) .

1.3.2 SCARs Classification

SCARs are classified as Type IV (delayed-type) hypersensitivity reactions according to the Gell and Coombs classification system, mediated by T cells and the

release of specific cytokines from regulatory immune cells (Uzzaman and Cho 2012). SCARs are T cell–mediated delayed-type (Type IV) hypersensitivity reactions. DRESS is associated with Type IV-b (Th2-mediated), while SJS and TEN are primarily Type IV-c reactions mediated by cytotoxic T cells that target drug-modified cells (Harr and French 2010).

1.3.2(a) Stevens-Johnson syndrome/Toxic epidermal necrolysis (SJS/TEN)

1.3.2(a)(i) Clinical Feature of SJS and TEN

SJS and TEN denote varying levels of severity within the same illness continuum. SJS and TEN are categorized based on the percentage of body surface area (BSA) affected by skin detachment. This syndrome, characterized by a skin detachment rate of less than 10%, is referred to as SJS. The most severe manifestations of this syndrome demonstrate skin detachment between 10% and 30%, categorized as SJS/TEN overlap, and skin detachment over 30% is classed as TEN (Roujeau 2013, Schwartz, Padmanabhan et al. 2016, Yamane, Matsukura et al. 2016).

Early symptoms of SJS/TEN include flu-like signs, mucosal discomfort, and skin pain, typically preceding the cutaneous eruptions. These eruptions often begin on the face, upper trunk, and proximal limbs, sparing the distal extremities (Lerch, Mainetti et al. 2018). SJS and TEN start with red or purplish target-like lesions that develop into flaccid blisters, eventually causing widespread skin necrosis and epidermal detachment (Williams, Mudhar et al. 2007). Mucosal membrane involvement is observed in around 85% to 95% of individuals with SJS and TEN. Additionally, SJS and TEN cases can also affect the conjunctivae and mucous membranes, including those of the nose, mouth, oropharynx, anorectal junction, vulvovaginal region, and urethral meatus (Tomy and Li 2008). The next parts will

discuss assessment results to help in SCAR research to identify causative medications and detect clinical trends.

1.3.2(a)(ii) Diagnosis of SJS and TEN

There is no definitive diagnostic test available for SJS and TEN. The diagnosis is contingent upon clinical symptoms, cutaneous signs, and histological testing. During the acute phase a common practice is to conduct histological investigations, including direct a immunofluorescence analysis on cryosections of the skin in order to facilitate a prompt diagnosis. This helps in excluding differential diagnoses of comparable conditions, Conditions such as bullous fixed drug eruption, autoimmune blistering diseases, acute generalized exanthematous pustulosis (AGEP), and staphylococcal scalded skin syndrome, the latter being a rarer cutaneous manifestation in adults, can resemble SJS/TEN. For an SJS/TEN diagnosis, a negative direct immunofluorescence test and confirmation of full-thickness epidermal necrosis are essential. To rule out autoimmune blistering disorders, the negative direct immunofluorescence test ensures that no immunoglobulin or complement deposits are present in either the upper or lower skin layers (Harr and French 2010). Once a diagnosis of SJS/TEN is confirmed, it is crucial to promptly identify and discontinue the medicine that is causing it.

1.3.2(a)(iii) Pathomechanisms of SJS/TEN

The exact pathomechanism of SJS/TEN is not fully understood, but immunological studies have provided important insights into its mechanisms and potential treatment approaches (Ko, Chung et al. 2011, Wei, Chung et al. 2012). Keratinocyte death in SJS/TEN is mainly caused by T cells using three pathways: Fas-Fas ligand, perforin-granzyme B, and and the granulysin-induced pathway. Granulysin is the most harmful because it kills skin cells extensively without needing direct contact. This widespread cell death leads to the skin peeling seen in SJS/TEN. Additionally, blister fluid contains various inflammatory molecules that contribute to the disease (Harr and French 2010, Duong, Valeyrie-Allanore et al. 2017).

Histological studies show that blister fluid in early SJS/TEN contains high levels of cytotoxic CD8+ T-cells expressing CLA, which bind to skin adhesion molecules like E- and P-selectin. These T-cells produce granzyme B, leading to drug-specific damage to keratinocytes. Additionally, elevated soluble FasL (sFasL) in patient serum may also trigger keratinocyte apoptosis(Abe et al., 2003).

Granulysin, found in high levels in the blood and blisters of SJS/TEN patients, plays a key role in keratinocyte apoptosis and epidermal necrosis. Its effects were confirmed in mice and are triggered by drug-specific cytotoxic T lymphocytes, especially after exposure to carbamazepine (CBZ). Granulysin may serve as a prognostic biomarker. The pathogenesis also involves *HLA*-mediated drug presentation to CD8+ T cells, with certain alleles like *HLA-B*15:02* increasing susceptibility by enhancing T-cell activation and granulysin release (Abe et al., 2009; Chung et al., 2008; Koga et al., 2008).

1.3.2(b) Drug reaction with eosinophilia and systemic symptoms (DRESS)

1.3.2(b)(i) Clinical Feature of DRESS

The term DRESS was initially employed in 1996. It is also referred to as drug-induced hypersensitivity syndrome. The identification of DRESS is challenging because of its diverse clinical presentation and involvement of organs, with or without visible skin symptoms (Kardaun, Sekula et al. 2013). In the early stages of DRESS, patients may experience fever, flu-like symptoms, lymph node swelling, itching, and burning pain days to two weeks before a rash appears. Skin signs include erythroderma, purpura, pustules, and facial or limb swelling, caused by tissue infiltration of eosinophils and lymphocytes. The liver is most commonly affected in DRESS syndrome, involved in about 80% of cases, leading to conditions like hepatic cytolysis or, rarely, liver failure. Kidney involvement presents as interstitial nephritis, while around 15% of cases show respiratory issues such as eosinophilic pneumonitis. Cardiovascular involvement may include myocarditis, pericarditis, and abnormal cardiac enzymes (Choudhary, McLeod et al. 2013, Duong, Valeyrie-Allanore et al. 2017).

In rare cases, the prognosis might be intricate when additional visceral organs, such as the muscle, neurological system, and pancreas, are affected. During the acute stage, it is highly recommended to do clinical tests to prevent misdiagnosis. These tests should include screening for blood abnormalities to assess organ involvement, hypereosinophilia, atypical lymphocytes, and viral reactivation (Duong, Valeyrie-Allanore et al. 2017).

1.3.2(b)(ii) Diagnosis of DRESS

Histological features of DRESS include non-specific eczematous lesions, keratinocyte necrosis, and infiltration by eosinophils, neutrophils, and monocytes, along with mild vasculitis. To reduce misdiagnosis, two diagnostic scoring systems

were developed one of which assesses HHV-6 reactivation—allowing confirmation of DRESS even without skin rash and highlighting its multi-organ drug-induced nature. (Kardaun, Sidoroff et al. 2007, Shiohara, Iijima et al. 2007). CD8+ T cells have a significant impact on the development of the DRESS cutaneous manifestation. Skin biopsies obtained from individuals with severe DRESS, exhibited a significant accumulation of CD8+T cells that were specific to the medication and expressed granzyme B. The use of HHV+ peripheral mononuclear cells in skin injury may have enhanced viral replication and transmission in CD4+ T cells (Beutler, Li et al. 2005, O'Meara, Borici-Mazi et al. 2011, Camous, Calbo et al. 2012, Pavlos, Mallal et al. 2012, Descamps, Avenel-Audran et al. 2013, Ogawa, Morito et al. 2013, Ortonne, Valeyrie-Allanore et al. 2015).

One study found that more than 50% of activated CD8+ T cells in cases with DRESS identified as HHV. On the other hand, the CD8+ T cells that penetrated the skin specifically identified as the Epstein-Barr virus (EBV) (Picard, Janela et al. 2010). Compared to SJS/TEN, DRESS has a relatively lower mortality rate ranging from 5% to 10%. The main factors leading to mortality are lesions in the heart, lungs, or myocardium, as well as hemophagocytosis. Autoimmune disorders are the primary long-term effects of DRESS. This excessive immune reaction is believed to be triggered by the continuous reactivation of a viral infection. Administering corticosteroids has been demonstrated to improve autoimmune disease in people with DRESS (Duong, Valeyrie-Allanore et al. 2017).

1.4 Main cause of SCARs

Seventy-five to eighty-five percent of SCARs are ascribed to drug-related factors (Lee, Martanto et al. 2013). Infections have been recognized as the principal

cause in roughly 15% of SJS/TEN patients. The most frequently cited non-pharmacological causes of SJS/TEN are infections with *Mycoplasma pneumoniae* and reactivation of the *Herpes simplex* virus. These cases are primarily encountered in adolescents. Researchers have encountered situations where they could not conclusively ascertain a precise reason. (Forman, Koren et al. 2002, Harr and French 2010).

1.5 Major histocompatibility complex

The major histocompatibility complex (MHC) molecules, also known as human leukocyte antigen (*HLA*) genes in humans, are situated on Chromosome 6. They cover a DNA segment of around 3,600 kilobases and are recognised as one of the most extensively variable areas (Pandey, 2020). Individuals exhibit variation in MHC molecules as a result of polygenicity and polymorphisms. Polygenicity arises due to the separate genetic encoding of class I and II molecules. Polymorphism arises from the presence of a highly polymorphic area on the chromosome housing the MHC genes. This leads to the existence of numerous variations, or alleles, of each gene within populations. This greatly reduces the probability of two unrelated people having an identical combination of class I and II molecules(Abualrous, Sticht, & Freund, 2021).

1.5.1 *HLA* Gene Structure

The MHC molecules are glycoproteins that are expressed on the cell surface and extend across the cell membrane. Class I molecules, commonly referred to as the α or heavy chain, comprise three intracellular immunoglobulin-like Ig-like domains and form a four-domain structure in association with β2-microglobulin (β2m). The genes encoding β2m and invariant chains in humans are located on chromosomes 15

and 5, respectively (Coico, 2021). Class II molecules consist of two chains, namely α and β. These are represented as a structure consisting of four domains. The peptide binding groove is located in the region of the molecule that is furthest away from the membrane. Class I molecules have the capacity to hold peptides that are 8-15 amino acids long, whereas class II molecules can hold longer peptides that are 11-30 amino acids long (Meydan, Otu, & Sezerman, 2013).

The majority of the variations seen across MHC molecules are located inside the peptide binding domain, which enables the binding of diverse peptides. The peptides attach to certain anchor residues and other different places along the groove, establishing between one and six connections with amino acids along the T-cell receptor (Choo, 2007). The peptides exhibit selective binding, however. A single *HLA* molecule may bind many peptides with varying sequences. CD8+ and CD4+ T-cells bind to the invariant regions of the class I and class II molecules respectively (Coico, 2021). Although there are differences in structure, expression and peptide binding, *HLA* class I and class II molecules share some common features Despite variations in structure, expression, and peptide binding, *HLA* class I and class II molecules exhibit several common characteristics. They are both polygenic, comprising of multiple independent genes, and highly polymorphic, they both bind selectively to peptides and are co-ordinately and co-dominantly expressed (Coico, 2021).

MHC molecules may have a variation of up to 20 amino acids among them. Polymorphisms impact the specific amino acids that form the lining of the pockets and the anchor residues inside the peptide binding groove. The residues located inside the peptide binding groove are often known as the sequence motif. These patterns allow us to anticipate the peptides that might potentially interact with each

protein. The identification of these motifs is achieved by profiling peptide sequences obtained from mass spectrometry data of monoallelic cell lines, together with the use of peptide binding affinity prediction tools. Subsequently, the motif data may be used to forecast the binding of peptides to different alleles. Predicting peptide binding of MHC class II molecules is more challenging compared to class I due to the MHC class II peptide binding groove having a more open structure and the peptides it binds being longer, resulting in more flexibility of peptide binding (Janeway Jr, Travers, Walport, & Shlomchik, 2001; van Deutekom & Keşmir, 2015).

The peptide binding groove is a lengthy hydrophobic crevice that is created between the α-helices and β-sheet platform. The size of this cleft is far greater than the inherent binding sites that proteins possess for tiny chemical compounds. The peptide binding groove has six subsites, designated as A-F. The size and stereochemistry of the subsites are dictated by the polymorphic residues located along the cleft (Wieczorek et al., 2017; Z.-H. Zeng et al., 1997). The peptide binding specificity is partly influenced by the interactions between anchor residues on the peptide side chains at two or more of these subsites (Rammensee, Bachmann, & Stevanovic, 2013). Figure 1.1 is a Simplified Diagram of the Position and Organisation of Human Leukocyte Antigen (*HLA*) Genes on Human Chromosome 6.

The *HLA* region in humans encodes more than 200 genes. These *HLA* genes form two distinct classes, class I (*HLA-A, -B, -C, -E, -F and -G*) and class II (*HLA-DRA, -DRB, -DQA1, -DQB1, -DPA1, -DPA2, -DPB1, - DPB2, -DMA, -DMB, -DOA and –DOB*. The most polymorphic *HLA* genes are the *HLA-A, -B, - C, -DRB* and -*DQB1* alleles) (Foroni et al., 2014; Mellins & Stern, 2014; Mosaad, 2015; Naruse et al., 2002).

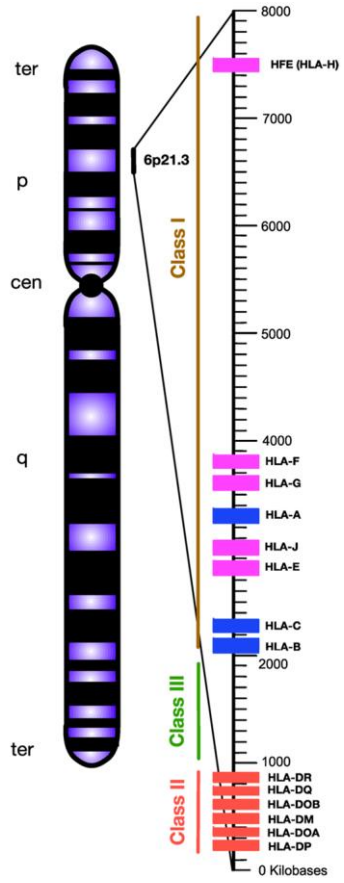

Figure 1.1 Simplified diagram of the position and organisation of human leukocyte antigen (HLA) genes on human chromosome 6. HLA-class I encompasses "classical" HLA-Ia and "non-classical" HLA-Ib loci, which are differentiated by blue and pink, respectively. HLA-class II are encoded by various HLA-II loci labelled by red. Unlike HLA-class I and II, the HLA-class III region does not encode HLAs. Instead, this densely packed region encodes for various inflammatory molecules, complement,

and heat shock proteins. Telomere, ter; p-arm, short arm; q-arm, long arm; Centromere, cen (Crux & Elahi, 2017).

Typically, the products of class I genes are responsible for displaying foreign peptides to cytotoxic T-cells, whereas class II genes are responsible for displaying foreign peptides to T helper cells (Alberts, Johnson et al.). Class I molecules are present on the cell membrane of all cells with a nucleus, but class II molecules are exclusively found on certain immune cells such as B lymphocytes, antigen presentation cells (monocytes, macrophages, and dendritic cells), and activated T-lymphocytes (Choo, 2007). The diversity of the alleles enables for the optimum binding of peptides produced from a wide range of environmental pathogens. The great level of variation seen in *HLA* genes is partly attributable to evolutionary processes.

HLA alleles are allocated a unique identification using a thorough and well-established nomenclature system. The identification always starts with the prefix *HLA*- and is then followed by the gene identifier (*A, B, C* for class I *HLA* genes, or *DRA, DRB, DQA, DQB* for class II *HLA* genes). The gene identification is delimited, followed by a "*" separator and a set of numbers separated into groups.

The first two numbers after the '*' separator give the allele group, originally defined by serotyping, and the next two numbers following ':' are unique for the specific *HLA* protein sequence. Further sets of digits are possible after additional colon separators i) to identify alleles different at the exon (DNA) level but causing no change to the protein sequence (synonymous substitutions), and then ii) for substitutions in introns e.g. '*HLAB*57:01:01:01'*. An optional suffix may also be

included to indicate the expression status. For example: Null alleles, given the suffix 'N', show no expression.

Alternative expression alleles may have the suffix 'L' for denoting low cell surface expression, compared to the normal 'S', meaning they are expressed as a soluble, secreted molecule that is not present on the cell surface, 'C' for alleles producing proteins present in the cytoplasm but not the cell surface, 'A' for showing aberrant expressions, or 'Q' for questionable expressions. For consideration of *HLA* associations with ADRs, a four digit resolution (i.e. resolved to the protein sequence level only) is generally considered sufficient (Marsh et al., 2010). Figure 2.3: show the standardized of *HLA* nomenclature.

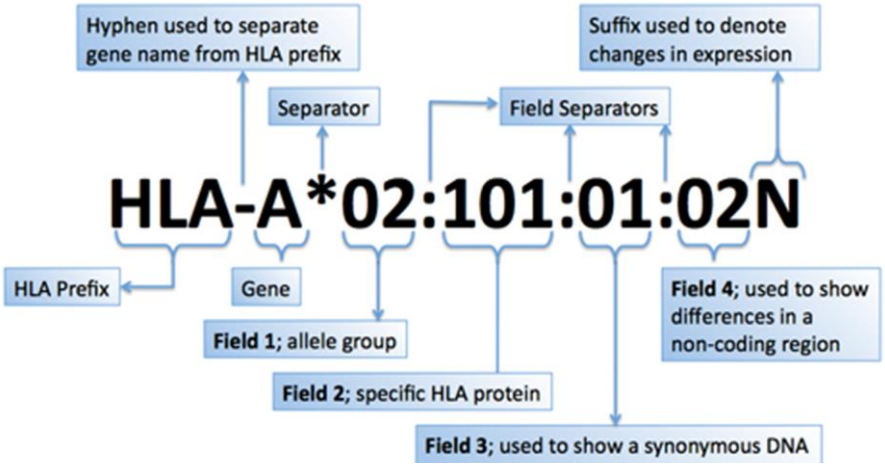

Figure 1.2: Standardized *HLA* nomenclature. Figure is adapted from https://hla.alleles.org/nomenclature/naming.html

The figure explanation of the *HLA* allele naming system. The *HLA* allele designation is structured as follows: the *HLA* prefix represents the gene e.g., *HLA*-A , followed by an asterisk (*) to separate the gene name from the allele number. The first field; Field 1 represents the allele group, while Field 2 indicates the specific *HLA* protein.

Field 3 shows a synonymous DNA substitution within the coding region. Field 4 is used to describe variations in the non-coding region. The hyphen separates different components of the allele name, and field separators colon divide fields, with a suffix N denoting changes in expression, such as N for a null allele. This structured naming system helps in the clear identification of *HLA* alleles, including mutations and expression patterns.

1.5.2 *HLA* protein function

HLA alleles have a crucial role in the functioning of the immune system. They have a crucial function in the pathways of antigen presentation, notifying the immune system of the host of infected cells (Matsuzaka & Yashiro, 2024). Proteins that originate from within the cell and reside in the cytosol undergo degradation by the proteasome. The resulting peptides are then transported into the rough endoplasmic reticulum (ER) by the TAP1/TAP2 complex. In the ER, these peptides bind to class I molecules along with β2-microglobulin (β2m) and are subsequently transported to the cell surface for recognition by CD8+ T cells and natural killer (NK) cells. Similarly, internal antigens, such as viral proteins, enter the cell and follow the endocytic pathway, where they are degraded and then loaded onto class II molecules, which are initially bound to an invariant chain that has been exported from the rough ER(Neefjes, Jongsma, Paul, & Bakke, 2011; Tiwari, 2005).

The invariant chain is a protein that initially blocks the binding site of MHC class II molecules to prevent them from picking up any peptides too early. As the MHC class II-invariant chain complex moves into acidic compartments within the cell, the invariant chain is broken down. This breakdown leaves a small piece called

CLIP (Class II-associated Invariant Peptide) still attached to the MHC class II molecule. CLIP needs to be removed so that the MHC class II molecule can bind the correct antigenic peptide. This replacement happens with the help of a molecule called *HLA-DM*, which swaps CLIP with a peptide from a degraded antigen. Once the antigenic peptide is bound, the MHC class II-peptide complex is moved to the cell surface, where it can be recognised by CD4+ T-helper cells. These cells are essential for triggering a targeted immune response (Simmonds & Gough, 2007).

HLA diversity is shaped by selective pressures over time. This includes long-term balancing selection, demonstrating that these alleles originate from an advantageous ancestral variation, and pathogen-derived selection (Meyer, C Aguiar, Bitarello, C Brandt, & Nunes, 2018; Spurgin & Richardson, 2010). There is a suggestion that populations have developed variety due to distinct selecting pressures in various geographic locations. This diversity may be influenced by the presence of different infectious organisms worldwide and the role of *HLA*s in the immune system (Choo, 2007). Fewer details are known about the roles played by the less polymorphic class I genes, *HLA-E*, *-F*, and *-G*. They have a structure similar to the previously mentioned polymorphic genes, but they do not present peptides to T-cells (Coico, 2021). There is a suggestion that *HLA-G* may have a role in preventing the destruction of cells by NK cells. NK cells do not express MHC class I, which means they cannot be detected by CD8+ T-cells. However, they can be detected by the inhibitory receptor, ILT2 (Immunoglobulin-like Transcript 2) on the NK cell.

The expression of these genes occurs in placental cells generated from the foetus, which then move into the uterine wall (Ferreira, Meissner, Tilburgs, & Strominger, 2017). Similarly, *HLA-E* may have a specific function in cell identification by NK cells via its binding of peptides that come from leader peptides

of other *HLA* class I molecules. The *HLA-E* peptide complex has the ability to bind the NKG2A inhibitor, which effectively hinders the cytotoxic action of the NK cell (Fisher, Doyle, Graham, Khakoo, & Blunt, 2022).

1.6 Molecular docking in silico analysis

Molecular docking has become an essential tool in computational biology and drug discovery, facilitating the understanding of molecular interactions and aiding the development of new therapeutics (Stanzione, Giangreco et al. 2021). By simulating the interactions between small molecules, such as potential drugs and biological targets like proteins, molecular docking helps predict the binding affinity and orientation of compounds within the active site of a target molecule (Maden, Sezer et al. 2022).

This predictive capability is invaluable in fields such as pharmacology, medicinal chemistry, and structural biology, where understanding the mechanisms of molecular binding is crucial for designing effective and targeted drugs. The core of molecular docking lies in finding the optimal fit between a ligand (the small molecule or drug candidate) and a receptor (typically a protein or enzyme). This process involves not only determining the best spatial orientation but also assessing the energy profile of the interaction to predict how strongly the ligand will bind to the receptor (Sliwoski, Kothiwale et al. 2014).

1.7 Research Problem Statement

A fundamental step in studying the genetic characteristics of the Iraqi population is to establish baseline frequencies of key *HLA* alleles in healthy individuals. Accordingly, a population frequency screening was conducted, involving healthy volunteers to establish baseline allele frequencies of *HLA-A, HLA-B,* and

HLA-DRB1 markers in the general population. These frequencies serve as a reference for understanding the genetic distribution within the Iraqi population. Such population-specific data are essential for interpreting genetic findings in patient groups and for advancing personalized medicine approaches within the Iraqi context.

SCARs are one of the most serious clinical issues, as SJS and TEN cause a high mortality rate. These reactions are especially concerning in patients treated with the aromatic antiepileptic drugs including CBZ, LTG and PHT. Genetic susceptibility to SCARs varies considerably between populations due to differences in *HLA* allele frequencies, with well-established examples such as *HLA-B*15:02* associated with carbamazepine. These associations have led to targeted genetic screening programs in several countries, substantially reducing drug-induced SCARs. In Iraq, there is almost no published data on genetic risk factors or the clinical spectrum of SCARs, partly because the clinical and laboratory parameters required for severity assessment (e.g., SCORTEN) are often missing from patient records, limiting the ability to describe the full severity distribution in our population. This lack of local evidence restricts clinicians' capacity to identify high-risk individuals and implement preventive strategies. The present study addresses part of this gap by investigating *HLA* alleles associated with antiepileptic drug–induced SCARs using a hospital-based case-control design, which is well suited for studying rare outcomes and estimating allele–disease associations but is not intended for estimating prevalence or incidence. Accurate prevalence estimation would require a population-based design, such as a descriptive cross-sectional or retrospective cohort study, which would also allow more precise sample-size calculation based on the expected proportion in the population. In contrast, the sample size for the current genetic association study was determined using expected

control allele frequencies and minimum detectable odds ratios, making it appropriate for the chosen case-control design. Furthermore, SCARs continue to present a significant clinical challenge in Iraq, with insufficient data on their demographic distribution and clinical presentation. Information regarding the most affected age groups, gender differences, incidence rates, and clinical severity including hospitalization duration and mortality rate is scarce or non-existent. This gap in epidemiological and clinical knowledge hampers healthcare professionals' understanding of the true burden of SCARs and limits the development of tailored management strategies for Iraqi patients. Without such localized clinical characterization, early detection and intervention may be delayed, resulting in increased morbidity and mortality. The genetic predisposition to SCARs has been well-documented in various ethnic groups, with specific *HLA* alleles including *HLA-B*15:02* and *HLA-A*31:01* defined as major risk factors. (Ayo et al., 2015). However, data are scarce concerning the link of the markers in *HLA-A*, *-B*, and *–DRB1* genes with SCARs in Iraqis. The absence of data based on genetic studies reduces precision medicine application to drugs that must be prescribed based on one's genetic make-up . Therefore important to define genetic risk factors among the Iraqi population to enhance on personalized medicine and patient safety.

An ethnic background plays a crucial role in genetic susceptibilities to SCARs, which different risk alleles were observed across Asians, Europeans, and other populations (Brown & Bayat, 2009). For example, *HLA-B*15:02* is a well-established risk factor for CBZ-induced SJS/TEN in Southeast Asian populations but is not prevalent or predictive in European or Japanese populations (Abe et al., 2003; Ahmed et al., 2021; Kaniwa et al., 2010). However, the identification of risk alleles for SCARs linked to AEDs among the Iraqi population has not yet been investigated.