ASSOCIATION OF HLA-A AND B ALLELES WITH ANTIEPILEPTIC DRUG-INDUCED SEVERE CUTANEOUS ADVERSE DRUG REACTIONS IN A MULTI–ETHNIC MALAYSIAN POPULATION

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ASSOCIATION OF HLA-A AND B ALLELES WITH ANTIEPILEPTIC DRUG-INDUCED SEVERE CUTANEOUS ADVERSE DRUG REACTIONS IN A MULTI–ETHNIC MALAYSIAN POPULATION

ABSTRACT

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), a type of severe cutaneous adverse drugs reaction, are an immune-mediated hypersensitivity reaction. Common high risk drugs causing SJS and TEN are aromatic antiepileptic drugs (AEDs) particularly Carbamazepine (CBZ), Phenytoin (PHT) and Lamotrigine (LTG). CBZ is a widely prescribed first-line treatment for epilepsy in Malaysia and therefore is the main causal drug for SJS/TEN cases. A strong association was discovered between *HLA-B*15:02* and CBZ-induced SJS/TEN in Taiwan Han Chinese, subsequently the association was replicated in other populations in Southeast Asia including Hong Kong, China, Thailand, Vietnam, India and Malaysia. Malaysia population is composed of heterogeneous multi-ethnic demographic from Austronesia (Malay), Southern India and Southern China. Studies from Malaysia reported the association in Malay and Chinese ethnic groups with one study on Malay CBZ-SJS/TEN that found *HLA-B*15:02* in 75% of the cases compared to 15.7% in general Malay population. While another study included a mixture of Malay and Chinese CBZ-SJS/TEN cases all of which were positive for *HLA-B*15:02* but without appropriate drug tolerant control. Furthermore, no study was performed in Malaysia's Indian ethnic group. Ethnicity specific *HLA-A* and *-B* allele association conferring susceptibility to CBZ, PHT and LTG-induced SJS/TEN was investigated in Malaysia three major ethnic groups in this study. AEDs-SJS/TEN cases and AEDs tolerant controls were recruited from two hospitals across Malaysia and *HLA-A* and *-B* genotyping were performed. The carrier frequencies of specific *HLA-A* and *-B* alleles were compared between 36 AEDs-SJS/TEN cases and 273 AEDs-tolerant controls, stratified by ethnicity and causal drugs. Significant association between *HLA-B*15:02* and CBZ-SJS/TEN was detected in the

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three major ethnic groups: Malay (87.5% versus 12.5%; p = 2.00 x 10^{-8}; Odds ratio (OR) = 49.0; 95% confidence interval (CI) = 9.4-256.8), Chinese (66.7% versus 12.3%; p = 0.0047; OR = 14.3; 95% CI = 2.4-86.0) and Indian (33.3% versus 3.5%; p = 0.042; OR = 13.8; 95% CI = 1.5-125.0). Combined analysis of all ethnic groups showed a significant association with OR_{CMH} of 26.6 (95% CI 12.8-55.3; P_{CMH} = 2.31 x 10^{-26}). For *HLA-A*, association of *HLA-A*31:01* with CBZ-SJS/TEN was discovered in Indians for the first time (p = 0.023; OR = 10.4; 95% CI = 1.6-65.8). This allele was not found in Malay and Chinese CBZ-SJS/TEN groups. Logistic regression analysis demonstrated that *HLA-A*31:01* has an independent effect after conditioning for *HLA-B*15:02* (P = 0.019; OR 11.7; 95% CI 1.5-9.1). No association was found in PHT and LTG-SJS cases for the three ethnic groups, probably due to limited sample size. In conclusion, this study replicated the association between *HLA-B*15:02* and CBZ-SJS/TEN in Malay, Chinese and Indian populations in Malaysia and further demonstrated a new association between *HLA-A*31:01* and CBZ-induced SJS/TEN in Indians.

Keywords: Severe cutaneous adverse drug reactions, Stevens-Johnson syndrome and toxic epidermal necrolysis, human leukocyte antigen allele.

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ASOSIASI ANTARA ALEL HLA-A DAN B DENGAN REAKSI KULIT TERUK AKIBAT UBAT ANTI-EPILEPTIK DI KALANGAN POPULASI MALAYSIA YANG BERBILANG ETNIK

ABSTRAK

Sindrom Stevens-Johnson (SJS) dan nekrolisis epidermis toksik (TEN), sejenis reaksi kulit teruk akibat ubat, adalah hypersensitiviti yang disebabkan sistem imun. Ubat penyebab umum yang berisiko tinggi mengakibat SJS dan TEN ialah ubat anti-epileptik aromatik (AEDs), terutama Carbamazepine (CBZ), Phenytoin (PHT) dan Lamotrigine (LTG). CBZ adalah ubat barisan pertama untuk epilepsi yang digunakan dengan meluas di Malaysia, oleh sedemikian ubat ini penyebab utama dalam kes SJS/TEN. Asosiasi yang kukuh telah ditemui di antara *HLA-B*15:02* dan CBZ-SJS/TEN di kalangan Taiwan Han China. Asosiasi ini juga dikesani berulang dalam populasi lain di kalangan Asia tenggara, termasuk Hong Kong, China, Thailand, Vietnam, India and Malaysia. Populasi Malaysia terdiri dari demografi heterogen yang berbilang etnik seperti Austronesia (Malayu), Cina selatan dan India selatan. Kajian dari Malaysia melapor asosiasi di kumpulan etnik Malayu dan Cina. Salah satu kajian mengesani *HLA-B*15:02* dalam 75% kes Melayu CBZ-SJS/TEN berbanding dengan 15.7% di kalangan populasi Melayu umum. Manakala satu kajian Malaysia yang meliputi campuran kes CBZ-SJS/TEN Melayu dan Cina mendapati semua pesakit positif kepada *HLA-B*15:02*, tetapi kajian ini kekurangan pesakit kawalan toleran yang sesuai. Tambahan pula, tidak ada kajian Malaysia yang menyelidik asosiasi di kalangan kumpulan etnik India. Alel-alel *HLA-A* dan *-B* dalam etnik tertentu berkaitan dengan kecenderungan mendapat CBZ, PHT dan LTG-SJS/TEN. Oleh sebab itu, faktor etnik adalah kemestian penting yang perlu dilitupi dalam kajian ini. Kes AEDs-SJS/TEN dan kawalan toleran AEDs direkrut dari dua hospital di Malaysia dan penjenisan genotip *HLA-A* dan *-B* dilaksanakan. Frekuensi pembawa alel *HLA-A* dan *-B* dibanding antara 36 kes CBZ-SJS/TEN kes dan 273 kawalan tolerant AEDs dan analisis berstrata

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dijalankan berasas faktor etnik dan ubat penyebab. Asosiasi signifikan antara *HLA-B*15:02* dan CBZ-SJS/TEN dikesan dalam ketiga-tiga kumpulan etnik: Melayu (87.5% lawan 12.5%; $p = 2.00 \times 10^{-8}$; Nisbah Ods (OR) = 49.0; 95% Selang Keyakinan (CI) = 9.4-256.8), Cina (66.7% lawan 12.3%; $p = 0.0047$; OR = 14.3; 95% CI = 2.4-86.0) dan India (33.3% lawan 3.5%; $p = 0.042$; OR = 13.8; 95% CI = 1.5-125.0). Analisis gabungan ketiga-tiga kumpulan etnik menunjuk hubungan signifikan ternyata dengan OR_{CMH} 26.6 (95% CI 12.8-55.3; $P_{CMH} = 2.31 \times 10^{-26}$). Asosiasi antara alel *HLA-A*31:01* dan CBZ-SJS/TEN di kalangan India dikesani pertama kali ($p = 0.023$; OR = 10.4; 95% CI = 1.64-65.8). Alel ini tidak dapat dikesani di kalangan kes Melayu dan Cina CBZ-SJS/TEN. Asosiasi tidak dapat dikesani dalam ketiga-tiga kumpulan etnik kes PHT and LTG-SJS kemungkinannya disebabkan oleh kekurangan saiz sampel. Kesimpulan, kajian ini mengulangi asosiasi antara *HLA-B*15:02* and CBZ-SJS/TEN dalam kalangan populasi Melayu, Cina dan India di Malaysia dan juga mengesani kaitan baru antara *HLA-A*31:01* and CBZ- SJS/TEN di kalangan India.

Kata kunci: reaksi kulit teruk oleh ubat, sindrom Stevens-Johnson, nekrolisis epidermis toksik, alel antigen leukosit manusia

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LIST OF SYMBOLS AND ABBREVIATIONS

AERS : Adverse event reporting system

ALDEN : Algorithm of drug causality for epidermal necrolysis

AEDs : Antiepileptic drugs

BD : Breslow-Day

CBZ : Carbamazepine

CIC : Clinical Investigation Centre

CPIC : Clinical Pharmacogenetics Implementation Consortium

CD : Cluster of differentiation

CMH : Cochran–Mantel–Haenszel

CI : Confident interval

CLA : Cutaneous lymphocyte-associated antigen

CYP : Cytochrome P450

CTL : Cytotoxic T cells

DNA : Deoxyribonucleic acid

DRESS : Drug reaction with eosinophilia and systemic symptoms

ER : Endoplasmic reticulum

FDA : Food and Drug Administration

GABA : Gamma amino butyric acid

HHV : Human herpesvirus

HLA : Human leukocyte antigen

IL : Interleukin

KIR : Killer cell immunoglobulin-like receptor

LTG : Lamotrigine

LTT : Lymphocyte transformation test

MPE : Maculopapular exanthema

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MHC : Major histocompatibility compatibility

NPV : Negative predictive values

NNT : Number needed to test

OR : Odds ratio

OXC : Oxcarbazepine

pHLA : Peptide-HLA complex

PBMC : Peripheral blood mononuclear cell

P-i : Pharmacological interaction with immune receptors

PHT : Phenytoin

PPV : Positive predictive values

RNA : Ribonucleic acid

RUCAM : Roussel Uclaf causality assessment method

SCARs : Severe cutaneous adverse drug reactions

SNP : Single nucleotide polymorphism

SEA : Southeast Asia

SJS : Stevens-Johnson syndrome

TCR : T cell receptor

TEN : Toxic epidermal necrolysis

UMMC : University Malaya medical centre

WHO : World Health Organization

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CHAPTER 1: INTRODUCTION

Severe cutaneous adverse reactions (SCARs) to drugs are a type of delayed hypersensitivity reaction that is idiosyncratic, unpredictable and dose-independent. SCARs account for 15-20% of all adverse drug reactions (Thong & Tan, 2011). It includes Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), which are characterized by blistering and erosion of skin and mucous membrane due to necrosis of keratinocytes. Although SJS and TEN are rare, it is associated with mortality rate as high as 5-15% and 40%, respectively (Roujeau et al., 1995). Another phenotype of delayed hypersensitivity reactions is drug reaction with eosinophilia and systemic symptoms (DRESS), which involved systemic organs. A milder form of cutaneous drug eruption (rash) is maculopapular exanthema (MPE) (Harr & French, 2010; Nayak & Acharjya, 2008).

One of the high-risk drugs in causing SJS/TEN are aromatic antiepileptic drugs (AEDs), Carbamazepine (CBZ), Phenytoin (PHT) and Lamotrigine (LTG). CBZ is a widely prescribed first-line treatment for epilepsy in Malaysia and hence the main causal drug for SJS/TEN cases (Choon & Lai, 2012; Thong & Tan, 2011). Population study from RegiSCAR group reported the risk of SJS/TEN in CBZ user as 1.4 per 10,000 new users (Mockenhaupt et al., 2005).

An important progression has been the discovery of the association of *HLA* alleles and these SCAR syndromes. The findings of *HLA* association have shed light on the predisposing genetic risk factor in SCARs and provide a means for prevention by screening prior to drug treatment. In line with the emerging approach of precision medicine where prescribed treatment takes into consideration individual genetic variability.

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Identifying the type of SCARs is important for identifying the *HLA* alleles associated with this hypersensitivity syndrome (Phillips et al., 2011). In SJS/TEN cases induced by Carbamazepine, strong association was discovered to *HLA-B*15:02* in Taiwan Han Chinese, subsequently the association was replicated in different populations across Asia, such as Thai, Vietnamese, Han Chinese from Hong Kong and Mainland China, Malays in Malaysia, west central Indians and northern Indians from India but with various effect size (Chang et al., 2011; Cheung et al., 2013; Chung et al., 2004; Mehta et al., 2009; Nguyen et al., 2015; Tassaneeyakul et al., 2010; Then et al., 2011; Wu et al., 2010). The association of *HLA-B*15:02* was extended to phenytoin and lamotrigine-induced SJS/TEN, although the strength of association is not as strong as found in CBZ-SJS/TEN. The associations between *HLA-B*15:02* and SJS/TEN secondary to phenytoin and lamotrigine were only reported in Han Chinese and Thai populations and remains unknown in other ethnicity (Cheung et al., 2013; Locharernkul et al., 2008). It became apparent that the exclusivity observed between *HLA-B*15:02* association to CBZ-SJS/TEN was specific to phenotype, ethnicity and to some extend the culprit drug.

The association between *HLA-B*15:02* and CBZ-SJS/TEN was not detected in Japanese, Koreans and Europeans. Instead, in Japan and Korea, CBZ-SJS/TEN was reported to be associated with *HLA-B*15:11* (Kaniwa et al., 2010; Kim et al., 2011; Ozeki et al., 2011). Interestingly, both *HLA-B*15:11* and *HLA-B*15:02* belongs to the same *B75* serotype subfamily. Recently, *HLA-A*31:01* allele was found in association with all phenotypes of CBZ-induced hypersensitivity reactions (including SJS/TEN, DRESS and MPE) in Japanese and Europeans (McCormack et al., 2011; Ozeki et al., 2011). However, later on meta-analysis study contradicted it, in which *HLA-A*31:01* was more strongly associated with CBZ-DRESS than CBZ-SJS/TEN in Han Chinese and Europeans (Genin et al., 2014). In Japanese, the association of *HLA-A*31:01* to all

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CBZ-hypersensitivity is confirmed in 2 more case-control studies (Kashiwagi et al., 2008; Niihara et al., 2012). Overall, these studies confirmed the association between *HLA-A*31:01* and CBZ-DRESS, but the association to CBZ-SJS/TEN and MPE is inconsistent.

The presence of multiple risk alleles in different ethnicity confer the need to investigate risk variants relevant to our population. Although two studies have investigated the association of *HLA-B*15:02* and CBZ-induced SJS/TEN in Malay and Chinese ethnic group in Malaysia, the sample sizes are small and appropriate ethnic-specific drug tolerant controls was not employed (Chang et al., 2011; Then et al., 2011).

Malaysia population is made up of three major ethnic groups; with Malays forming the largest group (67.4%) followed by Chinese (24.6%), Indians (7.3%) and other minorities (0.7%) according to the latest national census. The Malays in Malaysia are predominantly of Austronesian and Southeast Asian aboriginal (Proto-Malay) ancestries and are genetically similar to Singapore Malays and western Indonesian Malays (Deng et al., 2015). Whereas Malaysian Chinese are of Southern Han Chinese and Indians mostly originated from Southern India (Basu et al., 2003; Reich et al., 2009). However, for Indians, the allele frequency of *HLA-B*15:02* is not homogeneously distributed. *HLA-B*15:02* allele frequency in India population ranges from 0-6% depending on region, with as low as 0% in west coast Parsi, 1.6% in northern Indian, to 4% in west Bhil, and 6% in Pawra in Khandesh region, but not available for southern Indian (Gonzalez-Galarza et al., 2015).

Recent studies have demonstrated varying *HLA* allele frequency among different ethnicities could influent the susceptibility to drug hypersensitivity reactions (Grover & Kukreti, 2014; Yip et al., 2012). To factor in *HLA* allele frequency of different ethnicities, this study stratified the SCARs cases by ethnicity prior to perform the

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analysis and assessed the possibility of heterogeneity. Furthermore, no study was performed in Malaysian Indian ethnic group.

The study objectives are: 1) To confirm the association of *HLA-B*15:02* allele with CBZ, PHT and LTG-induced SCARs; 2) To investigate other ethnicity-specific *HLA-A* and *-B* allele associated with CBZ, PHT and LTG-induced SCARs in a multi-ethnic Malaysian population.

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CHAPTER 2: LITERATURE REVIEW

2.1 Severe cutaneous adverse drug reactions (SCARs)

Severe cutaneous adverse reactions to drugs are a type of delayed hypersensitivity reaction that is idiosyncratic, non-predictable and dose-independent. SCARs are associated with significant mortality and morbidity, long-term sequelae, high healthcare cost and it is a challenge in drug development. There were several drugs withdrawal from the market due to association with SCARs (Lasser et al., 2002).

SCARs cover a large variety of clinical phenotypes consists mainly of Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN) and drug reaction with eosinophilia and systemic symptoms (DRESS). A milder form of cutaneous adverse drug reactions is maculopapular exanthema (MPE), a rash that is mild, self-limited and usually resolved after the offending drugs are withdrawn. Unlike MPE, SCARs have serious morbidity, involving systemic manifestation and posed high mortality (Thong & Tan, 2011). The histopathology of MPE, DRESS and SJS/TEN is predominated with exocytosis and lymphocytes and macrophages infiltrate. In severe cases of SJS/TEN, extensive keratinocytes necrosis is observed. The severity of inflammatory infiltrate and epidermal manifestation increased from MPE to DRESS and SJS/TEN (Duong et al., 2017).

Early diagnosis of SCARs that helps in identification of culprit drugs are important in the acute stage of the reactions. A prompt recognition helps to improve the management of the disease as it progresses and limit long-term sequelae. Classification of SCARs is also important in identifying causal drug. Studies have shown interval between drug intake and SCARs onset differ for different SCARs type. SJS/TEN has shorter latency time compared to DRESS (Kardaun et al., 2007). Assessment scores and

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tools were developed to assist in investigation of SCARs to determine clinical patterns and identify causal drugs.

2.2 Classification of SCARs

The current commonly used classification is Severe Cutaneous Adverse Reactions (SCARs) study by Bastuji-Garin S. et al. and validation scores developed by a group of SCARs experts, RegiSCAR (Bastuji-Garin et al., 1993). Several studies have developed algorithms to assist causality assessment objectively. As a standardised approach is important to establish causality for physician to come to an accurate and reproducible diagnosis of SCARs. Some of the algorithms developed for drug causality assessment in cutaneous adverse drug reactions are Naranjo, the French pharmacovigilance causality score test, the RUCAM algorithm and algorithm of drug causality for epidermal necrolysis (ALDEN) which is validated for SJS/TEN (Begaud et al., 1985; Danan & Teschke, 2016; Naranjo et al., 1981; Sassolas et al., 2010). The most widely used algorithm worldwide to identify culprit drugs are Naranjo algorithm and the French pharmacovigilance causality score test. ALDEN algorithm is also gaining prominence as it is frequently applied in SJS and TEN cases to assess suspected drugs (Sassolas et al., 2010).

A multinational collaborative research consortium was formed in 1988 to study SCARs. The consortium, initially named EuroSCAR, is a European network focusing on SCARs that brought together experts from dermatology, genetics, immunology, pharmacology and epidemiology. It later changed its name to RegiSCAR when expending the scope of diseases and engaging new teams from other countries. The scope of SCARs type has been extended from the initial SJS/TEN and DRESS to include acute generalized exanthematous pustulosis (AGEP). The RegiSCAR group operates as a registry in collecting clinical data and biological samples, provide

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continuous surveillance on new drugs and guidelines for SCARs. They also aim to improve drug safety in order to reduce the medical and economic burden of SCARs in public health. To prevent misdiagnosis, RegiSCAR has defined consensus diagnosis criteria for each SCAR phenotype. RegiSCAR employed a rigorous systemic approach and clear diagnostic criteria to phenotype SCARs. SCARs cases were investigated by using standardized questionnaires to obtain detailed information on phenotypes, including collecting clinical photograph and skin biopsies, associated medical conditions and drug exposure. They reported that causality can be attributed to a dozen high risk medications and that misdiagnoses were frequent. Their studies show that overlapping SCARs phenotypes were rare. They established that SJS and TEN are variants of the same disease, and that SJS/TEN and DRESS are two distinct disease (Bastuji-Garin et al., 1993; Kardaun et al., 2007). Although SJS/TEN and DRESS are implicated by the same group of high risk medications. Besides that, there was also the difficulty in assessing drug causality due to incomplete reporting of drug exposure. From RegiSCAR findings, they constructed a specific algorithm for drug causality, algorithm of drug causality for epidermal necrolysis (ALDEN), that are used widely in recent SCARs studies (Sassolas et al., 2010). Using the collected data from their study, they are able to estimate medication risk, prognosis indexes, disease outcome and effects on treatments for SCARs. Other on-going investigations are phenotype determination, lymphocytes antigenic specificity and susceptibility genes and single nucleotide polymorphisms (SNPs) (Phillips et al., 2011).

Generally, for SCARs assessment, it relies mainly on cutaneous manifestation and clinical presentation, duration of the eruptions, associated symptoms and latency time between starting drug and eruption onset. The distribution and physical examination of the skin lesions coupled with skin biopsy for histological testing are all important for accurate and quick diagnosis of SJS/TEN, as diagnosis of SJS/TEN within

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7 days of onset is associated with improved survival. Currently, there is no validated curative treatment for SJS/TEN. The standard treatments strategies are symptomatic with the aim to prevent long-term sequelae (Duong et al., 2017; Palmieri et al., 2002).

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

2.2.1.1 Clinical Feature of SJS and TEN

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are considered variants of different severity from the same disease spectrum. SJS and TEN are classified by the extent of body surface area (BSA) with skin detachment. With less than 10% of skin detachment as SJS. The most severe forms with more than 30% of skin detachment as TEN and between 10-30% skin detachment as SJS/TEN overlap (Bastuji-Garin et al., 1993; Roujeau & Stern, 1994).

Early stage of SJS/TEN clinical manifestation includes fever, malaise, flu-like symptoms, eyes, ear, nose, throat events and skin pain prior to cutaneous manifestations by a few days. Symptoms of skin eruption first appears on face, pre-sternal area of upper trunk and extremities. The skin eruption is distributed more to the proximal part of the extremities while the distal part is relatively spared (Auquier-Dunant et al., 2002).

Cutaneous manifestations in SJS and TEN are characterised by rapid developing erythematous or purple-red macules and atypical target lesions with dark centre and indistinct border. The skin lesions progress to flaccid blisters and have a tendency to rapidly coalesced forming confluent epidermal necrosis, and large epidermal sheet detachment. However, if there is absence of obvious epidermal detachment, Nikolsky's sign, characterised by detachment of necrotic epidermis from the dermis under lateral mechanical pressure on erythematous zones, can be performed (Duong et al., 2017; Harr & French, 2010).

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In 80% of the case, haemorrhagic erosion of two or more mucous membranes preceding skin eruption occurs, usually in eyes, mouths, nasopharynx or genitalia. The haemorrhagic erosion in mouth has a greyish-white pseudo-membrane coating. Lesions occurring in eyes cause chemosis, hyperaemia, photophobia and erosions. In severe cases, it leads to ulceration in the corneal. However, it is noted that the severity of acute phase in ocular manifestation does not predict late phase complications. Common organ involvements associated with SJS and TEN are liver and kidney enzymes elevation and necrotic lesions in epithelial of bronchial and gastrointestinal track. Up to 73% of patients suffered long-term sequelae from mucosal involvement of oesophageal, lung and genital mucosa. In TEN patients, up to 50% develop long term ocular complications. Severe dry eyes and ingrowth of eyelashes are two of the most common complications (Harr & French, 2010; Magina et al., 2003; Yip et al., 2007),

2.2.1.2. Diagnosis of SJS and TEN

There is no specific diagnostic test for SJS and TEN. The diagnosis is based on clinical symptoms, cutaneous manifestation and histological test. In acute phase, to enable rapid diagnosis, histological examinations such as direct immunofluorescence analysis are usually performed on cryosection of the skin. This helps to exclude differential diagnosis of similar disease such as bullous fixed drug eruption, autoimmune blistering diseases, acute generalized exanthematous pustulosis (AGEP) and staphylococcal scalded skin syndrome, a rarer form of cutaneous manifestation in adults. A negative direct immunofluorescence test and full-thickness epidermal necrosis are mandatory signs for SJS/TEN diagnosis. A negative direct immunofluorescence test that show absence of immunoglobulin or complement deposit in the epidermis or dermal layer is used to rule out autoimmune blistering diseases (Harr & French, 2010).

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After SJS/TEN diagnosis is established, prompt identification and withdrawal of the causative drug is important.

2.2.2 Drug reaction with eosinophilia and systemic symptoms (DRESS)

2.2.2.1 Clinical Feature of DRESS

Drug reaction with eosinophilia and systemic symptoms (DRESS) was first described in 1996. It is also known as drug-induced hypersensitivity syndrome. DRESS is notorious to diagnose due to its heterogeneous clinical presentation and organ involvement with or without cutaneous manifestation (Kardaun et al., 2013). In the initial stage, fever, flu-like symptoms, lymphadenopathy, pruritus and burning pain can be seen preceding skin eruption up to 2 weeks. Cutaneous manifestation involve erythroderma, purpura, pustules, swelling of face and distal extremities. The organ involvement in DRESS symptoms are from the infiltration of specific eosinophils and lymphocyte into the tissues. Liver is the most frequently affected organ in DRESS. More than 80% of DRESS cases have liver involvement, seen as hepatic cytolysis and cholestasis or in rare case fulminant hepatic failure. Kidney involvement is manifested by interstitial nephritis. Respiratory system is affected in up to 15% of DRESS cases, characterised by cough, dyspnoea, eosinophilic pneumonitis and in rare cases, respiratory failure. In cardiovascular system involvement, it is manifested by cardiac enzyme abnormalities, myocarditis and pericarditis (Choudhary et al., 2013; Duong et al., 2017). In rare cases, other visceral organ such as muscle, nerves system and pancreas are affected, which complicates prognoses. In the acute phase, to prevent misdiagnosis, a few clinical investigations are strongly recommended such as testing for blood abnormalities to assess organ involvement, hypereosinophilia, atypical lymphocytes and virus reactivation (Duong et al., 2017). There is the complication of viral reactivation in DRESS, where the human herpesvirus (HHV) members, HHV6, is

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most reported. Reactivation of other type of HHV that were reported are Epstein Barr virus, HHV7 and cytomegalovirus. The reactivation was observed at 2 to 3 weeks after DRESS onset. A long period of relapses is described usually with the reactivation of HHV6 during the course of DRESS (Choudhary et al., 2013; Duong et al., 2017).

2.2.2.2 Diagnosis of DRESS

The histology features of DRESS are non-specific eczematous lesion, focal keratinocytes necrosis, eosinophil, neutrophil and monocytes infiltrate and mild vasculitis. Due to the high potential of misdiagnosis, two score have been developed to validate DRESS syndrome diagnosis with one of the score systematically assess HHV6 reactivation. Both these scores enable the validation of DRESS diagnosis without cutaneous eruption presentation and recommended to reconsider it as multi-organ drug-induced reaction (Kardaun et al., 2007; Shiohara et al., 2007). Cutaneous manifestation of DRESS is mainly mediated by CD8+ T cells. High level of drug-specific CD8+T cells expressing granzyme B was detected in skin biopsy of severe DRESS patients. The recruitment of HHV+ peripheral mononuclear cells to damaged skin may have enable virus transmission and replication in CD4+ T cells. One study reported that almost half of the activated circulating CD8+ T cells from DRESS patients recognised HHV whereas the visceral or skin infiltrate CD8+ T cells recognised EBV (Picard et al., 2010).

DRESS has lower mortality (5-10%) compared to SJS/TEN (10-40%). The causes of mortality are mainly due to lesions in lungs or myocardium and haemophagocytosis. The long-term sequelae seen in DRESS is predominantly of autoimmune disease. This excessive autoimmune response is suspected triggered by chronic virus reactivation. Corticosteroids treatment has been shown to improve autoimmune disease in DRESS patient (Duong et al., 2017).

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2.3 Epidemiology of SCARs

Epidemiology data on SCARs and their causal drugs in Asia is scarce, partly due to underreporting of SCARs event and difficulty in definitive diagnosis of SCAR types because of overlapping symptoms. Most studies reported on selected population group such as hospital inpatient population or specialist allergy and burn centers (Thong & Tan, 2011).

Based on studies that focus on cutaneous adverse drug reactions, a few prospective studies in hospital-based population have attempted to evaluate the incidence and prevalence. A 6-month prospective study in a France hospital reported 48 cases of inpatient cutaneous adverse drug reactions and the prevalence of cutaneous adverse drug reactions was reported as 3.6 per 1000 inpatient. In this group, 57% reported maculopapular eruption whereas 2% has SJS/TEN and one third of the cases have previous history of drug hypersensitivity. The main drug implicated was beta-lactam (Fiszenson-Albala et al., 2003). A study on network-based electronic drug allergy notification system for inpatient in a general hospital in Singapore reported 210 cases of drug hypersensitivity from 90910 admissions of which 5.2% has SCARs. They estimated the incidence of drug hypersensitivity at 4.2 per 1000 hospitalization. 75% of the cases were caused by antimicrobials and antiepileptic drugs (Thong et al., 2003). A Korea study based on their mandatory reporting system for immune-mediated drug hypersensitivity reactions found 100 new cases of drug hypersensitivity among 55432 admission. Among this group, 1% has SJS/TEN. They estimated the drug hypersensitivity incidence at 1.8 in 1000 hospitalization. The common causal drugs were antibiotics (32%) (Park et al., 2008).

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2.3.1 Epidemiology of SJS and TEN

The studies that focus on SCARs particularly SJS and TEN alone are more limited. Based on three studies from United States and Europe, incidence for SJS and TEN was estimated at 1.4-6 per million person per year (Chan et al., 1990; Thong & Tan, 2011). A study based on FDA Adverse event reporting system (AERS) database in US reported similar incidence with 1.9 cases per million for TEN (La Grenade et al., 2005). In a study from a district city in China, the prevalence for SCARs are 0.15 per 1000 hospitalization for SJS, 0.04 per 1000 hospitalization for TEN and 0.07 per 1000 for DRESS. The common causal drugs are antibiotics, followed by antiepileptic drugs (Li & Ma, 2006). In Taiwan, the incidence of SJS/TEN in Taiwan Han Chinese is 8 cases per million person-years. For CBZ-induced SJS/TEN incidence, Hung et. al. estimated it at 2.5 cases per 1000 new CBZ users based on 5 years retrospective data from Chang Gung Memorial Health System (Hung et al., 2005). In a post marketing report (2000-2006) by CBZ manufacturer, Novartis, the author reported that CBZ-induced SJS/TEN was 10 times higher in Asian countries compared to Caucasian countries (Farkas, 2007).

The incidence of SJS/TEN in this region, Southeast Asia, is still mostly undetermined. Most SJS/TEN epidemiology studies are derived from Thailand, Malaysia, Singapore and Philippines. In Malaysia, epidemiology data is limited to inpatient hospital based population. Two studies reported CBZ as the main causal drugs in all SJS/TEN cases (Kamaliah et al., 1998; Khoo & Foo, 1996). Another study reporting on a 10-year data from hospital registry shown incidence rate of cutaneous adverse drug reactions at 0.86% and SJS/TEN accounted for 30.1%. Carbamazepine is also found to be the main causative drugs in SJS/TEN (Choon & Lai, 2012). A different study captured data from primary care in a Malaysia general hospital reported the prevalence of cutaneous adverse drug reactions at 0.2% out of 69849 patients. Of all the

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cutaneous adverse drug reactions cases, the most common manifestation is maculopapular rash at 22.4%, followed by SJS at 9.7%, while TEN is 4.5% and DRESS is 3.7% (Talib et al., 2015). Talib et al. (2015) attributed the prevalence of cutaneous adverse drug reactions in their study (0.2%) lower than reported by Choon and Lai (2012) (0.86%) due to mild cases not referred for dermatology consultation hence it was not captured.

2.4 Main cause of SCARs

The cause of SCARs is predominantly drug related (75-85%) (Lee et al., 2013). However, in about 15% of SJS/TEN cases, infections have been reported as the cause. The most documented non-drug cause of SJS/TEN is *Mycoplasma pneumoniae* infection and Herpes simplex virus reactivation, which is mainly reported in children. There are also cases where no obvious cause was identified (Forman et al., 2002; Harr & French, 2010).

2.4.1 High risk drug in SCARs

Based on population registry of EuroSCAR and the international case-control SCARs studies, the majority of SCARs were attributed to a group of common high risk drugs such as anti-infective sulfonamides, allopurinol, oxicam-NSAIDs, nevirapine and aromatic antiepileptic drugs (AEDs) such as carbamazepine (CBZ), phenobarbital, phenytoin (PHT) and lamotrigine (LTG). In this study, I focus on three high risk aromatic antiepileptic drugs, carbamazepine (CBZ), phenytoin (PHT) and lamotrigine (LTG). Mockenhaupt et. al. conducted a case-control study on hospitalized patients investigating the medication risk for inducing SJS and TEN. Interestingly, treatment duration is found highly relevant for drugs that have long term medication usage such as in the case of AEDs. They observed that risk of drugs to induce SJS or TEN was

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confined to the first 4 weeks (4 to 28 days) of drug intake and subsequent declined within 8 weeks. The estimated risk associated with these aromatic antiepileptic drugs, CBZ, PHT and LTG are 1.4, 8.3 and 2.5 per 10,000 new users respectively. These findings have improved the assessment of drug causality in SJS and TEN (Mockenhaupt et al., 2005; Mockenhaupt et al., 2008). Similarly, the common high risk drug in DRESS (carbamazepine, anti-infective sulfonamides, phenobarbital, and phenytoin) are the same as those strongly associated to SJS/TEN. The time interval from drug intake to DRESS symptoms onset (index day) is reported to be around 2 to 6 weeks. Prevalence of DRESS is estimated at one in 1000 to one in 10,000 of new user of antiepileptic drugs (Kardaun et al., 2007).

The same group which is part of the RegiSCAR consortium constructed an algorithm, ALDEN, that helps to improve drug causality assessment. In the ALDEN score attribution part, the common causal drugs for SJS/TEN was categorized based on relative risk to SJS/TEN. High risk drugs such as those of AEDs group, carbamazepine, phenytoin, phenobarbital and lamotrigine were given higher positive value. While low risk drugs were given less value. This attribution score is an important evaluation component of the drugs involved and taking into account concomitant drugs that were in the patient during SCARs onset (Sassolas et al., 2010).

In cases where several concurrent drugs are implicated as possible culprit drug, allergological testing are another option in identifying causation. Due to the severity of SJS and TEN re-challenge of the culprit drug is not allowed. Patch testing has been reported by using dissolving 10% of native drug in petrolatum and taped to the patient's back for assessment at 48-hour and 96-hour exposure. However, patch test has varying specificity and sensitivity according to drug type. *In vitro* tests have been used to measure peripheral blood mononuclear cell (PBMC) activity in SCARs patients. For example, lymphocyte transformation test (LTT), an *in vitro* test that measure T-cells

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proliferation when exposure to the culprit drug, has been reported. But LTT has low relevance in SJS/TEN due to low sensitivity (50%) (Kano et al., 2007).

Interestingly, different causal drugs are associated with SCARs in different countries and population. Nonetheless, high risk drugs identified in EuroSCAR like carbamazepine and allopurinol are still the most common high risk drugs to associate with SJS/TEN in Asia populations. In a review on SJS/TEN causal drugs in Southeast Asia, CBZ and PHT-SJS/TEN made up of 26% of all cases compared to 12% in European countries. The differences can be attributed to several factors such as variation in prescribing patterns, standard practice, cost of medication, incidence of the drug's main indication and genetic background of the populations (*HLA* allele distribution and polymorphism of metabolizing enzyme) (Lee et al., 2013).

2.4.2. Main Causal Drug of SJS/TEN in Malaysia - Carbamazepine

Carbamazepine (CBZ), with the chemical formula of $C_{15}H_{12}N_2O$, is a dibenzazepine. It is a tricyclic compound with anticonvulsant, mood stabilising drug and specific analgesic indicated for epilepsy, trigeminal neuralgia and psychiatric disorders. It is a first-line antiepileptic drug for partial and generalised tonic-clonic seizures and has been used since 1965. CBZ acts as a voltage-gated sodium channels blocker by binding to activated sodium channels and prevents repetitive firing of action potential. It reduces postsynaptic response and blocks post-tetanic potentiation. CBZ also functions as an agonist for inhibitory neurotransmitter, GABA (gamma amino butyric acid) by decreasing the excitatory neurotransmitter, glutamate. 75-80% of the total plasma concentration of CBZ is bound to plasma protein with bioavailability at a range of 75–85%. CBZ is almost completely metabolised in the liver with only 5% excreted unchanged. The major metabolism route is conversion to carbamazepine 10,11-epoxide, catalysed by cytochrome P450 enzymes, *CYP3A4*, *CY2C8* and *CYP3A5*.

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The minor metabolism route is ring-hydroxylation to 2-hydroxy-CBZ and 3-hydroxy CBZ with *CYP2B6* and *CYP3A4* as the main catalysts. CBZ has autoinduction effect by stimulating the transcriptional upregulation of genes involved in its own metabolism, by autoinduction of *CYP3A4* and *CY2C6*. CBZ has an initial half-life of 25–65 hours. After autoinduction of CBZ is completed around 3 to 5 weeks at a fixed dose regimen, the initial half-life of 25-65 hours decreases to 12-17 hours. Adult therapeutic level for CBZ is 4 to 12 mg/mL. (Novartis Pharmaceutical Corporation, 2015; Tolou-Ghamari et al., 2013)

2.5 The *HLA* System

The major histocompatibility compatibility (MHC) complex in human is also known as Human Leukocyte Antigen (*HLA*). The main role of *HLA* molecules is in presenting pathogen derived peptides peptide to T cells and elicit T cell adaptive immune response. T cells can distinguish self-peptide from foreign peptide presented by *HLA* molecules.

2.5.1 Structure and function of *HLA* molecule

MHC is located on the short-arm of chromosome 6 (6p21.3) and encodes cell surface glycoproteins. MHC is divided into three main class: Class I, II and III. Class I is made up of 6 isoforms – *HLA*-A, B, C, D, E, F and G. *HLA* class I molecules is consisted of a highly polymorphic 43kDA α heavy chain with noncovalent bond to a non-polymorphic β2 microglobulin (12kDa) protein whose gene is located on chromosome 15. The α chain and β2 microglobulin forms a membrane bound heterodimeric glycoprotein anchored to the cell membrane by the α chain. The α heavy chain has 3 extracellular domains (α1, α2 and α3) and a cytoplasmic tail. α3 domain and β2 microglobulin are proximal to the cell membrane whereas α1 and α2 domains

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are more distal to the cell. α1 and α2 domains fold together into anti-parallel β pleated sheets on the bottom and flanked on the side by a pair of anti-parallel α helix wall. The structure creates a groove where into peptides binds. The endogenous peptides, usually 8 to 10 amino acid long, are bound in the peptide-binding groove through hydrogen bond and ionic interaction from each end of the peptide. Majority of the *HLA* class I polymorphisms are located in the amino acid residues of peptide binding groove. In total, the 8 exons spanned around 1089-1101bp and has 362-366 amino acid. *HLA* class I proteins are ligand for $CD8^+$ T-cell receptors (TCR) and killer cell immunoglobulin-like receptor (KIR) on natural killer cells, and usually present endogenous peptides. The endogenous peptides are derived from cytosolic self-antigen or intracellular pathogens. *HLA* class I are expressed on almost all nucleated cells (Choo, 2007; Marsh et al., 2000).

Class II is made up of 5 isoforms: *HLA*-DM, -DO, -DP, -DQ and -DR. *HLA* class II has one 33-35 kDa α chain and one 26-28 kDa β chain. Both chains are anchored to the membrane. α chain have 5 exons whereas β chain has 6 exons. *HLA* class II also forms a heterodimer with 2 domains from α chain (α1 and α2) and 2 domains from β chain (β1 and β2). The main role of *HLA* Class II main role is to present extracellular derived peptides to CD4+ T-cells by means of phagocytosis of exogenous proteins. *HLA* Class II molecules are expressed only in immune cells such as B-cells, macrophages, monocytes, dendritic cells and T-cells. MCH Class II also encodes genes in antigen processing such as Transporter associated with antigen processing gene (TAP). The Class III region is located between Class I and II. It encodes for gene involved in inflammatory and complement cascade (e.g. TNF, LTA, LTB, C2 and C4) and does not encodes any *HLA* molecules (Choo, 2007; Marsh et al., 2000).

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2.5.2 *HLA* nomenclature

The *HLA* nomenclature is standardized by WHO *HLA* Nomenclature Committee for Factors of the *HLA* System. It consists of an international committee that standardise the nomenclature regularly. The curation and maintenance of sequence are performed by IPD-IMGT/HLA database. In recent years, larger number of new alleles were reported and many new alleles are still being constantly discovered every year, although most alleles reported since 2000 have very low frequencies. The nomenclature undergoes radical updating to accommodate this. The naming starts with each locus that are followed by an asterisk (*HLA-A**). The subsequent allele name length depends on the sequence of the allele compared to its nearest relative. All alleles have a 4-digit name or first 2 sets of digits and the subsequent digits are assigned only when necessary. As illustrated in Figure 2.1, the numbering starts with field 1 - the first pair of digits is the allele group (*HLA-A*02*). Most of the alleles within same group have the same serological specificities. Field 2, the second pair of digits, is the subtypes within each allele group that differ in their amino acid residues (*HLA-A*02:01*). These two fields are sufficient to distinguish an *HLA* allele. Field 3's digits define allele with the same protein but has synonymous nucleotide substitutions in the exons (*HLA-A*02:01:01*). The last field shows the alleles with nucleotide substitutions in intronic regions or 5' or 3' untranslated regions. Each field is separated by a colon. Sometimes, a suffix is also added to indicate the protein status such as low expression (L), null (N), secreted (S), aberrant (A) and questionable (Q) (Figure 2.1). The alleles are named in the order that they are discovered, hence the numbering does not denote how closely related the *HLA* variants are. Usually, for *HLA* allele to obtain the minimum four-digit resolution, complete sequence of exons 2 and 3 for *HLA* class I, and complete sequence of exon 2 for *HLA* class II is sufficient (Marsh et al., 2000; Marsh et al., 2010).

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2.5.3 Polymorphism in *HLA*

In human, *HLA* system is the most polymorphic region. The polymorphisms in this region is not widely spread out, but are concentrated in the region encoding the peptide binding groove. In the case of *HLA* Class I, α1 and α2 domains contain variable amino acid residues and are the determinant of the antigenic specificities of the molecules. Of all the *HLA* gene, the most polymorphic is *HLA-B*, which has more than 4000 alleles (Marsh et al., 2000; Robinson, 2015).

The larger number of non-synonymous mutation in the peptide binding groove of MHC suggest strong selection. Resistance to disease is also proposed as one of the drive for variation in MHC. The high diversification of *HLA-B* allele is thought to be associated with its efficiency in detecting RNA viruses that evolve quicker than DNA viruses. The MHC region has been associated with many disease than any other regions of the human genome. MHC region is densely clustered with genes and have strong linkage disequilibrium that make it hard to pinpoint the exact causative variant and the function it has. Even though autoimmune diseases were discovered to associate with changes in the groove resides, taking the example of amino acid resides 57 of *HLA-DQB* in type I diabetes, it is still not fully understood how polymorphisms in *HLA* lead to peptides in the *HLA* grooves to mediate autoimmune disease (Trowsdale & Knight, 2013).

Aside from *HLA* allele association to diseases, its distribution to specific regions and extensive polymorphism have been used to infer human migration and genetic diversity among different populations. For example, the most polymorphic allele in *HLA-B* locus, the *HLA-B*15* allelic lineage has a non-symmetrical distribution in Asian population, where the alleles in northeast Asia tend to be more prevalent in global population whereas the alleles found in southeast Asian (SAE) population are more specific to SEA region. To illustrate: the most common allele in B*15 lineage, *HLA-*

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*B*15:01*, is more prevalent in northeast Asia, whereas *HLA-B*15:02* is more prevalent in the south of Asia spreading southwards into SEA. *HLA-B*15:13* is more specific and it is observed within the Indonesian archipelago of SEA. The distribution of *HLA-B*15:02* also support the human migratory route into SEA (Di & Sanchez-Mazas, 2011; Solberg et al., 2008).

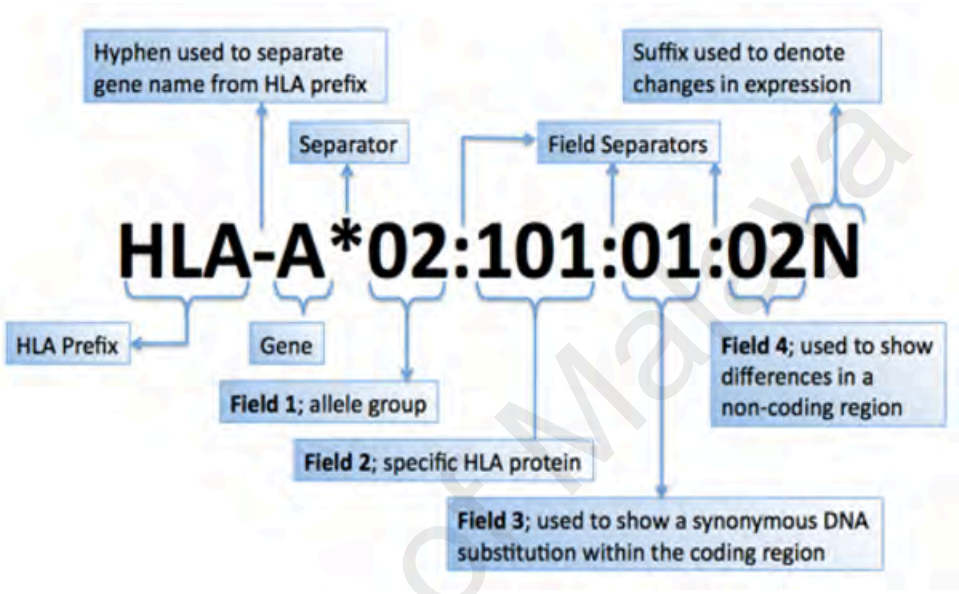

Figure 2.1: Standardized *HLA* nomenclature. Figure is adapted from Anthony Nolan Research Institute (http://hla.alleles.org/nomenclature/naming) (Robinson et al., 2015).

2.6 *HLA* allele: *HLA* association to SCARs-induced by antiepileptic drugs (AEDs)

2.6.1 *HLA-B* association to SJS/TEN-induced by antiepileptic drugs

The resemblances of SJS/TEN histopathological changes to allogeneic skin graft rejection of graft-vs-host disease elucidate an immunological mediated process (Roujeau et al., 1987). This is supported by SJS studies demonstrated findings of circulating immune complexes in serum samples and immunoglobulins in the vessels' wall of dermis of SJS patients (Wuepper et al., 1980). Genetic factors in SJS/TEN was further corroborated by occurrence of familial SJS in a pair of identical twin both caused by the same drugs. The mother and grandfather of the twins also have a history of cutaneous eruptions after ingestion of the same drugs. *HLA* phenotyping found a

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common haplotype (*A29, B44* and *DR7*) in the family (Fischer & Shigeoka, 1983). Immunogenic susceptibility in SJS was further supported by a study on patients with ocular lesion of SJS that has a significant increase of HLA-B44 antigen (Mondino et al., 1982). Taken together, these early studies established the link between *HLA* and a predisposed risk to drug hypersensitivity reactions.

Subsequently, a number of discoveries were made in association with specific *HLA* alleles and drug-induced SJS/TEN. The breakthrough discovery came from Chung et al. reporting the association of *HLA-B*15:02* and CBZ-SJS/TEN in Taiwan Han Chinese. *HLA-B*15:02* allele was found in 100% of CBZ-SJS/TEN cases and 3% of CBZ-tolerant controls and have a prevalent of 8.6% in the general population. It has the strongest strength of association reported thus far with an odds ratio of 2504 (95% CI 126-49,522) (Chung et al., 2004).

Subsequent studies further confirmed the association in other Asian populations such as China, Thailand, Vietnam, Singapore, India, Malaysia and Indonesia (Chang et al., 2011; Chong et al., 2014; Herlyani et al., 2017; Locharernkul et al., 2008; Mehta et al., 2009; Nguyen et al., 2015; Then et al., 2011; Wu et al., 2010). The association of *HLA-B*15:02* and CBZ-SJS/TEN was validated in Hong Kong Han Chinese and was also extended to include a weaker association to two other aromatic antiepileptic drugs, phenytoin and lamotrigine-induced SJS/TEN (Cheung et al., 2013). However, none of these association is as strong as that of CBZ-induced SJS/TEN. The association of *HLA-B*15:02* and PHT-SJS/TEN was also reported in Thai (Locharernkul et al., 2008; Tassaneeyakul et al., 2010). Two studies on west central and northern Indians from India demonstrate the association for *HLA-B*15:02* and CBZ-SJS/TEN, where the allele is present in 75% of cases but none in tolerant controls (Aggarwal et al., 2014; Mehta et al., 2009). But in contrast, the northern Indian study did not found association for *HLA-B*15:02* and PHT-SJS/TEN cases in which the allele is absent for both case

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and tolerant controls, reflecting the low prevalence of the allele in the population (2-6%) (Aggarwal et al., 2014). The association was also detected in Malaysian Malays, reporting the present of *HLA-B*15:02* alleles in 75% of CBZ-SJS/TEN cases, compared to a general Malay population frequency of 15.7% (Chang et al., 2011). The most recent study from this region came from Vietnam and Indonesia. Nguyen et al. reported the presence of *HLA-B*15:02* in 89.5% of CBZ-SCARs cases and 24% in tolerant controls, whereas it was noted that the prevalence of the *HLA-B*15:02* in the population is considerably high, 13.5% (Nguyen et al., 2015). In Indonesia, *HLA-B*15:02* allele was found in 57% of the CBZ-SJS/TEN cases and 26% of the tolerant controls (Herlyani et al., 2017). The association to *HLA-B*15:02* was demonstrated to be phenotype-specific as *HLA-B*15:02* does not shown association to MPE or DRESS induced by CBZ.

Conversely, the association was not found in Japanese and Koreans where the *HLA-B*15:02* frequency in the population is very low (<1%) (Kaniwa et al., 2010; Kim et al., 2011; Ozeki et al., 2011). The risk of *HLA-B*15:02* in predisposing to CBZ-SJS/TEN was not detected in all studies on Caucasian populations either. A European study, by RegiSCAR group, tried to replicate the association of *HLA-B*15:02* and CBZ-induced SJS/TEN in 12 European cases. They found four cases positive for *HLA-B*15:02*, of which all four patients have Asian ancestry and the other patients that are negative for *HLA-B*15:02* did not. In the other eight patients of European descent, no association to *HLA-B*15:02* or to another *HLA* allele was detected (Lonjou et al., 2006). The need to investigate genetic marker to SJS/TEN in Europeans prompted a genome wide association study of the largest sample size collected with enrollment of 563 SJS/TEN cases. The strongest associated SNP was found located close to *HLA-B* locus. Still no other locus reached the genome-wide association threshold. This study lead to the conclusion that *HLA-B*15:02* is not a marker for CBZ-induced SJS/TEN in Europeans population (Genin et al., 2011). This was also partly explained by the

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ethnicity-specific variation in allele frequency of *HLA-B*15:02*. In European populations, the allele frequency of *HLA-*15:02* is very low (<1%). This variation has also been correlated to the variation in incidence of CBZ-induced SJS/TEN.

In Japan and Korea, CBZ-SJS/TEN was associated with a different *HLA-B* allele, *HLA-B*15:11* (Kaniwa et al., 2010; Kim et al., 2011). Interestingly, both *HLA-B*15:11* and *HLA-B*15:02* alleles belongs to the same B75 serotype subfamily. A recent case study on CBZ-SJS/TEN in a *HLA-B*15:02*-negative Thai patient was shown to have another HLA-B75 members, *HLA-B*15:21* (Jaruthamsophon et al., 2017). The study further investigated the structural similarities between *HLA-B*15:02* and *HLA-B*15:21* alleles in terms of CBZ binding and presentation to cytotoxic T-cells.

2.6.2 *HLA-A* association to SJS/TEN-induced by antiepileptic drugs

Recently, *HLA-A*31:01* allele was found in association with all CBZ-induced hypersensitivity reactions (including MPE, DRESS and SJS/TEN) in Japanese and Europeans (Amstutz et al., 2013; McCormack et al., 2011; Ozeki et al., 2011). However, this association has not been consistently replicated. *HLA-A*31:01* was first reported associated to CBZ-maculopapular exanthema (MPE) in Han Chinese (Hung et al., 2006). Subsequent studies demonstrated that *HLA-A*31:01* has a stronger association to CBZ-DRESS compared with a much weaker association to CBZ-SJS/TEN in Han Chinese and Europeans (Genin et al., 2014). Due to inconsistent findings, association of *HLA-A*31:01* with CBZ-SJS/TEN still requires further investigation in European and Asian populations.

Across these studies, it is evident that *HLA* association to AEDs-induced SJS/TEN is both phenotype- and ethnicity specific (Table 2.1). Numerous studies in different Asian populations point to the possibility that besides *HLA-B*15:02* allele, other members of HLA-B75 family also predisposed risk to CBZ-SJS/TEN.

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Table 2.1: Ethnicity and phenotype specific association between *HLA* allele and AEDs-SCARs.

AEDs	***HLA* allele**	**Population/ Ethnicity**	**Phenotype**	**Reference**
Carbamazepine	*HLA-B*15:02*	Han Chinese, Thai, Malays, Indians, Vietnamese, Indonesian	SJS/TEN	(Chang et al., 2011; Chung et al., 2004; Herlyani et al., 2017; Mehta et al., 2009; Nguyen et al., 2015; Tassaneeyakul et al., 2010)
	*HLA-A*31:01*	Caucasians, Japanese	DRESS, MPE, SJS/TEN	(McCormack et al., 2011; Ozeki et al., 2011)
		Koreans	DRESS, SJS/TEN	(Kim et al., 2011)
		Han Chinese	DRESS, MPE	(Genin et al., 2014; S. I. Hung et al., 2006)
	*HLA-B*15:11*	Japanese, Koreans	SJS/TEN	(Kaniwa et al., 2010; Kim et al., 2011)
Phenytoin	*HLA-B*15:02*	Han Chinese, Thais	SJS/TEN	(Cheung et al., 2013; Locharernkul et al., 2008)
	*HLA-B*15:13*	Malays	SJS/TEN	(Chang et al., 2016)
Lamotrigine	*HLA-B*15:02*	Han Chinese	SJS/TEN	(Cheung et al., 2013; Zeng et al., 2015)

AEDs, antiepileptic drugs; SCARs, severe cutaneous adverse reactions; SJS, Steven-Johnson syndrome; TEN, toxic epidermal necrolysis; DRESS, Drug reaction with eosinophilia and systemic symptoms; CBZ, Carbamazepine; PHT, Phenytoin; LTG, Lamotrigine

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2.7 Pathogenesis of carbamazepine-induced Stevens-Johnson syndrome/Toxic epidermal necrolysis

2.7.1 SCARs Classification

The heterogeneous manifestation of SCARs feature is due to different regulatory cells that secrete cytokines. SCARs are classified under type IV hypersensitivity reactions according to the Gell and Coombs classification. Type IV reaction is a delayed-type of drug hypersensitivity mediated by T-cell with four subgroups according to the type of responsive T-cells and clinical presentation. IVa is mediated by type 1 T helper cells; IVb is mediated by T helper 2 cells, interleukins 4, 5 and 13 and eotaxin cytokines as found in DRESS; IVc is mediated by cytotoxic T-cells as found in SJS and TEN; and IVd is mediated by T cells and neutrophils through chemokine interleukin 8 (IL8/CXCL8) and granulocyte-macrophage colony-stimulating factor. CBZ-induced SJS and TEN is classified under IVb drug hypersensitivity reactions, as it is mediated by drug-specific cytotoxic T-lymphocytes (Harr & French, 2010).

2.7.2 Pathomechanism of SJS/TEN

The pathomechanism of SJS/TEN is not fully understood. Several studies on immunological mechanism of SJS/TEN have given valuable insight into the pathomechanism of SJS/TEN and in developing new methods for the treatment of SJS/TEN (Ko et al., 2011; Wei et al., 2012). The effect of wide spread keratinocytes apoptosis seen in SJS/TEN, which could not be explained by direct cell to cell contact of CTL alone, suggested the involvement of cytotoxic molecules. Analysis of blister fluid of SJS/TEN patients found various pro-inflammatory and anti-inflammatory cytokines. The massive T-cell meditated cell death observed in epidermal necrosis is attributed to three pathways, Fas-Fas ligand interaction, perforin-granzyme B pathway and granulysin-induced pathway. The most detrimental effect is by granulysin, which is responsible for the extensive keratinocyte necrosis without direct cell-to-cell contact.

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This forms the basis of epidermal detachment seen in SJS/TEN (Duong et al., 2017; Harr & French, 2010).

In the initial phase, histological findings showed abundant CTL in blister fluid. The clonal expansion of CD8+ T-lymphocytes is an immune response to a major histocompatibility class-I restricted drug presentation. These CD8+ T-lymphocytes was found to express cutaneous lymphocyte-associated antigen (*CLA*). *CLA* is a ligand recognized by adhesion molecules E-and P selectin, which are expressed by inflamed skin cells (Nassif et al., 2004). It was demonstrated that T-cells from blister fluid exerted drug-specific cytotoxic activity towards B-lymphocyte and keratinocytes through secretion of granzyme B. Another study showed that the sera of SJS/TEN patients, containing elevated level of soluble FasL (sFasL), were able to induce keratinocytes apoptosis (Abe et al., 2003; Murata et al., 2008). Another molecule, granulysin, has also been detected in high level in serum and blister fluid of SJS/TEN patients. The activity of granulysin was demonstrated when injecting intradermally into mice, it showed feature of SJS/TEN (Abe et al., 2009). Other study supported this with *in vitro* assay showing that drug-specific cytotoxic T lymphocytes (CTL) expressed large amount of granulysin after triggered by CBZ leading to extensive keratinocytes apoptosis and eventually necrosis seen in the epidermal layer. One study suggested utilising granulysin as a biomarker in the prognosis of SJS/TEN (Chung et al., 2008).

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2.7.3 HLA-drug interactions models

2.7.3.1 Hapten concept

Previous studies have proposed several models to explain the phenomena of T cells recognition of small compounds like drugs and how T cell mediate the immune response. The model, first proposed, is hapten model. Small chemically reactive drug molecules formed covalent bonds with larger protein or peptide, eliciting a humoral immune response.

Pro-hapten, after metabolized or intracellular processing, became chemically active are able to elicit B and T cells response to the drug-modified peptide or protein. This is demonstrated in the case of penicillin hypersensitivity, where penicillin binds to serum albumin and undergo intracellular processing generating modified peptides that are able to elicit immune response (Figure 2.2) (Pichler et al., 2011).

2.7.3.2 Altered repertoire model

Another hypothesis in pathogenesis of drug hypersensitivity is the altered peptide repertoire model. Drugs bind non-covalently to the *MHC* binding pocket altering the chemistry of the binding cleft and endogenous peptide repertoire. The new endogenous peptide presented leads to activation of cytotoxic T-cells. This model was conceptualized from the crystallographic structure of *HLA-B*57:01* bound with Abacavir and peptide. Abacavir hypersensitivity syndrome has been found with strong association to *HLA-B*57:01* allele. The peptide is presented by the *HLA-B*57:01* to TCR while the drug is bound below the peptide and not in contact with the TCR-peptide interaction. Abacavir is found to bind noncovalent to *HLA-B*57:01* molecule, causing changes in the peptides binding ability of *HLA-B*57:01* molecule causing alteration in the repertoire of endogenous peptides presented to TCR (Figure 2.2) (Illing et al., 2012).

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2.7.3.3 Pharmacologic interaction with immune receptors

The most relevant model to SJS/TEN induced by AEDs is the drug pharmacological interaction with immune receptors model: p-i concept. The drug or its metabolites binds directly to immune receptors such as HLA molecules or T-cell receptors (TCR) with noncovalent bonds without peptide ligand or intracellular processing. P-i reactions is attributed to the consequence of reactive T-cells. The component involved are the drug, HLA molecules on antigen presenting cells and T-cell receptor that subsequent leads to T cell stimulations. The p-i concept does not require metabolism of the drug or any processing. This is thought to be the case of CBZ-induced SJS/TEN. CBZ binds directly to HLA-B*15:02 molecules (Figure 2.2) (Pichler et al., 2015).

Functional studies on HLA-B in the pathogenesisof SJS/TEN was carried out when it was observed that approximately up to 25% of CBZ-SJS/TEN patients of Asia ancestry do not have HLA-B*15:02 allele, hence it cannot be explained by HLA molecules alone. The studies investigated the role of HLA-B molecules binding to CBZ and activating cytotoxic T lymphocytes (CTL) mediated cell death. Ko et al. found T cells from CBZ-SJS/TEN patients that are also HLA-B*15:02 positive have TCR clonotype of Vβ-11-ISGSY. This clonotype was present in 17 from the 19 CBZ-SJS/TEN patients while it was absent in all the CBZ-tolerant controls, suggesting requirement for cofactors that co-stimulate to develop a strong T cells stimulation (Ko et al., 2011). The mechanism of CBZ on T-cells response has also been compared to Abacavir. The CBZ-induced peptide repertoire shift is smaller than that observed in abacavir (Illing et al., 2012). The smaller shift also explained the more restricted TCR repertoire observed in reactive drug-specific T cells found in CBZ-SJS patients.

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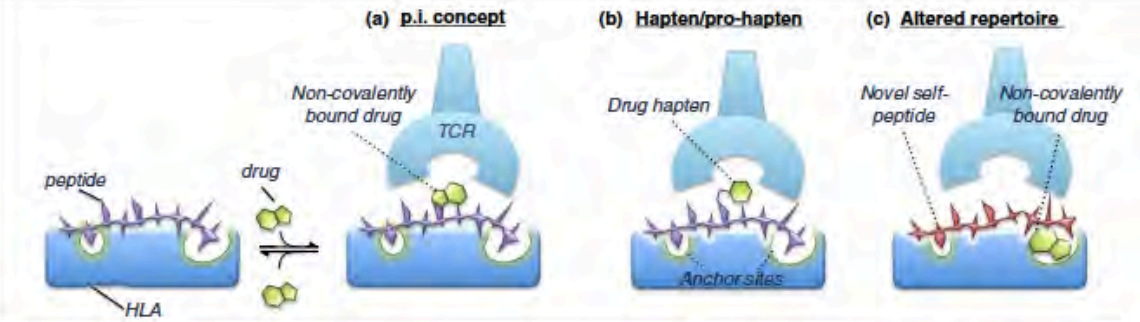

Nature of interaction	Non-covalent	Covalent	Non-covalent
Stability of complex	Labile (or interaction primarily with TCR?)	Equivalent to canonical pHLA	Equivalent to canonical pHLA
Maintained on washing of cells at physiological pH/mediated by drug pulsed cells	No	Yes	Yes
Site of immunogenic complex formation	Cell surface	ER or cell surface	ER *(or cell surface?)*
Active antigen processing pathways required for generation	No	Yes (intracellular hapten formation) or No (cell surface hapten formation)	Yes
Influence on peptide repertoire	Minimal	Introduction of neo-epitopes	Change in peptide-binding motif

Figure 2.2: Models of interaction between human leukocyte antigen (HLA), small molecule (drug), peptide repertoire and T-cell receptor. (a) p.i. concept: The drug binds directly and noncovalently to HLA-peptide complex or to TCR on cell surface without intracellular antigen processing. The impact of drug on the peptide repertoire is less than the other two interaction models. (b) Hapten/pro-hapten concept: The drug or its reactive metabolite acts as hapten and binds covalently to endogenous peptides where the covalent modification to endogenous peptides generated neo-epitopes that are then presented on cell surface. These novel peptide-HLA–drug complexes are recognised as foreign by TCR. The immunogenic complexes generated are antigen-processing dependent. (c) Altered repertoire model. The drug interacts with antigen-binding groove in HLA molecules, altering its conformation and thereby altering its binding to peptide ligand residues. This resulted in selection of peptides with novel HLA binding motif. The peptide-HLA (pHLA) complex is stable and require antigen processing pathway. Adapted from (Illing et al., 2016)

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CHAPTER 3: METHODOLOGY

3.1 Patient recruitment

3.1.1 Cases

Patients with CBZ, PHT and LTG-induced SCARs were recruited by retrospective review of medical records from University Malaya Medical Centre (UMMC) (from 1991 to 2015) and Hospital Sultanah Bahiyah, Kedah (from May 2012 to December 2015). This study was approved by UMMC medical ethics committee (No: 950.49) and Ministry of Health medical research and ethic committee (No.: nmrr-13-1157-16170).

Drug causality of SJS/TEN was identified based on temporal relationship of drug intake and reactions onset. And assessed with Algorithm of drug causality for epidermal necrolysis (ALDEN). All cases were classified as probable or very probable with score of ≥6 (Sassolas et al., 2010).

Diagnosis of SJS and TEN was based on clinical presentation and confirmed by dermatologists according to criteria by Roujeau and Stern (1994). SJS, SJS/TEN and TEN were distinguished by extend of body surface area detachment (SJS <10%; SJS/TEN overlap 10-30%; TEN >30%) (Bastuji-Garin et al., 1993). In this study, different severity of SJS, SJS-TEN overlapped syndromes were grouped collectively as SJS/TEN.

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3.1.2 Drug tolerant controls

Drug tolerant controls were patients receiving CBZ, PHT and LTG for at least three months without any adverse reactions. All tolerant controls were recruited from UMMC Neurology clinic. Tolerant control medical information and history of AEDs use were collected.

All participants in case and control groups were not of mixed ethnicity, as determined by the ethnicity of biological parents and grandparents. Written informed consent was obtained from all participants. Approximate 6ml of venous blood sample was obtained from each participant. In the event that blood sampling was not feasible, buccal swab was obtained.

3.2 *HLA-A* and -*B* allele genotyping

Genomic DNA was extracted from blood samples using QiaAmp DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. *HLA-A* and -*B* genotyping was performed with WAKFlow HLA typing kit (Wakunaga Pharmaceutical Co. Ltd, Japan) and analysed by Luminex 200 (Luminex, Austin, TX, USA) following the manufacturer's instructions.

3.3 Statistical Analysis

Comparisons of carrier frequencies of a particular *HLA-A* or -*B* allele between groups were performed with Fisher's exact test. P-value is two-tailed. P-value < 0.05 indicated statistical significance. Corrected *Pc*-value for multiple testing was performed with Bonferroni correction. The strength of association was calculated using 2x2 contingency table, and presented as odds ratio, OR and 95% confidence interval, CI. Haldane's modification was used to counter for zero count cells in OR calculation. Sensitivity and specificity, positive and negative predictive values (PPV and NPV) were

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also calculated from 2x2 contingency tables. Demographic characteristics of case and control in terms of age and sex were compared using non-parametric test, e.g. Mann-Whitney test for continuous data and Chi-square test of independent for dichotomous data. Association analysis of combined samples was performed using Cochran–Mantel–Haenszel 2×2×3 test (CMH) controlling for the differences among ethnic groups, and Breslow–Day test was used to evaluate the heterogeneity of OR among the ethnic groups. To study the effect of *HLA-A* and *-B* alleles on each other, logistic regression with the allele as a covariate under dominant model were performed. All statistical test was performed with PLINK (www.cog-genomics.org/plink2) (Purcell et al., 2007). Linkage disequilibrium analysis was performed with Haploview (www.broadinstitute.org/haploview/haploview) (Barrett et al., 2005).

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CHAPTER 4: RESULTS

4.1 Demographic characteristic of cases and tolerant controls

The characteristics of cases and tolerant controls are illustrated in Table 4.1. A total of 36 patients with AEDs-SJS/TEN, 3 AEDs-DRESS and 273 AEDs-tolerant controls were recruited in this study. Both cases and tolerant controls are categorised according to causal drugs and further sub-grouped by ethnicity. In total, there is 28 CBZ-SJS/TEN (16 Malay, 6 Chinese and 6 Indian), 5 PHT-SJS (1 Malay, 2 Chinese and 2 Indians) and 3 LTG-SJS (1 Malay, 1 Chinese and 1 Indian) (Table 4.1). The three DRESS cases were consisted of 2 PHT-DRESS (1 Malay, 1 Chinese) and 1 CBZ-DRESS (1 Malay). For the AEDs tolerant controls subjects, they were grouped by the anti-epileptic drugs that they were tolerant to: 227 CBZ (57 Indian, 64 Malay, 106 Chinese), 61 PHT (31 Malay, 30 Chinese) and 136 LTG (36 Indian, 44 Malay, 56 Chinese). Hence the AEDs tolerant controls overlapped. This is to prevent sampling bias. There is no significant difference in age and sex between the SJS/TEN groups and the AEDs-tolerant controls groups. Demographic characteristics of DRESS cases were not compared to the AEDs-tolerant controls due to small sample size (Table 4.1). The following association analysis workflow for *HLA-A* and *-B* allele with AEDs-SCARs in the three ethnic groups is shown in Figure 4.1.

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Table 4.1: Demographic characteristics of cases with AEDs-SCARs and AEDs-tolerant controls.

		DRESS cases (*n*=3)	SJS/TEN cases (*n*=36)	AEDs-tolerant controls (*n*=273)	*P*-value*
Sex, *n* (%)					
	Female	2 (66.7)	18 (50.0)	132 (48.4)	
	Male	1 (33.3)	18 (50.0)	141 (51.6)	0.86
Age (years), mean (range)		53.7 (30-66)	38.1 (9-71)	37.5 (13-78)	0.99
Race, *n* (%)					
	Malay	2 (66.7)	18 (50.0)	83 (30.4)	
	Chinese	1 (33.3)	9 (25.0)	119 (43.6)	
	Indian	-	9 (25.0)	71 (26.0)	
Causal AED, *n* (%)					
	CBZ	1 (33.3)	28 (77.8)	227 (83.2)[a]	
	PHT	2 (66.7)	5 (13.9)	82 (30.0)[a]	
	LTG	-	3 (8.3)	136 (49.8)[a]	

* Comparisons were preformed between SJS/TEN cases and AEDs-tolerant controls. DRESS cases were not compared due to limited case number.
[a] AEDs-tolerant controls categorised into CBZ, PHT and LTG were overlapped (n/273).
AEDs, Antiepileptic drugs; SJS, Steven-Johnson syndrome; TEN, toxic epidermal necrolysis; DRESS, Drug reaction with eosinophilia and systemic symptoms; CBZ, Carbamazepine; PHT, Phenytoin; LTG, Lamotrigine

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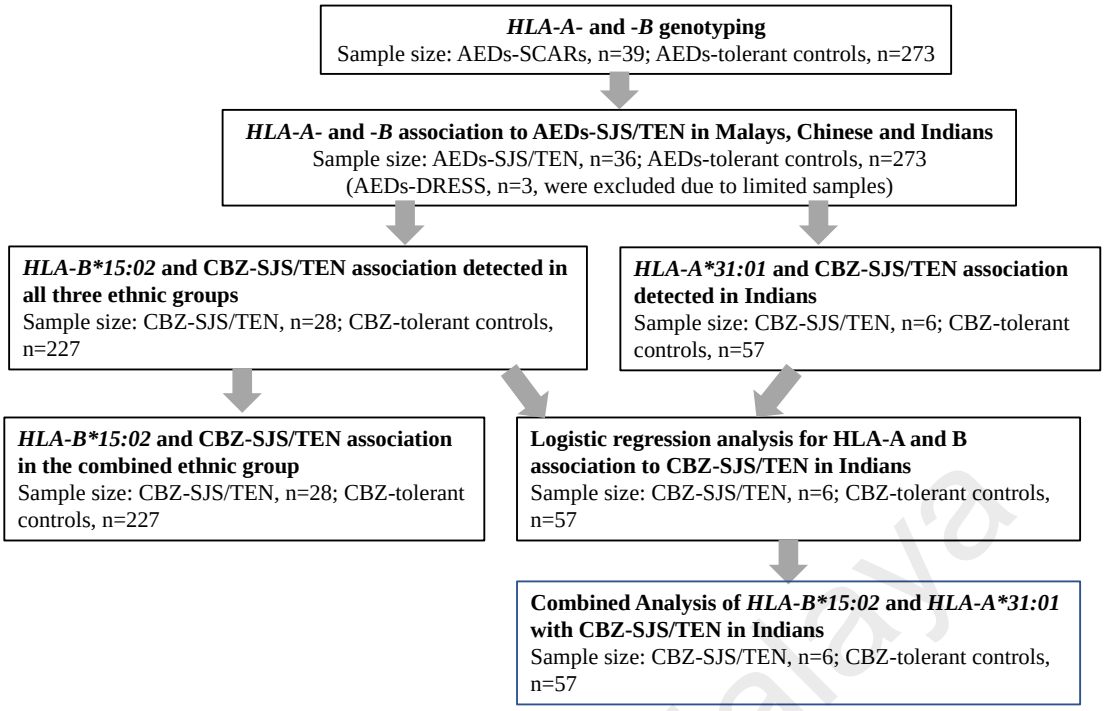

Figure 4.1: Association analysis workflow of *HLA-A* and *-B* with AEDs-SCARs in Malays, Chinese and Indians.

4.2 *HLA-B* association to SCARs-induced by antiepileptic drugs

4.2.1 *HLA-B* alleles of AEDs-SJS/TEN cases

Specific *HLA-B* alleles that were present in AEDs-SJS/TEN cases and their allele frequencies were shown in Appendix A, B and C, categorised according to ethnicity.

In the Malay CBZ-SJS/TEN cases, 16 *HLA-B* alleles were detected and *HLA-B*15:02* allele has the highest allele frequency (46.9%). Similarly, in Chinese CBZ-SJS/TEN cases, 7 alleles were detected and *HLA-B*15:02* has the highest allele frequency (33.3%). The same was found for Indian CBZ-SJS/TEN cases, in the 9 *HLA-B* alleles detected, *HLA-B*15:02* allele frequency was the highest (16.7%). However, in Indians, in addition to *HLA-B*15:02*, two other alleles, *HLA-B*07:05* and *HLA-B*51:01*, were also found at 16.7% in CBZ-SJS/TEN cases. Other than *HLA-B*15:02*, the other *HLA-B* alleles were not used in case-control comparison because of low

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frequency (less than 10% allele frequency in either case or tolerant control group) (Appendix A, B and C).

4.2.2 *HLA-B*15:02* association to AEDs-SJS/TEN cases

Of the 28 CBZ-SJS/TEN cases and 227 CBZ-tolerant controls, association analysis was performed in the respective ethnic groups. In terms of carrier frequency, *HLA-B*15:02* was detected in 87.5% of Malay, 66.7% of Chinese and 33.3% of Indian patients. Significant association was found in Malay (87.5% versus 12.5%; $p = 2.00 \times 10^{-8}$; Odds ratio (OR) = 49.0; 95% confidence interval (CI) = 9.4 – 256.8), with a sensitivity of 87.5% and specificity of 87.5%, Chinese (66.7% versus 12.3%; p = 0.0047; OR = 14.3; 95% CI = 2.4 – 86.0) with a sensitivity of 66.7% and specificity of 87.8% and Indian (33.3% versus 3.5%; p = 0.042; OR = 13.8; 95% CI = 1.5-125.0) with a sensitivity of 33.3% and specificity of 96.5% (Table 4.2).

Of the 8 cases of PHT and LTG induced SJS, *HLA-B*15:02* was detected in only 1 of the Malay PHT-SJS subject and none was found in LTG-SJS cases. No other *HLA-B* allele reach statistical significant in AEDs-SJS/TEN (Table 4.2). A post-hoc power analysis was performed for *HLA-B*15:02* in PHT and LTG-SJS/TEN cases. Based on 10% of the controls carried the risk allele, the current sample size has 19% power for Malay cohort and 30% power for Chinese cohort. Both Malay and Chinese cohort requires a minimum of 7 cases to reach 80% power. While for Indian, based on 3.5% of risk allele in controls the current sample size has 24% power. It requires 9 cases to reach 80% power.

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Table 4.2: Association between *HLA-B*15:02* and CBZ, PHT and LTG-SJS/TEN.

	*HLA-B*15:02* carrier frequency N/total in group (%)		OR (95% CI)	*P*-value
	SJS/TEN cases (*n*=28)	AED-tolerant controls (*n*=227)		
Carbamazepine				
Malay	14/16 (87.5)	8/64 (12.5)	49.0 (9.35 - 256.8)	2.00×10^{-8}
Chinese	4/6 (66.7)	13/106 (12.3)	14.3 (2.38 - 86.0)	0.0047
Indian	2/6 (33.3)	2/57 (3.5)	13.8 (1.51 - 125.0)	0.042
Phenytoin	(*n*=5)	(*n*=82)		
Malay	1/1 (100.0)	6/31 (19.4)	11.8 (0.43 - 323.8)	0.22
Chinese	0/2 (0.0)	2/30 (6.7)	2.28 (0.08 - 61.8)	1
Indian	0/2 (0.0)	1/21 (4.8)	2.73 (0.08 - 86.9)	1
Lamotrigine	(*n*=3)	(*n*=136)		
Malay	0/1 (0.0)	6/44 (13.6)	1.97 (0.07-53.9)	1
Chinese	0/1 (0.0)	8/56 (14.3)	1.90 (0.07-50.7)	1
Indian	0/1 (0.0)	2/36 (5.6)	4.6 (0.15-144.7)	1

$P < 0.05$ is considered significant
AEDs, Antiepileptic drugs; SJS, Steven-Johnson syndrome; TEN, toxic epidermal necrolysis; CBZ, Carbamazepine; PHT, Phenytoin; LTG, Lamotrigine

4.2.3 Association between *HLA-B*15:02* and CBZ-SJS/TEN in the combined ethnic group

A combined association analysis of *HLA-B*15:02* allele with CBZ-SJS/TEN in the three ethnic groups were performed by pooling into a stratified analysis using Cochran-Mantel-Haenszel test. In total, there is 28 CBZ-SJS/TEN cases and 227 CBZ tolerant controls. The overall association was found to be highly significant (P_{CMH} = 2.31×10^{-26}, OR_{CMH} = 26.6; 95% CI = 12.8 – 55.3). Breslow–Day test did not show significant evidence for odds ratio heterogeneity between ethnic groups (P_{BD} = 0.26) (Table 4.3).

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Table 4.3: Association between *HLA-B*15:02* allele and CBZ-SJS/TEN stratified by ethnicity.

Subgroup (Ethnicity)	OR	95% CI	OR_{CMH}	95% CI	P_{CMH}	P_{BD}
Malay	49.0	9.35 - 256.8				
Chinese	14.3	2.38 - 86.0				
Indian	13.8	1.51 - 125.0				
			26.6	12.8 - 55.3	2.31×10^{-26}	0.26

OR_{CMH}: Cochran Mantel-Haenszel odds ratio; P_{CMH}: *p*-value for Cochran Mantel-Haenszel test
P_{BD}: Breslow-Day test *p*-value

4.2.4 *HLA-B* allele and AEDs-DRESS cases

Of the 3 DRESS cases, 2 were induced by PHT (1 Malay, 1 Chinese) and 1 was induced by CBZ (1 Malay). One of 2 of the PHT-DRESS patient (Malay) was positive for *HLA-B*15:02*. The CBZ-DRESS patient was also positive for *HLA-B*15:02*. Due to limited sample, no association analysis was conducted for DRESS cases. Allele frequency of the other *HLA-B* alleles detected in CBZ-DRESS cases were shown in Appendix A and B.

4.3 *HLA-A* association to SCARs-induced by antiepileptic drugs

4.3.1 *HLA-A* alleles of AEDs-SJS/TEN cases

Specific *HLA-A* alleles that were present in AEDs-SJS/TEN cases and their allele frequencies were shown in Appendix D, E and F according to ethnicity. In Malay CBZ-SJS/TEN cases, a total of 15 *HLA-A* alleles were detected and *HLA-A*11:01* has the highest allele frequency (21.9%). In Chinese CBZ-SJS/TEN cases, 9 *HLA-A* alleles were found and *HLA-A*11:01* also presented with the highest allele frequency (25.0%). Whereas in Indian, 8 *HLA-A* alleles were detected and the highest frequency were found for *HLA-A*31:01* (Appendix D, E and F).

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4.3.2 *HLA-A* association to AEDs-SJS/TEN cases

*HLA-A*31:01* was detected in 3 of the 6 Indian CBZ-SJS/TEN subjects with a significant association (carrier frequency: 50% versus 8.8%, p = 0.023; OR = 10.4; 95% CI = 1.6-65.8). *HLA-A*31:01* was not present in both Malay and Chinese CBZ-SJS/TEN cases, and the carrier frequency in Malay and Chinese CBZ-tolerant controls was detected at 6.3% and 2.8%, respectively (Table 4.4). *HLA-A*02:06* was found with significant association in Indian CBZ-SJS/TEN subjects against CBZ tolerant controls (33.3% versus 1.8%, p = 0.022; OR = 28.0; 95% CI = 2.1-379.3) (Table 4.5).

*HLA-A*11:01* detected with high frequency in Malay and Chinese CBZ-SJS/TEN cases, were also found with similar frequency in the CBZ-tolerant controls of both ethnic groups (17.2% in Malay CBZ-tolerant controls and 23.6% in Chinese CBZ-tolerant controls). Thus, comparisons were not conducted for *HLA-A*11:01* as the allele frequency are similar for both case and control groups.

In PHT and LTG-induced SJS cases, no allele was statistically significant. *HLA-A*31:01* was not detected in either PHT or LTG induced SJS cases (Table 4.4).

4.3.3 *HLA-A* allele in AEDs-DRESS cases

*HLA-A*31:01* was not detected in all 3 cases of DRESS (2 PHT-DRESS and 1 CBZ-DRESS). For the CBZ-DRESS case, *HLA-A*24:02* was detected (Appendix D). Due to limited sample, no association analysis can be conducted for DRESS cases.

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Table 4.4: Association of *HLA-A*31:01* alleles and CBZ-SJS/TEN.

Ethnicity	*HLA-A*31:01* carrier frequency N/total (%) CBZ-SJS/TEN cases	CBZ-Tolerant control	OR (95% CI)	*P*-value
Malay	0/16 (0)	4/64 (6.3)	0.4 (0.02 - 7.96)	0.58
Chinese	0/6 (0)	3/106 (2.8)	2.3 (0.1 - 48.9)	1.00
Indian	3/6 (50.0)	5/57 (8.8)	10.4 (1.6 - 65.8)	0.02

SJS, Steven-Johnson syndrome; TEN, toxic epidermal necrolysis; CBZ, Carbamazepine

4.4 Logistic Regression analysis for *HLA-A* and *HLA-B* alleles associated with CBZ-SJS/TEN in Indians

To assess whether the association of *HLA-A* alleles detected is independent of *HLA-B*15:02* status in the Indian cohort, the subsequent association was performed conditioning for HLA-B*15:02. We found *HLA-A*02:06* have a dependent effect on *HLA-B*15:02* demonstrated by diminished association of *HLA-A*02:06* after adjusting for *HLA-B*15:02* (P = 0.112). The pairwise LD between *HLA-A*02:06* and *HLA-B*15:02* is found to be moderate (D' = 0.644; r^2 = 0.306). In contrast, *HLA-A*31:01* demonstrated an independent effect from *HLA-B*15:02* as the association persisted even after conditioning for *HLA-B*15:02* (P = 0.019). This is also supported by the low LD observed between *HLA-A*31:02* and *B*15:02* (D' = 0.141; r^2 = 0.009) (Table 4.5).

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Table 4.5: Conditional logistic regression for the association between *HLA-A* allele and CBZ-SJS/TEN in Indians.

Indians	Carrier frequency, n/total (%)		Odds Ratio (95% CI)	*P*-value
	CBZ-SJS/TEN case	CBZ-tolerant control		
*HLA-A*02:06*	2/6 (33.3)	1/57 (1.8)	28.0 (2.07 – 379.27)	0.012
*HLA-A*02:06* [a] Conditioned for *HLA-B*15:02*			12.3 (0.56 – 268.80)	0.112
*HLA-A*31:01*	3/6 (50.0)	5/57 (8.8)	10.4 (1.64 – 65.80)	0.013
*HLA-A*31:01* [a] Conditioned for *HLA-B*15:02*			11.7 (1.49 – 91.11)	0.019

[a] The conditioning allele is treated as a covariate.
SJS, Steven-Johnson syndrome; TEN, toxic epidermal necrolysis; CBZ, Carbamazepine

4.5 Combined analysis of *HLA-B*15:02* and *HLA-A*31:01* with CBZ-SJS/TEN in Indians

In a combined analysis of both risk alleles, i.e. *HLA-A*31:01* and *HLA-B*15:02*, three CBZ-SJS/TEN cases carried either risk allele and one patient carried both the risk alleles (4/6, 66.7%) whereas only 7 in 57 (12.3%) of CBZ-tolerant patients were carriers for either risk marker (*P* = 0.0068; OR 14.3; 95% CI 2.2-92.9), giving a sensitivity and specificity of 66.7% and 88.7%, respectively (Table 4.6).

Table 4.6: Combined analysis of *HLA-B*15:02* and *HLA-A*31:01* in CBZ-SJS/TEN in Indians

	*Combined carrier frequency** N/total (%)		OR (95% CI)	*P*-value	P_C-value
	CBZ-SJS/TEN case	CBZ-Tolerant control			
Indian	4/6 (66.7)	7/57 (12.3)	14.3 (2.2 - 92.9)	0.0068	0.0204*

*carried *HLA-A*31:01* or/and *HLA-B*15:02*
*adjusted for multiple comparisons (n=3)

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CHAPTER 5: DISCUSSION

5.1 Overview: Population structure of Malaysia

The Malaysian population is unique where it is multi-ethnic and composed of three major ethnic groups, Malays (67.4 %), Han Chinese (24.6%) and Indians (7.3%), in addition to other minority ethnic group (0.7%) (such as Ibans, Bidayuh, Kadazan and Dusun) (Department of Statistics, 2014-2016). Malaysia's multi-ethnic population consisted of subpopulation from different genetic lineage contributing to a diverse and complex genetic makeup. The diverse ethnic composition of Malaysia population is illustrated by historical migratory events from neighbouring countries that shaped the population. Malaysia is situated along a major trading route between the East and West. In the 6th to 7th century, Melaka strait became an important maritime trading route and the sea traffic contributed immensely to establishing this region as a major entreport for international traders from India, China and Southeast Asia (Bellwood, 1993). This historical event shaped the interaction between Malay, India, Arab and China with the indigenous tribes (*Orang Asli*) in Malaysia. During the European colonisations (starting from 16th century), large groups of immigrant labours were brought in such as Chinese and Indian to work in tin mines and rubber plantations. The trading ports also attracted Malay subethnic groups from the nearby Indonesia archipelago for example from Sumatra, Java, Sulawesi and Kalimantan (Saw, 2007). Gene inflow from Chinese, Indians and Arabs traders with the contemporary Malays and *Orang Asli* tribes plus the European colonists have significant effect on the genepools (Norhalifah et al., 2016).

Population genetic structure studies revealed that among the three ethnic groups researched, Indians is more differentiated than Chinese and Malays, while the Chinese and Malays are closer (Hatin et al., 2011; Teo et al., 2009). The Malaysia Malay ethnic group are comprised of four major ancestral lineages (Austronesian, Proto-Malay, East Asian and South Asian). The predominant lineages are of Austronesian and Southeast

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Asian aborigines in which Proto-Malays and aboriginal Taiwanese (Ami and Atayal) made up the largest component (Deng et al., 2015; Ko et al., 2014). The Chinese ethnic group comprised of Han Chinese descendants from southern provinces of China such as Guangdong and Fujian (Teo et al., 2009). Population structure studies show that Singaporean Chinese, which is of the same ethnic group as Malaysian Chinese, clustered together with Chinese sub-ethnic groups from Southeast Asia and Southern China (Teo et al., 2009). Indian ethnic group of Malaysia are mainly consisted of descendants from *Telugas* and Tamils from India south eastern region with a minority of Pathans and Sikhs from the northern part of India (Teo et al., 2009). Singaporean Indian, which is also of the same ethnic composition as Malaysian Indian, were shown to be closer to Central and South Asia population and European population. Substantial genetic variability was noted within the Indian cohort that was attributed it to sub-ethnicities that existed in Indian population (Teo et al., 2009). The aspect of Indian population's sub-ethnicities leading to genetic variability is further discussed in section 5.2.

5.2 *HLA-B* association to CBZ-induced SJS/TEN

As the association of *HLA-B* allele and CBZ-SJS/TEN has not been researched in the Indian ethnic group of Malaysia, this aspect is of much interest. So far, there were two published studies on association of *HLA-B*15:02* and CBZ-SJS/TEN in Indians. One study sampled from Modasa, a city in Northwest India in the state of Gujarat (Mehta et al., 2009) while the second study were from Chandigarh, a city in the North India (Aggarwal et al., 2014). However, in India, the allele frequency of *HLA-B*15:02* is not homogeneously distributed (Gonzalez-Galarza et al., 2015). Indian populations are genetically diverse due to different waves of immigration resulting in subdivided castes and tribes which is further subdivided by geography, language and religion

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forming a non-panmictic population, in which the mating within the breeding population is not random. This is supported by evidence that groups in India inherited different proportions of ancestry from Ancestral North Indians and Ancestral Southern Indians, two genetically diverse ancient populations (Reich et al., 2009). The most recent immigration is Indo-European speakers that entered India primarily from the northwest establishing the Hindu caste system and after initial admixture, the indigenous Dravidian-speakers were displaced and migrated southward. Population structure studies showed admixture of Central and West Asians population with Northern Indian population as evidenced by lower genetic differentiation between Central Asian and Northern Indian population than with Southern Indian populations. Indian population can be broadly classified into Northern Aryans and Southern Dravidians (Basu et al., 2003).

*HLA-B*15:02* allele frequency in Indian population ranges from 0-6% depending on region (0% in west coast Parsi, 1.6% in northern Indian, to 4% in west Bhil, and 6% in Pawra in Khandesh region) (Gonzalez-Galarza et al., 2015), but not available for southern India. This study fills a gap in the prevalence of *HLA-B*15:02* among Malaysia Indian ethnic groups, which are predominantly Southern Indian.

Earlier in the course of this study, in the Indian cohort of 5 CBZ-SJS/TEN cases and 52 CBZ-tolerant controls, I reported a significant association between *HLA-B*15:02* and CBZ-SJS/TEN (40% vs. 3.8%, OR 16.7, 95% CI 1.70–163.0, p = 0.0349), despite a low carrier frequency of *HLA-B*15:02* in Indians (3.8%) (Khor et al., 2014). It is also the first reported association between *HLA-B*15:02* and CBZ-SJS/TEN in cohort of predominantly Southern Indians. At that time of the study, meta-analysis was carried out between my Indian cohort with Mehta et al. from Northwest India (Mehta et al., 2009) as it was the only published study on CBZ-SJS/TEN in Indians. The combined studies shown strong association (OR=38.54; 95% CI=6.83–217.34, p < 1.03

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x 10^{-4}) confirming *HLA-B*15:02* as a valid pharmacogenetic marker for CBZ-SJS/TEN in the Indian population. At the end of this study, with the increase in sample size (addition of 1 CBZ-SJS/TEN and 5 CBZ-tolerant controls in Indians) I further confirm the association between *HLA-B*15:02* and CBZ-SJS/TEN in Indians.

Furthermore, this study replicated the association between *HLA-B*15:02* and CBZ-SJS/TEN in Malay and Chinese. The association was confirmed between *HLA-B*15:02* and CBZ-SJS/TEN separately in each ethnic group as well as in the combined analysis of all the three ethnic groups, whereby Breslow-Day test demonstrated non-significant heterogeneity of OR between the ethnic groups. The reason the association was analysed separately according to ethnicity is to avoid population stratification. Allele frequencies are known to vary among different ethnic population due to different genetic background, ancestral migration patterns, social history or mating practices. This is even more striking for *HLA* alleles whose frequencies differ greatly by ethnicity (Gonzalez-Galarza et al., 2015). These frequency discrepancies may cause confounding issues if not controlled for. One way to reduce stratification is to match control to case by sampling the control from the same source population from which cases are sampled, hence keeping allelic differences to a minimum. Another approach is structure assessment by subdividing into homogenous population (ethnicity), hence the subgroups are matched appropriately. The association test is performed in each matched subgroup before each subgroup is statistically combined to obtain the total test for disease association. Thus, the analysis for association between *HLA-B*15:02* and CBZ-SJS/TEN in the three ethnic groups were conducted in this manner. A post-hoc power analysis was performed for *HLA-B*15:02* in CBZ-SJS/TEN cases. Based on 10% of the controls carried the risk allele (*HLA-B*15:02*) and a sample of 16 cases, a 4:1 ratio of controls to cases have 99% power for Malay, whereas in the Chinese cohort based on the same frequency of 10% of risk allele in controls in a sample of 6 cases, a 17:1 ratio

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of controls to cases have 86% power. As for *HLA-B*15:02* in Indian CBZ-SJS/TEN cases, based on 3.5% of the controls carried the *HLA-B*15:02* allele and a sample of 6 cases, a 9:1 ratio of controls to cases have 61% power. Therefore, this study has more than 80% power to detect an effect size in the Malay and Chinese cohort. However, in the Indian cohort, the power did not reach 80% due to a lower *HLA-B*15:02* frequency in controls.

Nonetheless, this present study investigated by far the largest number of AEDs-SCARs cases and AEDs tolerant controls representing the three major ethnic groups in Malaysia. Other than *HLA-B*15:02*, no other *HLA-B* allele was significantly associated with CBZ-SJS/TEN in this study (Gonzalez-Galarza et al., 2015).

5.3 *HLA-A* association to CBZ-induced SJS/TEN

A significant association was detected between *HLA-A*31:01* and CBZ-SJS/TEN in Indian ethnic group, which is made up of southern Indians. In the Indian cohort, *HLA-A*31:01* test has a 50% sensitivity and 91.2% specificity for detecting CBZ-SJS/TEN. The association between *HLA-A*31:01* and CBZ-induced hypersensitivity reactions was previously reported in Europeans, Japanese and Hans Chinese populations. *HLA-A*31:01* had been associated with different CBZ-induced hypersensitivity phenotypes ranging from mild rash to SJS/TEN and DRESS in a few populations but the association to CBZ-SJS/TEN was comparatively much weaker (Amstutz et al., 2014; Genin et al., 2014). The different association detected may also be influenced by background allele of the population, as seen in the prevalence of *HLA-B*15:02*, where carrier frequency is higher among the south Asian population (12-15.7% in Malay, 14.5% in Han Chinese, 13.5% in Vietnamese, 18% in Thai), in contrast, in Indian it is at 3.5% and it is much lower in European (< 1%), Japanese (1-2%), and Korean (1-2%) populations. Whereas *HLA-A*31:01* has a more average

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background carrier frequency in these populations (2.8% in Han Chinese, 7.1-12% in Japanese, 5.4% in Koreans and 2-4.1% in Caucasian) and the allele frequency of *HLA-A*31:01* (ranges at 1.9-18.9%) observed in Indian is also higher compared to *HLA-B*15:02* frequency (2-6%) (Gonzalez-Galarza et al., 2015).

However, *HLA-A*31:01* was not detected in any of the 3 AEDs-DRESS patients, even though several studies have consistently demonstrated that *HLA-A*31:01* is more strongly associated with CBZ-DRESS than with CBZ-SJS/TEN. Similarly, in this Malay and Chinese CBZ-SJS/TEN cohorts, the association between *HLA-A*31:01* and CBZ-SJS/TEN was not detected and the carrier frequency was found at 6.3% and 2.8% in Malay and Chinese tolerant controls, respectively. The reported association of *HLA-A*31:01* and CBZ-induced hypersensitivity are more diverse and not exclusive to one phenotype of drug hypersensitivity reactions unlike the association of *HLA-B*15:02* with CBZ-SJS/TEN. The mechanism behind *HLA-A*31:01* association to various CBZ-hypersensitivity phenotypes is not known. As most of the research on CBZ-hypersensitivity reactions were on *HLA-B*15:02* interaction with CBZ, peptides and TCR (this topic is discussed in section 5.7.2), studies investigating *HLA-A*31:01* interaction in CBZ-hypersensitivity is very limited. So far there is only one case study that show activation of CBZ-specific $CD8^+$ T-cells restricted by *HLA-A*31:01* and CBZ-specific $CD4^+$ T cells restricted by *HLA-DRB1*04:04* (Lichtenfels et al., 2014). Illing et al. suggested that *HLA-A*31:01*, sharing 2 of the 3 key amino acid residues (Ile95 and Leu156 but not Asn63) involved in CBZ binding to *HLA-B*15:02*, may play a role in the interaction with CBZ (Illing et al., 2013). However, it is still unclear why *HLA-B*15:02* is specific to CBZ-SJS/TEN whereas *HLA-A*31:01* predispose to various CBZ-hypersensitivity. The molecular mechanism of *HLA-A*31:01* in CBZ-hypersensitivity and its interaction with TCR will required further study.

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But given previous findings, our data increased the potential clinical utility of *HLA-A*31:01* genetic screening test in predicting CBZ-SJS/TEN in Southern Indians. A post-hoc power analysis for *HLA-A*31:01* in Indian CBZ-SJS/TEN cases was performed based on a frequency of 8.8% of risk allele in controls and a sample of 6 cases, a 9:1 ratio of controls to cases have 66% power. Therefore, combined analysis of both *HLA-A*31:01* and *HLA-B*15:02* risk variants improved the predictive power in identifying patient with increased risk for CBZ-SJS/TEN in Indian population where *HLA-B*15:02* frequency is lower and not homogeneous. Although my study is limited by small CBZ-SJS/TEN cases in Indians, and further confirmation of this association is needed, the findings support genetic testing for both *HLA-B*15:02* and *HLA-A*31:01* in Indian patients *prior to* CBZ treatment.

5.4 *HLA* association to PHT and LTG-SJS/TEN

No *HLA-A* and *-B* allele reach statistical significant in PHT and LTG-SJS/TEN cases. Post-hoc power analysis shown the current sample size lacked power to detect an effect size. A minimum of 9 cases for Indians and 7 for Malay and Chinese is required to reach 80% power. As evidenced from other studies, the strength of association between *HLA-B*15:02* in PHT and LTG-SJS/TEN was weaker compared to CBZ-SJS/TEN and it was not detected in other population besides Han Chinese. Therefore, to detect an association, a bigger sample size for each ethnic group will be necessary.

PHT, an aromatic antiepileptic drug, is also a neutral tricyclic ring compound similar to carbamazepine and lamotrigine. These three drugs have similar mechanism and pharmacological action demonstrated by sharing of a common pharmacophore. They act on voltage-gated sodium channels and interact with the same amino acid residues in the inner pore of neuronal Na^+ channel. They have similar affinity to Na^+ channel which is voltage-dependence with low affinity at resting state and high affinity

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for open-inactivated states (Lipkind & Fozzard, 2010). However, there is no study on PHT and LTG interaction with *HLA-B*15:02* molecule. Nonetheless, *HLA-B*15:02* association to PHT and LTG-SJS/TEN has been reported in Han Chinese and Thai (Cheung et al., 2013; Locharernkul et al., 2008). PHT-SCARs were also reported in association with *HLA-B*15:13* in Malays (Chang et al., 2016). In my cohort, there is two CBZ-SJS/TEN patients carrying *HLA-B*15:13* allele, but none in PHT-SJS/TEN cases. This is due to small sample size and ethnic-specific genetic background (5 PHT-SJS/TEN cases, of which only one is of Malay ethnicity). *HLA-B*15:13* has high prevalent in Malaysian Malays and Indonesian (6.9-12.5% and 11-13.3% respectively), but low in other South Asian population (0.1-0.3% in Han Chinese, 1.8% in Thai).

Lamotrigine (LTG), a tricyclic aromatic antiepileptic drug similar to CBZ and PHT, is a one of the common high risk drug in SJS/TEN. Risk of SJS/TEN is reported at 2.5 per 10,000 new users of LTG (Mockenhaupt et al., 2005). LTG-SJS/TEN has been reported in association with *HLA-B*15:02* in Han Chinese. However, due to small sample size and lower incidence of LTG-SJS/TEN, the strength of association between *HLA-B*15:02* and LTG-SJS/TEN (OR 4.98) was found consistently weaker than to that of CBZ-SJS/TEN (OR 80.70-113.39) (Grover & Kukreti, 2014; Yip et al., 2012; Zeng et al., 2015). In a LTG-SJS/TEN study of European ancestry, *HLA-B*15:02* was not detected and no *HLA* allele reached statistical significant, but the author implicated involvement of other *HLA* alleles (*HLA-B*58:01, A*68:01, Cw*07:18, DQB1*06:09,* and *DRB1*13:01*) (Kazeem et al., 2009). In Koreans, another allele, *HLA-B*44:03*, was detected in association to LTG-SJS/TEN (Park et al., 2016). Similarly, *HLA-B*15:02* was not detected in my LTG-SJS/TEN cases (of the 3 LTG-SJS/TEN cases, it is consisted of 1 Malay, 1 Chinese and 1 Indian). This is due to limited sample size and ethnicity-specific genetic background.

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*HLA-A*24:02* was recently reported to be a shared predisposing risk factor for SJS/TEN induced by aromatic antiepileptic drugs, CBZ, PHT and LTG in southern Han Chinese, in addition to confirming association of *HLA-B*15:02* in CBZ-SJS/TEN. Interestingly, *HLA-A*24:02* was found in CBZ-SJS/TEN cases that were negative for *HLA-B*15:02*, suggesting *HLA-A*24:02* is an independent risk factor. In my cohort, two *HLA-B*15:02*-negative Chinese AEDs-SJS/TEN patients (1 CBZ-SJS/TEN and 1 LTG-SJS/TEN) has *HLA-A*24:02*. The authors hypothesized that both *HLA-B*15:02* and *HLA-A*24:02* have an accumulative effect and contributed to AEDs-SJS/TEN (Shi et al., 2017).

5.5 Cross-Reactivity in SCARs

Aromatic antiepileptic drugs (AEDs), in particular carbamazepine, phenytoin, phenobarbital, and lamotrigine, are some of the common high rick medications associated with severe cutaneous adverse reactions. Cross-reactivity between these aromatic antiepileptic drugs is not uncommon (Hirsch et al., 2008). A large-scale study investigating risk predictors of AEDs-induced rash found that one of the strongest predictors is a history of rash with another AED (Arif et al., 2007). This finding is supported by another study showing a significant association between carbamazepine-, phenytoin-, and oxcarbazepine–induced hypersensitivity skin reactions with a previous history of AEDs-induced rash (Alvestad et al., 2008). *In vitro* lymphocyte toxicity assay study found 80% of the cases that had hypersensitivity to one drug showed cross-reactivity to another aromatic antiepileptic drug (Shear & Spielberg, 1988). Study on AEDs-SJS/TEN have found *HLA-B*15:02* associated to CBZ-SJS/TEN and the association was shared by PHT and LTG-SJS/TEN, albeit weaker.

Here, I describe a patient in my cohort with SJS induced by LTG after a history of CBZ-induced SJS. The patient is a 63-year-old Indian woman with a diagnosis of

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right-sided trigeminal neuralgia diagnosed since 2002. Carbamazepine was prescribed and resulted in complete pain relief. Fourteen days later, the patient developed a generalized rash on the trunk and limbs and was diagnosed with carbamazepine-induced SJS (ALDEN score 6). The second hypersensitivity event occur ten years later. The patient was started on LTG due to uncontrolled pain. On day 20, while on LTG 100 mg a day, the patient developed a second episode of SJS (ALDEN score 8). *HLA-A* and *-B* allele genotyping detected *HLA-A*02:11* and *A*24:17* and *HLA-B*40:06* and *B*51:06*. These alleles have not been reported in association with AEDs-induced SJS/ TEN (Khor et al., 2016).

Negative results for known *HLA*-alleles associated with AEDs-induced severe cutaneous adverse drug reactions, such as in this case, does not predict against cross-reactivity. Although these reactions are unpredictable, identification of predisposing risk factors prior to drug selection can reduce the probability of a hypersensitivity reaction. Patients with a history of severe cutaneous adverse reactions to aromatic AEDs such as carbamazepine, phenobarbital, phenytoin, and lamotrigine are strongly recommended to be managed with newer AEDs that has a lower risk of severe cutaneous adverse reactions.

This case show that in patient with history of AEDs-SJS/TEN, the adverse reaction can recur with another aromatic AED. Therefore, precautions should be taken to avoid these aromatic AEDs in patients with previous history of AED-SJS/TEN. Although *HLA*-genotyping helps to predict AEDs-SJS/TEN reactions, negative results for known *HLA*-alleles (*HLA-B*15:02* and *HLA-A*31:01*) does not predict absence of risk to SJS/TEN especially patient has a previous history of AED-induced severe cutaneous adverse drug reactions. In addition, for those with positive *HLA-B*15:02,* although the recommendation is to avoid carbamazepine, caution should also be taken when considering another aromatic AEDs.

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5.6 *HLA* Screening: Cost-effectiveness and AEDs dosing guideline

5.6.1 *HLA-B*15:02* screening

In health care, adverse reaction to drugs is one of the main cause of morbidity and mortality. Drug-related morbidity and mortality costed an estimated 136 billion USD annually in US, which is higher than compared to common diseases such as diabetes or cardiovascular disease (Johnson & Bootman, 1995). The United States Food and Drug Administration (FDA) changed the CBZ label to include warning and strongly recommend but did not mandate *HLA-B*15:02* screening prior to starting CBZ in Asians in December 2007 (https://www.fda.gov/drugs/drugsafety/postmarketdrugsafety informationforpatientsandproviders/ucm107834.htm) (Ferrell & McLeod, 2008). A prospective study in Taiwan demonstrated that *HLA-B*15:02* screening prior to CBZ treatment reduced incidence of SJS/TEN from 10 expected SJS/TEN cases to 0 (P. Chen et al., 2011). Subsequently, Taiwan implemented *HLA-B*15:02* screening mandatory prior to starting CBZ treatment. This was follow by Hong Kong. Singapore in April 2013 recommended *HLA-B*15:02* screening as standard of care. In Malaysia, the screening patient for *HLA-B*15:02* is strongly recommended as standard of care as well. Prevention measurement of AEDs-induced SJS/TEN in Southeast Asia is mostly involving limiting exposure of susceptible patient that are carrier of *HLA-B*15:02* by prescribing alternative medication. *HLA-B*15:02* screening prior to starting CBZ treatment is strongly advocated. The cost-effectiveness and usefulness of *HLA-B*15:02* screening prior to CBZ treatment has been demonstrated to be cost-effective in population where prevalence of *HLA-B*15:02* is high by several studies from Taiwan, Hong Kong, Thailand and Singapore (Chen et al., 2014b; Dong et al., 2012; Rattanavipapong et al., 2013).

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5.6.2 *HLA-A*31:01* screening

*HLA-A*31:01* has been shown to associated with CBZ-hypersensitivity reactions in several populations including Han Chinese, European and Japanese, but *HLA-A*31:01* screening has not been implemented as of now. It was included in CBZ drug label as information only. However, unlike *HLA-B*15:02* whose prevalent is mostly restricted to Southeast Asia population, *HLA-A*31:01* is more widely distributed in many different population (Gonzalez-Galarza et al., 2015). Many studies are in favor of *HLA-A*31:01* screening as it is more useful in populations where *HLA-B*15:02* is rare.

No prospective study on *HLA-A*31:01* was published at the moment. There were 3 systematic review examining the association of *HLA-A*31:01* and CBZ-hypersensitivity reaction in a few ethnic groups (Caucasian, Japanese, Koreans and Han Chinese). Odds ratio (OR) ranging from 3.9 to 14 was reported with the highest OR found for association to CBZ-DRESS in Caucasian. The number needed to test (NNT) to prevent one case of CBZ-hypersensitivity reactions was reported with NNT of 47 for Caucasian and 67 for Japanese based on an estimated incidence of CBZ-hypersensitivity reaction of 10% and 2.9% respectively (Amstutz et al., 2014; Genin et al., 2014; Grover & Kukreti, 2014). In Han Chinese, the NNT to prevent one case of CBZ-DRESS was 5000 as the incidence of CBZ-DRESS was estimated at 0.05%. One of the studies demonstrated the combined *HLA-A*31:01* and *HLA-B*15:02* test in Han Chinese population has NNT of 455 (Genin et al., 2014).

A cost-effectiveness study on *HLA-A*31:01* screening conducted in the United Kingdom showed that routine testing is cost-effective with a predicted reduction of estimated 780 to 700 CBZ-hypersensitivity cases per 10,000 patient (Plumpton et al., 2015).

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5.6.3 *HLA* genotyping and AEDs dosing guideline

Guideline on the use of pharmacogenetic test such as *HLA-B*15:02* in CBZ dosing has been published by Clinical Pharmacogenetics Implementation Consortium (CPIC), an international consortium aiming to facilitate the use of pharmacogenetic test in clinical practice. The consortium addresses the barrier faced in implementing pharmacogenetic testing in clinical practice due to difficulty of translating the test results into an actionable decision in drug prescribing for clinician. CPIC curates and systematically grade the published evidence and clinical recommendations to establish their guidelines by categorising dosing recommendations into strong, moderate and optional (Table 5.1). In the case of *HLA-B* genotyping test for CBZ dosing, the consortium follows FDA recommendation that "patients with ancestry in at-risk populations should be screened for the presence of *HLA-B*15:02* allele prior to starting carbamazepine" (https://www.fda.gov/drugs/drugsafety/postmarketdrugsafety informationforpatientsandproviders/ucm107834.htm) (Ferrell & McLeod, 2008). In addition, CPIC further stated the highest risk patients are from Han Chinese descent, followed by Vietnam, Cambodia, the Reunion Islands, Thailand, India (specifically Hindus), Malaysia, and Hong Kong. The other populations not stated are categorized as low frequency. But they emphasize that patient may be unaware of presence of distant Asian ancestry in their family. Hence, recommended strongly that patient should be tested for *HLA-B*15:02* regardless of ancestry or age. If the test is positive, alternative medication should be used. The consortium justified the denial of CBZ to *HLA-B*15:02*-positive patient although they acknowledge that the number needed to test (NNT) to prevent one case of CBZ-SJS/TEN in Asian for *HLA-B*15:02* test is 461(Yip et al., 2012), which is quite a large number of *HLA-B*15:02*-positive patients that would not develop CBZ-SJS/TEN. However, they argued the benefit outweighed the risk considering the severity and long-term sequelae of SJS/TEN. The consortium also

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recommends that consideration should be taken for other AEDs with aromatic ring that are similar structurally and therapeutically to CBZ, such as phenytoin, lamotrigine, oxcarbazepine and eslicarbazepine acetate, for choosing alternative to CBZ (Leckband et al., 2013). In agreement with this, current practice strongly discourage choosing aromatic AEDs with similar structure that are also associated with risk to SJS/TEN as alternative to CBZ. Instead, newer AEDs with no risk of SJS such as sodium valproate or levetiracetam are recommended as CBZ alternative. CPIC guidelines are updated periodically and available on CPIC website (cpicpgx.org) and PharmGKB website (http://www.pharmgkb.org)

Recent emerging evidences on *HLA-A*31:01* association to multiple CBZ-hypersensitivity reactions (including SJS/TEN, MPE and DRESS) detected in a large number of populations (such as Japanese, Caucasian, Han Chinese and South Korean) has prompted update to screening recommendations. A systematic review reported that NNT to prevent one case of CBZ-hypersensitivity reactions for *HLA-*31:01* screening (67 for Japanese and 47 for Caucasian) is lower compared to *HLA-B*15:02*. But the author noted that if NNT is specifically for preventing SJS/TEN only, *HLA-B*15:02* screening is more favorable (Leckband et al., 2013; Yip et al., 2012). Another study strongly recommended *HLA-B*15:02* testing while moderately recommended *HLA-A*31:01* testing for all CBZ-naive patient prior to CBZ treatment explaining the reduced recommendation of *HLA-A*31:01* is because of smaller number of studies and citing uncertainty in the sensitivity and PPV due to lack of prospective study on the *HLA-A*31:01* in reducing CBZ-hypersensitivity reactions (Amstutz et al., 2014).

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Table 5.1: CPIC Guidelines - Classification and definition of the strength of therapeutic recommendations.*

Classification of recommendations	CPIC definition
Strong	The evidence is high quality and the desirable effects clearly outweigh the undesirable effects.
Moderate	There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.
Optional	The desirable effects are closely balanced with undesirable effects and there is room for differences of opinion as to the need for the recommended course of action.

*Adapted from Clinical Pharmacogenetics Implementation Consortium Guidelines for *HLA-B* Genotype and Carbamazepine Dosing (www.pharmgkb.org/guideline/PA166105008) (Leckband et al., 2013)

5.7 Other risk factors implicated in AEDs-SJS/TEN

5.7.1 T-cell receptor

Meta-analyses of published studies on the association between *HLA-B*15:02* and CBZ-SJS/TEN showed that there are approximately 25% of CBZ-SJS/TEN cases negative for *HLA-B*15:02* (Genin et al., 2014; Grover & Kukreti, 2014; Yip et al., 2012). It seems *HLA-B*15:02* is important but not necessary for CBZ-SJS/TEN. Functional study on the T-cell receptors (TCR) repertoire in recognition of CBZ presented by HLA molecules in the context of CBZ-SJS/TEN disease revealed other key risk factors besides *HLA-B*15:02*. Specific TCR clonotype, VB-11-ISGSY, was present in 84% of SJS/TEN patients while absence in all of the tolerant controls, revealing that TCR usage in CBZ-SJS/TEN patients is restricted (Ko et al., 2011). Illing et al. observed the shift in peptide repertoire caused by CBZ binding to *HLA-B*15:02* molecule is smaller and it corroborates with restricted TCR usage observed in CBZ-SJS/TEN patients (Illing et al., 2012).

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5.7.2. Antigen binding site in HLA-B molecule

Another study investigated the presentation of CBZ by HLA molecules demonstrated that *HLA-B*15:02*-β2-microglobulin-pepetide complex has high affinity for chemical with 5-carboxamide on tricyclic ring such as CBZ. They found 3 key residues (Asn63, Lle95 and Leu 156) in the peptide binding groove of *HLA-B*15:02* that are necessary for CBZ presentation which lead to CTL activation (Wei et al., 2012). The key residue, Asn63, are also shared by other alleles of the HLA-B75 family explained why alleles in this family are able to present CBZ while other family (HLA-B62 and HLA-B72) could not. Mutagenesis of these three sites abolished CBZ binding to *HLA-B*15:02* recombinant protein and CTL recognition of CBZ (Wei et al., 2012). Other allele of HLA-B75 family that was reported with association to CBZ-SJS/TEN is *HLA-B*15:11* in Japanese (Kaniwa et al., 2010) and *HLA-B*15:21* in a pooled Asian cohort (Jaruthamsophon et al., 2017). Another HLA-B75 family allele, *HLA-B*15:08*, was not statistically associated with CBZ-SJS/TEN but it was detected in *HLA-B*15:02*-negative CBZ-SJS/TEN patients of Indian ethnicity (Mehta et al., 2009). Consistent with my study's observation, one of the Indian CBZ-SJS/TEN patients is positive for *HLA-B*15:08.* It was not detected in CBZ-SJS/TEN cases of Chinese and Malay. *HLA-B*15:08* is found more prevalent in Indian than Chinese and is absence in Malay. Whereas for *HLA-B*15:21* frequency, it is higher in Malays than in Chinese and Indians (Gonzalez-Galarza et al., 2015). But it was not detected in any of the cases in this study.

Studies on the antigen-binding groove of HLA-B75 family suggested that HLA-B75 family members have similar conformation that enable the binding and presentation of CBZ. Wei et al. (2012) supported this by showing antigen presenting cell transfected with B75 family members was able to induce *in vitro* CBZ-specific CTL cytotoxicity (Wei et al., 2012). *In-silico* molecular conformation modelling show the

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key binding site for CBZ is Arg62 in *HLA-B*15:02* molecule while Asn63 attributes to molecular recognition. In fact, the structure of antigen binding groove is not key determinant of CBZ binding, the selectivity is attributed to amino acid side chain (Jaruthamsophon et al., 2017). The strongest binding site was found on the side chain of Arg62 which form a hydrogen bond with ketone group of the 5-carboxamide of CBZ (Wei et al., 2012). It has been proven that CBZ binds directly and noncovalently to *HLA-B*15:02* molecules and lead to an altered peptide repertoire capable of eliciting T-cells response. Taken together the findings, it is apparent that HLA molecules is susceptible to antigen selection modulation by drugs that in turn elicits altered T-cell immunity. Studies on pathogenesis of *HLA-A*31:01* and CBZ-hypersensitivity is more limited. So far, no binding sites of CBZ in *HLA-A*31:01* molecule has been postulated.

5.7.3 Drug metabolism variants

Besides *HLA* gene, *CYP2C9*2* and *CYP2C9*3*, a variant from cytochrome P450 enzymes, a drug metabolizing enzyme, was associated with PHT-SJS/TEN. The association linked functionally to impairment in drug metabolism that caused delayed clearance of plasma phenytoin. The identification of patients with poor metabolizer variant can help prevent PHT toxicity and reduce risk of PHT-induced SCARs (Chung et al., 2014).

CYP2C9 genotypes were interpreted into phenotype predicting metabolizer status by Clinical Pharmacogenetics Implementation Consortium (CPIC) (cpicpgx.org). CPIC dosing recommendation linked *CYP2C9* variants (*CYP2C9*2* and *CYP2C9*3*) to phenytoin toxicity by categorised *CYP2C9*2* and *CYP2C9*3* genotype into intermediate (carrier with only one copy of **3* or **2*) and poor metabolizer (carrier with two copy of **3* or **2*) whereas carrier of two wildtype *CYP2C9*1* alleles is considered extensive metabolizer (Caudle et al., 2014). PHT dosing is therefore recommended

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based on metabolizer phenotype of either 25% (intermediate) or 50% (poor) reduction in recommended starting maintenance dose. *CYP2C9*2* and **3* variants differ in different populations. In Han Chinese, allele frequency of *CYP2C9*2* and **3* is 1% and 5.5% respectively. While in Southern Indian, allele frequency of *CYP2C9*2* is 2-5% and **3* is 10% (Caudle et al., 2014). The frequency is not known in Malay. However, due to small sample size in my PHT-SJS/TEN cohort (n=5; 1 Malay, 2 Chinese and 2 Indians), *CYP2C9*2* and **3* variant was not investigated in this study.

5.8 Implication and future direction

5.8.1 Implication of *HLA* association in CBZ-SJS/TEN

Due to high mortality, morbidity and long-term sequelae of SCARs, the goals in SCARs research is in prevention and exploring risk markers with strong predictive value for SCARs. The risk of aromatic antiepileptic drugs in causing SCARs affect many epilepsy treatment decision such as the choice of prescribed AEDs and how soon drug treatment is initiated. Therefore, evidence on association between *HLA* alleles (*HLA-A*31:01* and *HLA-B*15:02*) that strongly predict SJS/TEN with high PPV and NNV have a profound effect on clinical practice.

Even though risk marker of *HLA-B*15:02* for CBZ-SJS/TEN in Han Chinese and certain Asian populations have 100% negative predictive value, it is not generalized across other ethnicities such as Caucasian, Japanese and Koreans (Yip et al., 2012). Given the strong association of *HLA-B*15:02* and CBZ-SJS/TEN in most Asia populations, screening prior to CBZ treatment is imperative. In Malaysia, *HLA-B*15:02* is a strong predictor for CBZ-SJS/TEN for all three major ethnic groups, Malay, Chinese and Indian. And *HLA-B*15:02* screening prior to CBZ treatment is implemented in clinical practice. However, further study still need to be done on the factors that are important in ensuring *HLA-B*15:02* screening is cost-effective and

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implemented in clinical practice such as frequency of *HLA-B*15:02* in other ethnic minority group, the disease prevalence of CBZ main indication, the rate of CBZ utilization and rapid detection time for *HLA-B*15:02* test.

5.8.2 Future direction

Recently, with even more alleles implicated with association to SJS/TEN induced by CBZ, PHT and LTG in different population, high resolution *HLA* genetic screening will be very much preferred in the future. However, that will be create more uncertainties which may make it difficult for clinician to decide suitable alternative AEDs. Such as the risk of SJS/TEN for a *HLA-B*15:02* or *HLA-A*31:01*-positive patient started on other aromatic AEDs (OXC, LTG or PHT). If the patient is positive for one of the allele in HLA-B75 family that was implicated to associate with SJS/TEN, what is the risk of SJS/TEN and should CBZ be avoided. As such, studies on the pathogenesis on other *HLA* alleles implicated in SJS/TEN is needed to understand the association observed for these alleles and how drugs are capable of triggering T-cell immune response through these HLA molecules.

Although *HLA* screening has successfully utilized predictive risk marker into clinical practice, *HLA* mandatory screening has its pitfalls. If *HLA-A*31:01* screening is to be implemented in clinical practice, further study on *HLA* test with short turnaround time (rapid test kit), policy adherence, and possible unintended knock-on effects will be needed. As exemplified in Hong Kong, mandatory screening of *HLA-B*15:02* before CBZ treatment has led to the reduction in CBZ prescription and switching to other AEDs such as LTG and PHT that does not require pre-screening. Hence, although the incidence of CBZ-SJS/TEN decreased, the overall SJS/TEN incidence did not as patient was put on other AEDs associated with SJS/TEN that does not require screening (Chen et al., 2014a).

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5.9 Study Limitation

Our data did not detect association of *HLA-B*15:02* or other *HLA-A* or *-B* allele to PHT and LTG-induced SJS/TEN in all three ethnic groups. This is due to limited sample size, particularly after categorised into respective ethnic groups. As evidenced from other studies, the strength of association between *HLA-B*15:02* and PHT/LTG-induced SJS/TEN is weaker compared to CBZ-induced SJS/TEN. That is the same for AEDs-DRESS cases where there is only 3 (2 PHT-DRESS and 1 CBZ-DRESS). Given the small sample size in each of these group and taking into account the association is ethnicity-specific, further studies with larger sample size in each ethnic group will be needed to detect the association with sufficient power.

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CHAPTER 6: CONCLUSION

The current study replicated and confirm the association between *HLA-B*15:02* and CBZ-SJS/TEN in Malay, Chinese and Indian populations in Malaysia. And a new association between *HLA-A*31:01* and CBZ-SJS/TEN in Indians were also detected. Combining both *HLA-A*31:01* and *HLA-B*15:02* improve the predictive power in identifying patient with increased risk for CBZ-SJS/TEN. This is relevant for Indian population where *HLA-B*15:02* frequency is lower and not homogeneous. This study's findings also support clinical utility of *HLA-A*31:01* screening and also added to the growing evidence on *HLA-A*31:01* as a risk predictor for CBZ-SJS/TEN.

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LIST OF PUBLICATION AND PAPERS PRESENTED

Publications

1. Khor, A. H., Lim, K. S., Tan, C. T., Wong, S. M., & Ng, C. C. (2014). *HLA-B*15:02* association with carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in an Indian population: a pooled-data analysis and meta-analysis. *Epilepsia, 55*(11), e120-124.

2. Khor, A. H., Lim, K. S., Tan, C. T., Kwan, Z., & Ng, C. C. (2016). Cross-reactivity in AED-Induced Severe Cutaneous Adverse Reaction: A Case Report. *The Journal of Investigational Allergology and Clinical Immunology, 26*(5), 329-331.

3. Khor, A. H., Lim, K. S., T., T. C., Kwan, Z., Tan, W. C., Wu, B.-C. W., & Ng, C. C. (2017). *HLA-A*31:01* and *HLA-B*15:02* association with Stevens-Johnson syndrome and toxic epidermal necrolysis to Carbamazepine in a multi-ethnic Malaysian population. *Pharmacogenetics & Genomics, 27*(7), 275-278.

4. Shi, Y. W., Min, F. L., Zhou, D., Qin, B., Wang, J., Hu, F. Y., . . . Liao, W. P. (2017). *HLA-A*24:02* as a common risk factor for antiepileptic drug-induced cutaneous adverse reactions. *Neurology, 88*(23), 2183-2191.

Presentations

1. Khor, A. H., Lim, K. S., Tan, C. T., Wong, S. M., & Ng, C. C. (2014). *HLA-B*1502* allele association with carbamazepine-induced severe cutaneous reaction in Malaysian Indian, a pooled-sample analysis and meta-analysis. Poster presented at the 10th Asian and Oceanian Epilepsy Congress, Grand Copthorne Waterfront Hotel, Singapore.

2. Khor, A. H., Lim, K. S., Tan, C. T., & Ng, C. C. (2015). Association of *HLA-B* alleles and aromatic anti-epileptic drugs induced Stevens - Johnson syndrome and toxic epidermal necrolysis in Malaysian population. Poster presented at the Golden Helix Symposia: Next-generation Pharmacogenomics, Connexion at Nexus, Bangsar South, Kuala Lumpur.

3. Khor, A. H., Lim, K. S., Tan, C. T., & Ng, C. C. (2016). Association of *HLA-A* and *B* alleles and Carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Malaysian population. Poster presented at the 11th Asian and Oceanian Epilepsy Congress, Hong Kong Convention and Exhibition Center, Hong Kong.

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