Frequency of the HLA-B*1502 allele contributing to carbamazepine-induced hypersensitivity reactions in a cohort of Malaysian epilepsy patients

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Summary

We describe the association of the HLA-B*1502 allele in 27 epilepsy patients (19 Malays, 8 Chinese) treated with carbamazepine (CBZ) at the UKM Medical Center (UKMMC), 6 with CBZ-Steven Johnson Syndrome (CBZ-SJS), 11 with CBZ-induced rash, 2 with suspected phenytoin-induced rash and 8 negative controls. Our study showed that 10 (6 Malay, 4 Chinese) patients were positive for HLA-B*1502. Out of the 10 patients, six were confirmed to have CBZ-SJS (p =0.0006), while four patients developed a skin rash. However there were 6 Malay patients and 1 Chinese patient that developed a skin rash after CBZ administration who were not positive for the allele, indicating that there might be more that one allele associated with CBZ-induced hypersensitivity. Another 2 patients were suspected of having phenytoin-induced rash, instead of CBZ, and these patients did not have HLA-B*1502. In conclusion, this study confirmed the association of HLA-B*1502 with CBZ-SJS among Malaysian epilepsy patients, however there might be other genes that could be responsible for the CBZ-induced rash. *(Asian Pac J Allergy Immunol 2011;29:290-3)*

Key words: *HLA-B*1502 allele, carbamazepine, Steve-Johnson syndrome, epilepsy, drug hypersensitivity, pharmacogenomics*

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Introduction

It has been shown that the HLA-B*1502 allele is strongly associated with CBZ-induced hypersensitivity reactions including Steven Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) in Taiwan and Hong Kong Han Chinese[1,2]. Another study in the Thai population also showed a strong association between HLA-B*1502 and CBZ-induced SJS (100% in 6 cases)[3]. A recent study in Malaysia however showed that HLA-B*1502 was also present in 75% (12/16) of Malay patients with CBZ-induced SJS/TEN[4]. Although there is only limited data on the frequency of HLA-B*1502 across populations, current estimates suggest that the HLA-B*1502 allele is present in 10-15% or more subjects in parts of China, Thailand, Singapore, Malaysia, Indonesia, Myanmar, the Philippines, Taiwan and Vietnam, and in 2-8% of subjects in India and less than 1% of Japanese, Koreans and Caucasian subjects[5]. Data from the WHO Uppsala Monitoring (WHO-UMC) and Novartis CBZ-SJS/TEN 2000-2006 reports showed that the incidence of CBZ-induced hypersensitivity reactions was high in some Asian countries, such as Malaysia and Thailand, which also had the highest incidence of SJS/TEN induced by CBZ[5,6]. The US Food and Drug Administration (FDA) has made it mandatory to screen for HLA-B*1502 in patients with ancestry from areas in which there is a high frequency of this allele before commencing treatment with CBZ[7]. Therefore, an initial screening for HLA-B*1502 polymorphism in epileptic patients will be beneficial to prevent complications of hypersensitivity reactions arising from the prescription of CBZ. However such screening is not yet available in Malaysia. This case series was the result of a preliminary screening of the allele in epilepsy patients who were prescribed with CBZ, some of whom had developed CBZ-induced hypersensitivity reactions.

Table 1. Summary of results for epileptic patients tested for HLA-B*1502 by multiplex PCR and DNA sequencing and clinical data on the drug hypersensitivity reactions. The results show that the presence of HLA-B*1502, as detected by multiplex PCR and DNA sequencing, correlates with patients (2, 3, 6, 10, 16, 17, 18, 19, 24, 25) who developed CBZ-induced hypersensitivity after taking CBZ for less than 8 weeks, while other patients (5, 9, 11, 14, 15, 21 and 23) developed a skin rash but were not positive for the allele. [1]Patient 8 was prescribed both CBZ and PHT but the development of skin rash was suspected because due to PHT instead of CBZ as the rash persisted after discontinuation of CBZ. The rash subsided after discontinuation of both PHT and CBZ. [2]Patient 13 also developed skin rash after being prescribed both CBZ and PHT, which subsided after discontinuation of both PHT and CBZ.

Patient	Sex	Race	Age (years)	Multiplex PCR				DNA Sequencing	Clinical Diagnosis	Treatment/Associating drug hypersensivity
				Primer 1	Primer 2	Primer 3	Primer 4	HLA Polymorphism		
Epi 1	M	Malay	35	+	-	-	+	*5801	Partial epilepsy	PHT, CBZ, Lamotrigine, Sodium Valproate / Nil
Epi 2	F	Chinese	25	+	+	+	+	***1502**	Epilepsy	CBZ/ SJS
Epi 3	M	Malay	15	+	+	+	+	***1502**	Epilepsy	CBZ/ rash
Epi 4	M	Malay	11	+	+	-	-	*15	Epilepsy	CBZ/ Nil
Epi 5	M	Malay	17	+	-	+	+	*4001	Generalized epilepsy	CBZ/ rash
Epi 6	F	Malay	28	+	+	+	+	***1502**	Epilepsy	CBZ/ SJS
Epi 7	F	Chinese	10	+	-	-	-	*35	Epilepsy due neurofibromatosis	CBZ/ Nil
Epi 8	F	Chinese	48	-	-	-	+	*4102	Partial epilepsy due to primary brain lymphoma	CBZ, PHT/ rash [1]
Epi 9	M	Malay	5	+	-	-	-	*44032	Epilepsy	CBZ/ rash
Epi 10	M	Chinese	2	+	+	+	+	***1502**	Generalized epilepsy	CBZ/ rash
Epi 11	M	Malay	6	+	-	-	-	*1801	Partial epilepsy	CBZ/ rash
Epi 12	M	Malay	9	+	-	+	-	*1531	Epilepsy secondary to spastic quadriplegia	Sodium Valproate, CBZ/Nil
Epi 13	M	Malay	1	-	-	+	+	*44	Left frontal lobe dysplasia with intractable seizure	CBZ, PHT/ rash [2]
Epi 14	M	Malay	17	+	-	+	-	*35	ALL with epilepsy	CBZ/ rash
Epi 15	F	Chinese	18	+	-	-	+	*35	Epilepsy	CBZ /rash
Epi 16	F	Malay	9	+	+	+	+	***1502**	Trigeminal neuralgia	CBZ/ SJS
Epi 17	F	Malay	7	+	+	+	+	***1502**	Neonatal focal seizure	CBZ/ SJS
Epi 18	M	Chinese	11	+	+	+	+	***1502**	Partial seizure	CBZ/SJS
Epi 19	F	Malay	20	+	+	+	+	***1502**	Epilepsy	CBZ/rash
Epi 20	F	Malay	22	+	-	-	-	*5811	Epilepsy	CBZ./ Nil
Epi 21	F	Malay	49	+	-	-	-	*4813	Trigeminal neuralgia	CBZ/ rash
Epi 22	M	Chinese	4	+	-	+	-	*5101	Global developmental delay with epilepsy	CBZ/ Nil
Epi 23	F	Malay	27	+	+	-	-	*4812	Epilepsy	CBZ/rash
Epi 24	M	Malay	14	+	+	+	+	***1502**	Cerebral palsy with epilepsy	CBZ/ SJS
Epi 25	F	Chinese	29	+	+	+	+	***1502**	Epilepsy	CBZ/ rash
Epi 26	M	Malay	27	-	+	+	+	*15	Epilepsy	CBZ/ Nil
Epi 27	F	Malay	22	+	+	+	+	*1825	Epilepsy	CBZ/ Nil

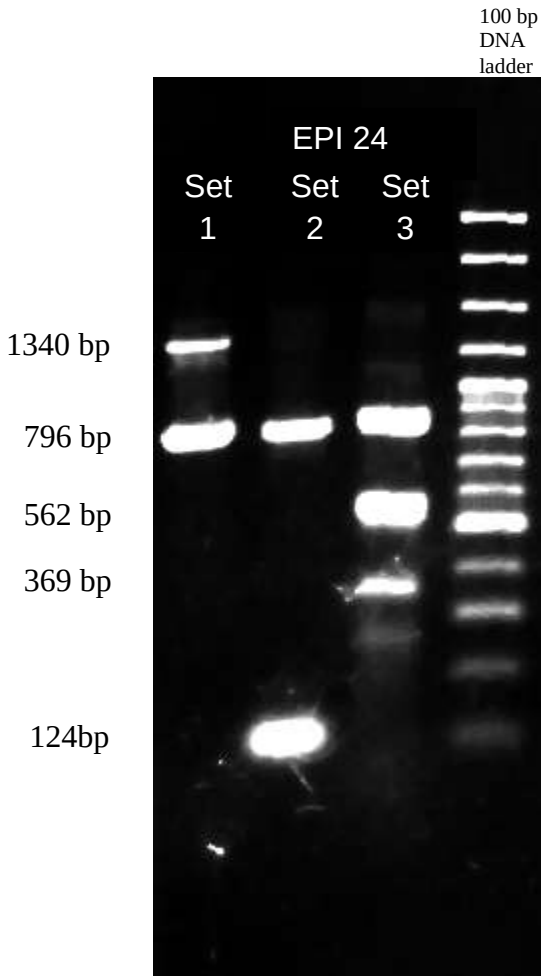

Figure 1. Representative multiplex PCR results for the detection of HLA-B*1502 for patients 24. Multiplex PCR were run on all samples with multiplex Set 1 with internal control (796 bp) and PCR product of primer 1 (1340 bp); multiplex Set 2 consisted of internal control (796 bp) and PCR product of primer 2 (124 bp) and multiplex Set 3 consisted of internal control (796 bp), PCR product of primer 3 (562 bp) and primer 4 (369bp). The results showed that patient 24 tested positive for HLA-B*1502 allele with the presence all four PCR products.

A total of 27 epileptic patients (19 Malays and 8 Chinese) were involved in this study, with six patients (patient 2, 6, 16, 17, 18 and 24) confirmed to have developed SJS. There were 11 patients (3, 5, 9, 10, 11, 14, 15, 19, 21, 23, 25) who had developed a rash while taking CBZ which resolved after CBZ was discontinued, while two patients (8 and 13) developed a rash while taking both CBZ and phenytoin (PHT) and the rash resolved after both CBZ and PHT were discontinued. Eight negative controls (patient 1, 4, 7, 12, 20, 22, 26 and 27) were also included among the sample cohort. All patients signed an informed consent form and the study was approved by the UKM Medcal Centre (UKMMC) Ethics Committee. Five milliliters of peripheral blood was drawn from the subjects into EDTA blood containers (vacutainer) for DNA extraction using the conventional 'salting out' method with slight modification. Multiple sequence-specific primer (SSP) based on the PCR was used to detect the HLA-B*1502 polymorphism. Primer pairs were designed by Man et al (2007) with modifications specifically aimed at amplifying each polymorphic sequence that was detected to provide the desired level of genotyping resolution[2]. For DNA sequence validation, primers for Exon 2 and Exon 3 were designed and protocols optimized in-house. Data were expressed as positive or negative for HLA-B*1502, as shown in Table 1., together with DNA sequencing data which were sent to the IMGT/HLA website (http://www.ebi.ac.uk/imgt/hla/) to retrieve the HLA subtype of the patients. Fisher's exact test was used to calculate the association of the HLA-B*1502 allele to the presentation of the hypersensitivity reactions (either SJS or skin rash) compared to control.

The HLA-B*1502 status by multiplex PCR and DNA sequencing, patient characteristics, diagnosis, treatment and the associated hypersensitivity reactions are shown in Table 1. Ten out of the 27 patients, or 37.0% of our cohort of epilepsy patients, demonstrated the HLA-B*1502 allele with the presence of all four PCR bands and these were further validated by DNA sequencing. Patients 2, 6, 16, 17, 18 and 24 with CBZ-induced SJS who were HLA-B*1502 positive had developed the rash within 8 weeks of taking CBZ. Meanwhile, four patients 3, 10, 19 and 25 (14.8%) also developed rashes while taking CBZ, but did not progress to SJS. Out of the 10 patients positive for the HLA-B*`1502 allele, six were Malays and four were Chinese. However, there were six Malay and one Chinese patients (5, 9, 11, 14, 15, 21 and 23) who developed skin rashes after CBZ administration but were negative for the HLA-B*1502 allele (Table 1.). All the adverse reactions subsided after discontinuation of the CBZ. There were two patients (8 and 13) with suspected PHT-induced hypersensitivity who tested negative for the HLA-B*1502 allele. Fisher's exact test showed that the HLA-B*1502 allele is strongly associated with the CBZ-induced SJS when compared with both control (p =0.0003) and CBZ-induced rash (p =0.0345) respectively. However the HLA-B*1502 allele is

not significantly associated with CBZ-induced rash when compared to control (p =0.1032).

Consistent with the original Taiwanese report[1], all six of our patients with CBZ-induced SJS were positive for HLA-B*1502, among whom four patients were of Malay ethnicity. This study also confirmed a recently reported Malaysian study which showed a 75% association of CBZ-induced SJS/TEN with the HLA-B*1502 allele in the Malay ethnic population, which forms the majority of the population in Malaysia[4]. These findings suggested that the association of the HLA-B*1502 allele with SJS/TEN is not confined to Han Chinese and Thais as reported previously[3], but is also seen in the Malays, who make up the largest ethnic group in Malaysia. It is interesting to note that some patients who developed a skin rash after CBZ administration were not positive for the HLA-B*1502 allele. This suggests that there might be other alleles such as Cw*0801 that might have a stronger association ith the CBZ-induced hypersensitivity[1], especially in non-Han Chinese populations. Further studies on such populations need to be performed to validate our findings.

Our preliminary study highlighted the need of a bigger study to investigate the association of HLA-B*1502 and other genes with CBZ-induced hypersensitivity among the different ethnic groups in Malaysia. In conclusion, detection of HLA-B*1502 provides vital prescribing information when treating patients with epilepsy.

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