Association between HLA-DQB1, -DPB1 alleles and risk of carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy

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Abstract:

Adverse drug reactions are a serious problem for health and one of the main reasons for treatment failure with antiepileptic drugs. In this study, we determined the HLA-DQB1 and -DPB1 alleles in the blood samples of 44 epileptic patients receiving carbamazepine treatment, including 21 patients with hypersensitivity and 23 patients with tolerance, using the next-generation sequencing method. We identified 19 HLA-DQB1 alleles, of which 13 were in the hypersensitive group and 15 were in the tolerant group. The frequency of the HLA-DQB1*03:01:01 allele was highest in both groups. Although the frequency of the HLA-DQB1*03:03:02 allele in the hypersensitive group was higher than that in the tolerant group (p=0.0431), this difference was not statistically significant after applying the Bonferroni correction (p_c=0.5249). However, the evidence of *in silico* interaction between CBZ and HLA-DQB1*03:03 at the peptide-binding cleft was suggested. For HLA-DPB1, 16 alleles were found in epileptic patients, with the HLA-DPB1*05:01:01 allele having the highest frequency; however, no allele was associated with the risk of hypersensitivity to carbamazepine in epileptic patients. Our study demonstrates that the HLA-DQB1*03:03:02 allele tended to behave as a susceptibility factor for carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

Keywords: **carbamazepine, epilepsy, HLA-DPB1, HLA-DQB1*03:03:02.**

Classification numbers: **3.2, 3.5, 3.6**

1. Introduction

Epilepsy is a common neurological disorder, affecting around 50 million people and accounting for 0.5% of the global disease burden [1]. The annual estimated proportion of epilepsy worldwide is 4-7 per 1,000 people; in Vietnam, this prevalence is 4.4 per 1,000 people [1, 2].

Carbamazepine (CBZ) is the first-line treatment for epilepsy, modulating voltage-gated sodium channels, inhibiting the action potential, and reducing synaptic transmission. Despite being an effective anticonvulsant, CBZ can cause hypersensitivity reactions of varying frequency and severity. The majority of hypersensitivity reactions are relatively mild skin rashes, which are usually ordered by the doctor to be discontinued to resolve the symptoms of hypersensitivity [3]. However, CBZ also causes serious and potentially severe cutaneous adverse drug reactions such as toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome (SJS), hypersensitivity syndrome (HSS), drug rash with eosinophilia and systemic symptoms (DRESS) [4].

Human leukocyte antigen (HLA) molecules play a crucial role in adaptive immunity. Specifically, HLA class II molecules have been linked to various diseases, including autoimmune diseases, infections, allergies, and epilepsy [5-8]. In 2016, a previous study conducted a study on the genetic association of HLA class II alleles

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DRB1, DQA1, and DQB1 with mesial hippocampal sclerosis. Their results showed that the allele HLA DRB1*13:02 may act as a susceptibility factor in patients with pharmacoresistant temporal lobe epilepsy associated with mesial hippocampal sclerosis [7]. Another previous study analysed HLA typing results and found that class II HLA genes, particularly DRB4*03:01N, a null allele, may have a strong protective role in epilepsy patients in the Middle East [8]. However, there is limited data on the role of HLA class II in the Vietnamese population, especially in those with epilepsy treated with CBZ. Therefore, this study aimed to identify the association between HLA class II, specifically HLA-DQB1 and -DPB1, and the risk of CBZ-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

2. Materials and methods

2.1. Biological materials

A total of 44 blood samples from epilepsy patients treated with CBZ were used in this study, comprising 2 groups: 21 hypersensitive patients and 23 tolerant patients provided by Tam Anh Hospital (Hanoi) with characteristics including age, gender, CBZ dose, drug allergy or tolerance status, and clinical features. The inclusion criteria were as follows: (i) The patients were examined and diagnosed with epilepsy; (ii) They had clear indications for treatment with CBZ; (iii) They had taken CBZ for less than 3 months and had allergic manifestations (hypersensitivity group) or more than 3 months and non-allergic manifestations (tolerance group); (iv) They had not been administered other drugs with a high allergy risk. The exclusion criteria were: (i) Patients with clinically significant allergic conditions before drug administration or allergic conditions present within 24 hours of drug administration; (ii) Pregnant women; (iii) Patients who had not undergone sufficient tests necessary for the study; (iv) Patients who refused to participate in the study.

The patients were informed of the study's purpose and agreed to provide the samples. All enrolled patients or their guardians signed an informed consent form. The study was approved by the Institutional Ethical Review Board of Hanoi Medical University, code 118/GCN-HDDDNCYSH-DHYHN on July 8, 2020. Blood samples were collected in EDTA anticoagulant tubes and stored at -25°C until use.

2.2. Amplification of HLA-DQB1 and -DPB1 genes by long-range polymerase-chain-reaction

Total DNA was extracted from whole blood samples using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Total DNA concentration and quality were measured by Qubit™ 4 Fluorometer (Invitrogen, USA), and then DNA samples were stored at -20ºC until further experiments.

The HLA-DQB1 and -DPB1 genes were amplified using the QIAGEN Long-Range PCR Kit (Qiagen, USA) with locus-specific long-range PCR primers provided by Omixon Holotype HLA 96/7 RUO (Omixon, Hungary), according to the manufacturer's instructions. The HLA-DQB1 and -DPB1 loci were amplified in 25 µl of a reaction mixture consisting of 2 µl of primer mix, 2.5 µl of PCR long-range buffer (10X), 1.25 µl of mixed dNTPs (10 mM each), 0.4 µl Taq polymerase, and 20-30 ng/µl DNA. The thermal cycle used for amplification was 95°C for 3 mins; 35 cycles (95°C for 15 s; 60°C for 30 s; 68°C for 9 mins) and 68°C for 10 mins. The Qubit™ 4 Fluorometer (Invitrogen, USA) was used to measure the nucleic acid's ultraviolet light absorption density of PCR products at 260 nm (A_{260}). The PCR products were then diluted to a final concentration of 100 ng/µl for library construction.

2.3. Library preparation and next-generation sequencing

The library was constructed in 4 main steps: (1) DNA fragmentation to form short fragments approximately 300 bp in length by restriction enzyme; (2) DNA fragment repair and adenylation by adding an A nucleotide; (3) The DNA fragments were attached to two heads by adapter 1 and adapter 2 with specific sequencing; and (4) Library size selection using AMPure XP beads (Beckman Coulter, USA) performed with the Omixon Holotype HLA 96/7 RUO kit (Omixon, Hungary) according to the manufacturer's procedure.

Library DNA concentrations were quantified by measuring the A_{260} value using a Qubit™ 4 Fluorometer (Invitrogen, USA) for optimal use of the output of the Illumina MiSeq. All samples were barcoded,

aggregated, and then sequenced on the MiSeqFGx instrument (Illumina, San Diego, CA) using the Miseq Reagent Kit v3.

Sequencing data was generated from the Illumina Miseq platform as FASTQ files. The raw reading results of the sequencing system (FASTQ file format) were checked by FASTQC software to evaluate the quality criteria and analysed using Omixon HLA Twin v2.1.3 software (Omixon, Hungary), which assisted in aligning reads with specific HLA loci to generate the best-matched alleles at each read. Interpretation of the HLA genotyping format results was performed using Omixon Target software v1.7 (Omixon Biocomputing Kit, Budapest, Hungary) and Omixon Target software (Omixon) v1.8 with updated reference sequences on the IMGT/HLA database.

2.4. In silico analysis

HLA-DQB1*03:03 protein sequence was collected from the HLA database (https://www.ebi.ac.uk/ipd/imgt/hla/alleles/) and a protein structure model was built using SWISS-MODEL software (https://swissmodel.expasy.org/interactive/) [9], with the structure of the HLA-TCR complex (SMTL ID: 6px6.1) as the template. The appropriate model selection was based on the structural evaluation measures of SWISS-MODEL, especially with two important parameters, GMGE and QMEANDisCo Global [9-14]. The structure of carbamazepine was collected from the LIGANDs Database Open and eXtensible (LigandBox) with LigandBoxID of D00252. The chemical binding sites between the target (HLA-DQB1*03:03 molecule) and the ligand (CBZ) were predicted using SwissDock (http://www.swissdock.ch/) based on EADock DSS, and results were visualised by UCSF Chimera (https://www.cgl.ucsf.edu/chimera/) [13-15].

2.5. Statistical analysis

Differences in clinical and subclinical characteristics of the groups and frequency of HLA alleles were statistically analysed using GraphPad Prism version 8.4.2 for Windows (GraphPad Software, San Diego, California, USA). Continuous variables that followed a normal distribution were represented as mean and standard deviation. In contrast, the Mann-Whitney U test was used if the data did not follow a normal distribution. For categorical variables, statistical comparisons were performed using Fisher's exact test.

Hardy-Weinberg equilibrium tests were conducted for each locus using the Basic Statistics of Gene[rate] Tools (one-locus summary; HLA-net, University of Geneva, Switzerland; https://hla-net.eu/tools/basic-statistics/) [16]. Alleles at each locus were considered in Hardy-Weinberg equilibrium if the observed and expected (estimated) frequencies did not differ significantly ($p>0.05$). The comparison of allele frequencies in this study was performed using Fisher's exact test. The Bonferroni-Dunn method for multiple testing correction was applied when the p values were significant.

HLA-DQB1 allele frequencies in Vietnam were based on information from the Allele Frequency Net Database (www.allelefrequencies.net) [17]. The HLA-DQB1*03:03 allele frequencies data of this study were compared to those in previous research on the Vietnamese population using Fisher's exact test.

The HLA allele-related risk estimation was expressed as an odds ratio (OR) and 95% confidence interval (CI) from logistic regression. A p-value and p-correction (p_c) less than 0.05 were considered to be statistically significant.

3. Results

3.1. Clinical and subclinical characteristics of the patient groups with epilepsy

The clinical and subclinical data of CBZ-treated patients with epilepsy are presented in Table 1. Statistical comparison results showed that, in the hypersensitivity group, the average dose used by patients with severe cutaneous adverse drug reactions (SJS/TEN) was higher than that of mild cutaneous adverse drug reactions (MCADR) ($p=0.0394$), and the hypersensitivity group had a significantly lower dose of the drug than the tolerant group ($p<0.0001$). Furthermore, the hypersensitivity group's haemoglobin content (g/l) was lower than that of the tolerant group ($p=0.0233$). The remaining characteristics between the two groups were not significantly different ($p>0.05$).

Table 1. Clinical and subclinical characteristics of Vietnamese patients with epilepsy.

Characteristics	MCADR (*n*=16)	SJS/TEN (*n*=5)	*p*-value	Hypersensitive cases (*n*=21)	Tolerant cases (*n*=23)	*p*-value
Average dose (mg/day)	315.6	500.00	**0.0394**†	359.53	595.65	**<0.0001**†
Age (year)						
Min-max	7-71	16-40		7-71	13-65	
Average	33	27	0.4834†	32	28	0.5484†
Gender (*n*, %)						
Male	9 (42.86%)	2 (40.00%)	0.6351††	11 (52.38%)	14 (60.87%)	0.7613††
Female	7 (43.75%)	3 (60.00%)		10 (47.62%)	9 (39.13%)	
Time of allergy appearance (day)	21	24	0.4332†	19		
Skin lesions (n, %)						
Maculopapular eruptions	9 (56.25%)	0 (0.00%)		4 (19.05%)		
Skin rashes and/or itches	7 (43.75%)	0 (0.00%)		8 (38.09%)		
Blisters and erosions, in which skin detachment	0 (0.00%)	5 (100.00%)		9 (42.68%)		
Subclinical features (X±SD)						
ALT (IU/ml)	23.86±12.14	42.44±26.61	0.0728†	28.28±17.83	24.97±13.09	0.5257†
AST (IU/ml)	24.82±9.85	45.42±36.79	0.1842†	29.72±20.59	22.20±7.50	0.1618†
Urea (mmol/l)	4.24±1.04	3.97±1.53	0.9837†	4.17±1.14	4.71±4.70	0.2594†
Creatinine (µmol/l)	59.73±11.45	51.38±7.22	0.1471†	57.74±11.05	63.90±13.77	0.2308†
Glucose (mmol/l)	5.39±0.52	7.36±3.90	0.3031†	5.86±2.00	5.46±0.63	0.6876†
Potassium (mmol/l)	3.65±0.28	3.77±0.29	0.6423†	3.67±0.28	3.72±0.24	0.5864†
RBC (T/l)	4.77±0.76	4.53±0.48	0.5582†	4.71±0.69	4.73±0.46	0.5333†
Hb (g/l)	133.30±10.05	128.20±9.86	0.2471†	132.10±10.01	139.17±13.58	**0.0233**†
PLT (G/l)	248.60±79.76	216.20±80.12	0.4950†	240.90±79.09	252.96±49.43	0.6541†
WBC (G/l)	5.95±1.99	7.06±1.75	0.1297†	6.22±1.95	7.46±2.39	0.0710†

*MCADR: mild cutaneous adverse drug reactions; ALT: alanine aminotransferase; AST: aspartate transaminase; RBC: red blood cell; Hb: haemoglobin; PLT: platelet; WBC: white blood cells; X: average; SD: standard deviation; † Mann-Whitney U test; †† Fisher's exact test, the p-value in bold was statistically significant.

3.2. Frequency of the HLA-DQB1 and -DPB1 alleles in carbamazepine-treated patients with epilepsy

There is no significant deviation from Hardy-Weinberg equilibrium for loci of HLA-DQB1 and -DPB1 in both groups (p>0.05). In total, we identified 19 HLA-DQB1 alleles in 44 Vietnamese epilepsy patients treated with CBZ (Table 2). Among these 19 alleles, HLA-DQB1*03:01:01 was the most frequent allele in both hypersensitivity and tolerance groups, with frequencies of 0.4048 and 0.5090, respectively. Additionally, the HLA-DQB1*03:03:02 allele appeared higher in the hypersensitivity group than in the tolerant group (p=0.0431); however, the difference was not significant after applying Bonferroni correction (p_c=0.5249).

For HLA-DPB1, 16 alleles were found in epileptic patients (Table 3), of which the HLA-DPB1*05:01:01 allele had the highest frequency. None of the 16 alleles were found to be associated with CBZ-induced hypersensitivity in epileptic patients.

Table 2. Frequency of the HLA-DQB1 allele in epilepsy patients treated with carbamazepine.

No.	HLA-DQB1 allele	Hypersensitive case (n=21)		Tolerant cases (n=23)		OR (95% CI)	p-value	p_c-value
		NA	*AF*	*NA*	*AF*			
1	02:01:01	1	0.0238	3	0.0652	0.35 (0.03-2.45)	0.6178	0.5771
2	02:02:01	1	0.0238	1	0.0217	1,10 (0.06-21.27)	>0.9999	0.9989
3	03:01:01	17	0.4048	15	0.3261	1,40 (0.58-3.30)	0.5090	0.9338
4	03:02:01	-	-	4	0,0870			
5	03:03:02	8	0.1905	2	0.0435	5.18 (1.11-25.05)	**0.0431**	0.5249
6	03:276N	1	0.0238	-	-			
7	03:358N	-	-	2	0.0435			
8	04:01:01	1	0.0238	2	0.0435	0.57 (0.04-4.78)	>0.9999	0.6868
9	04:02:01	1	0.0238	-	-			
10	05:01:01	4	0.0952	3	0.0652	1.51 (0.38-6.26)	0.7049	0.8525
11	05:01:24	3	0.0714	2	0.0435	1.69 (0.33-9.86)	0.6664	0.7979
12	05:02:01	2	0.0476	3	0.0652	0.72 (0.12-3.67)	>0.9999	0.8000
13	05:03:01	-	-	2	0.0435			
14	05:11:01	1	0.0238	-	-			
15	05:66:01	1	0.0238	-	-			
16	06:01:01	1	0.0238	4	0.0870	0.26 (0.02-1.68)	0.3631	0.5268
17	06:02:01	-	-	1	0.0217			
18	06:03:01	-	-	1	0.0217			0.5771
19	06:09:01	-	-	1	0.0217			0.9989

*: NA: number of alleles; AF: allele frequencies. The p-value obtained with Fisher's exact test, the bolded value was statistically significant. p_c: p-value after applying Bonferroni correction.

Table 3. Frequency of the HLA-DPB1 allele in epilepsy patients treated with carbamazepine.

No.	HLA-DPB1 allele	Hypersensitive case (*n*=21)		Tolerant cases (*n*=23)		OR (95% CI)	p-value	p_c-value
		NA	*AF*	*NA*	*AF*			
1	02:01:02	5	0.1190	3	0.0652	1.94 (0.45-7.651)	0.4710	0.7529
2	02:02:01	2	0.0476	1	0.0217	2.25 (0.25-33.24)	0.6039	0.6848
3	03:01:01	4	0.0952	2	0.0435	2.32 (0.51-12.58)	0.4192	0.6848
4	04:01:01	3	0.0714	5	0.1087	0.63 (0.16-2.66)	0.7158	0.7550
5	04:02:01	1	0.0238	-	-			
6	05:01:01	15	0.3571	15	0.3261	1.15 (0.45-2.71)	0.8238	0.9989
7	104:01:01	1	0.0238	2	0.0435	0.54 (0.04-4.78)	>0.9999	0.6868
8	105:01:01	-	-	1	0.0217			
9	107:01	-	-	1	0.0217			
10	13:01:01	5	0.1190	6	0.1304	0.90 (0.28-3.23)	>0.9999	0.9067
11	135:01	1	0.0238	-	-			
12	14:01:01	4	0.0952	3	0.0652	1.51 (0.38-6.26)	0.7049	0.8525
13	19:01:01	1	0.0238	1	0.0217	1.11 (0.06-21.27)	>0.9999	0.9989
14	21:01	-	-	3	0.0652			
15	235:01	-	-	1	0.0217			
16	31:01:01	-	-	2	0.0435			

*: NA: number of alleles; AF: allele frequencies; the p-value obtained with Fisher's exact test; p_c- p-value after applying Bonferroni correction.

Table 4. Comparison of HLA-DQB1*03:03 allele frequencies in some Vietnamese groups.

Group	Sample size	Genotyping method	AF	(4)		(5)		References
				p-value	*OR (95% CI)*	*p-value*	*OR (95% CI)*	
Hypersensitive (1)	21	NGS	0.19	0.2311	1.69 (0.80-3.62)	0.3270	1.59 (0.69-3.83)	
Tolerant (2)	23	NGS	0.04	0.1149	0.33 (0.08-1.18)	0.1236	0.31 (0.07-1.21)	
Epilepsy (3)	44	NGS	0.11	>0.9999	0.92 (0.46-1.74)	0.8471	0.87 (0.42-1.88)	
Vietnam Kinh DQB1 (4)	2076	SSOP	0.12					[18]
Vietnam Kinh (5)	101	NGS	0.13					[19]

*: (3)=(1) + (2); AF: allele frequencies.

Previous studies have reported data on the HLA-DQB1*03:03 allele using various research methods. To compare the frequency of this allele in Vietnamese groups, we searched information from the Allele Frequency Net Database and compared it to our own study. The results indicated that the frequency of the HLA-DQB1*03:03 allele in our study was consistent with previous publications (Table 4).

So far, we have not found any published data on the frequency of the HLA-DPB1 allele in the Vietnamese population, especially in Vietnamese patients with epilepsy.

*3.3. Prediction of the carbamazepine interaction site on HLA-DQB1*03:03 molecular structure*

The molecular structure of HLA-DQB1*03:03 was not available in the Protein Database bank (http://www.rcsb.org/pdb/), so SWISS-MODEL was used to model the structure of HLA-DQB1*03:03 based on the structure of the HLA-TCR complex (SMTL ID: 6px6.1) with sequence similarity up to 92.82% (Fig. 1A) [20]. The quality of the structure was confirmed using the SWISS-MODEL structural evaluation metrics [10]. The values of parameters GMQE=0.70 and QMEANDisCo Global=0.80±0.05 indicated that the model was sufficiently reliable and of high quality [11, 12]. The model quality was also evaluated based on the local similarity of the predicted protein to the corresponding target of each model residue (Fig. 1B) [21]. The results showed that the predictive model had good quality (quality scores ranged from 0.8 to 1.0), however, there were two areas (positions 70-80 and 135-145) with low quality (scores lower than 0.6). Besides these, the QMEAN Z-Scores were also used to evaluate and validate the model [22]. The QMEAN Z value was equal to -0.98 and the set of Z values for various parameters including the interaction between the second carbon atoms (Cβ) was equal to -0.28; interaction between all atoms was 0.14; the solvation, -0.05, and the torsion, -0.97, were both small and close to 0, which indicated good quality of the model (Fig. 1C). Fig. 1D shows a histogram of the normalised QMEAN score based on protein size, relating the model quality score to the values obtained for experimental constructs of similar size. When compared to the non-redundant set of PDB structures, the HLA-DQB1*03:03 model's structure was found to be in the range |Z-score|<1, confirming reliability. In addition, Ramachandran analysis revealed that 95.86% of the amino acid residues were in regions corresponding to the quadratic structure of the Ramachandran plot, 0.00% of the radicals were in the outliers, and only 0.30% of the side chains of the sequence with low energy were A120 SER, further showing that the model had good quality (Fig. 1E) [23, 24]. Based on the above parameters, the HLA-DQB1*03:03 model was considered reliable.

Following the construction of the structural model of HLA-DQB1*03:03, molecular docking analysis was performed to evaluate the possibility of interaction between DQB1*03:03 protein and CBZ. The chemical

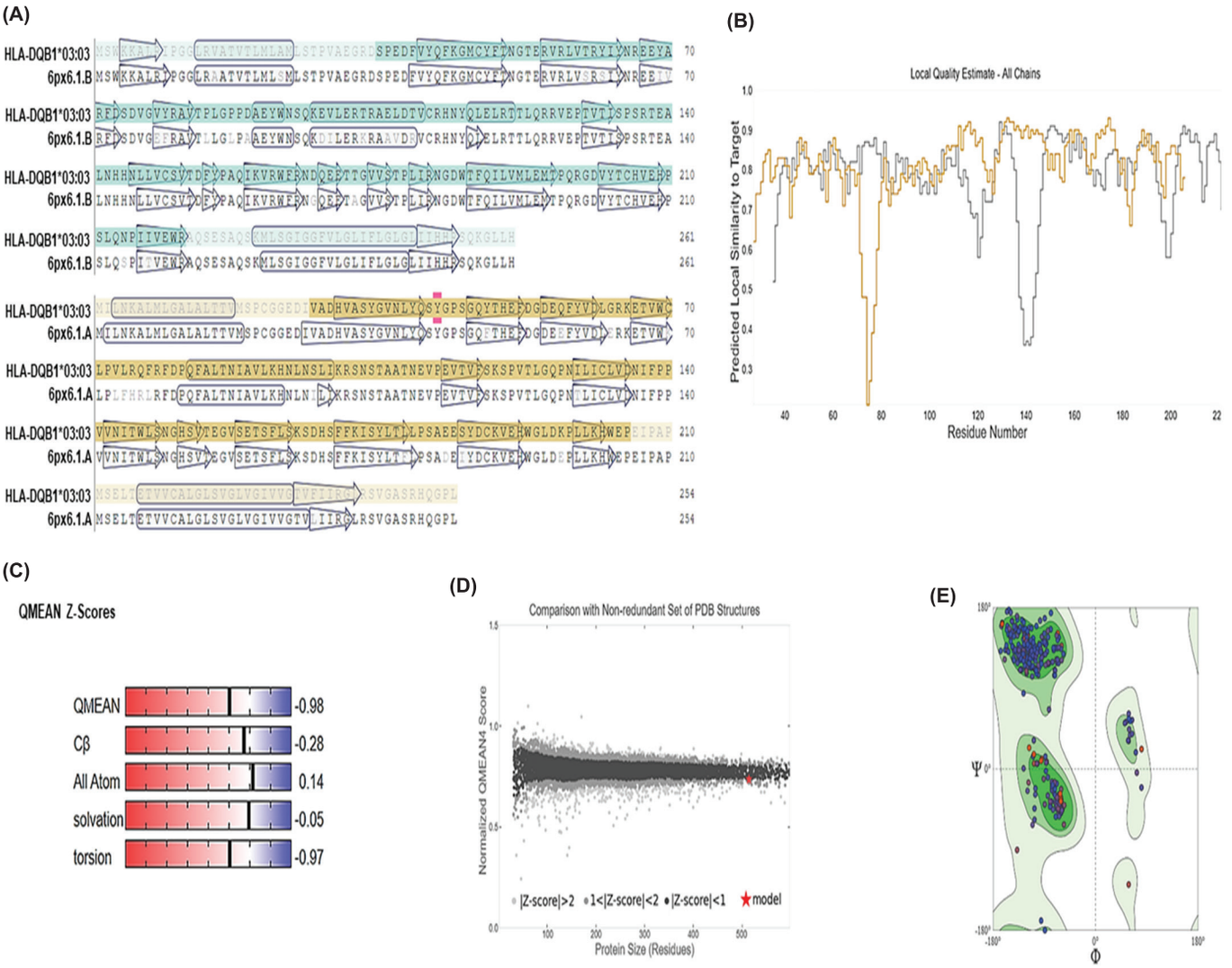

Fig. 1. HLA-DQB1*03:03 structure modelling using SWISS-MODEL Interactive Workspace. (A) Sequence alignment of target (HLA-DQB1*03:03) and template (HLA-TCR complex, SMTL ID: 6px6.1) by SWISS-MODEL; **(B)** Local quality estimate graph showing the values of the predicted local similarity to target (y-axis) plotted against the protein residue number (x-axis), this quality estimation was carried out on both chains of the target protein, with the yellow and grey lines representing chains (A) and (B), respectively; **(C)** QMEAN Z-Scores of the model; **(D)** Comparison with a non-redundant set of PDB structure (The x-axis shows protein length, represented by the number of residues). The y-axis was the normalized QMEAN score. Each dot represented an experimental protein structure, and the |Z-score| indicated the standard deviation from the mean. DQB1*03:03 model is represented as a red star; **(E)** Ramachandran plot (the white area was the outlier, where there were almost no Φ/Ψ pairs; the light green area was the area created by the first contour line, where there were 99.7% of the Φ/Ψ pairs; the green area was the area created by the 2nd contour line, where 95.0% of the number of Φ/Ψ pairs were present, and the dark green area was the area of interest, created by the 3rd contour line, where there were 80.0% the number of Φ/Ψ pairs).

binding sites were predicted using the SwissDock software, and the results were visualised using the UCSF Chimera software [13-15]. In the results, 38 clusters were obtained with cluster rank, of which 6/38 clusters expressed CBZ interacting with HLA-DQB1*03:03 at the peptide binding cleft. The cluster with the lowest molecular FullFitness and binding Estimated ΔG values were selected with indices of -1789.0076, kcal/mol and -6.703787 kcal/mol, respectively (Fig. 2A). CBZ binds to hydrophobic amino acids at the binding site and neutralises the potential on the β chain encoded by the HLA-DQB1*03:03:02 gene (Figs. 2B and 2C).

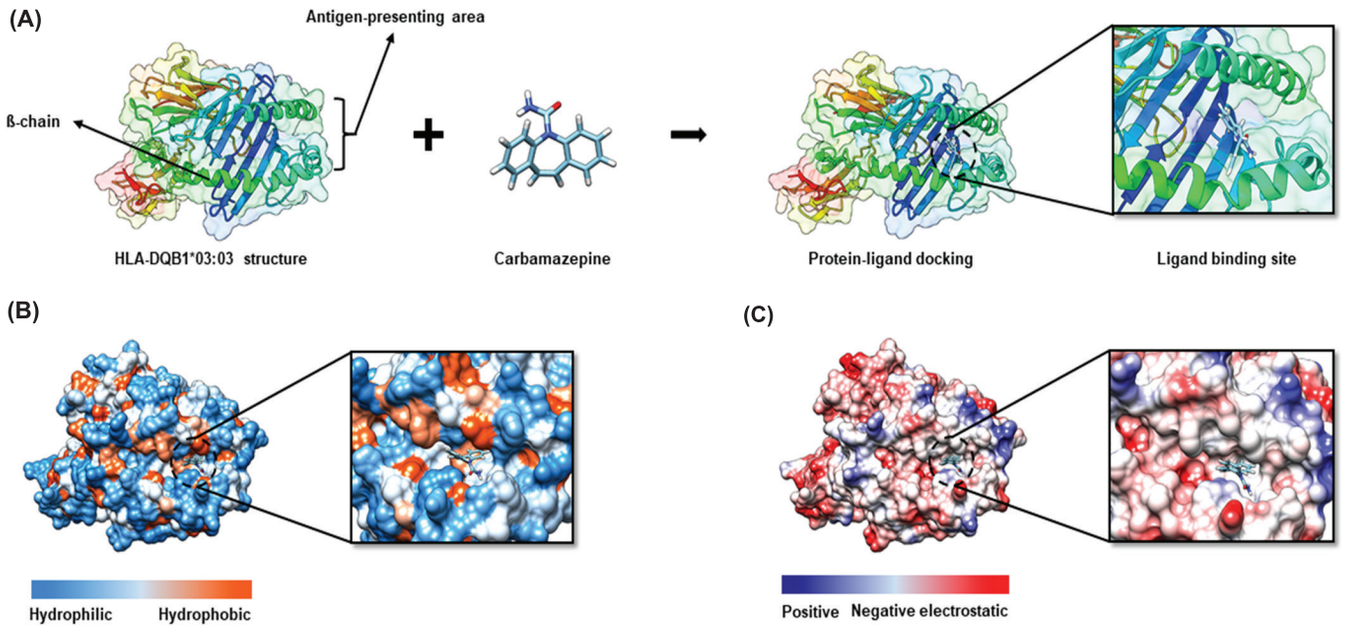

Fig. 2. Simulation of interaction between DQB1*03:03 protein and carbamazepine by molecular docking analysis. (A) The ribbon structure of the HLA-DQB1*03:03 molecule and its interaction site with carbamazepine; **(B)** The hydrophobic surface of the HLA-DQB1*03:03 molecule and interaction site with carbamazepine (the surface colour is mapped from red to blue to denote the most hydrophobic and hydrophilic regions, respectively); **(C)** The electrostatic surface of the HLA-DQB1*03:03 molecule and interaction site with carbamazepine (red the negative electrostatic potential energy values and in blue the positive values).

4. Discussion

CBZ is one of the most commonly prescribed antiepileptic drugs for the treatment of seizures. Patients who develop symptoms of hypersensitivity to the drug are often advised by their physician to discontinue its use to resolve the symptoms. For tolerant patients, the daily dose of CBZ may be increased, and the duration of dosing may be prolonged. Long-term use of antiepileptic drugs can lead to potentially toxic effects, especially haematological side effects. In this study, we found that patients in the SJS/TEN group who were taking carbamazepine had a significantly higher daily dose (p=0.0394) compared to the MCADR group. The observed severe drug reactions may be due to the high dose. This contradicts the results of a previous study by K.W. Chong, et al. (2014) [25], which showed that the SJS/TEN group had a lower median dose compared to the minor drug reactions group (0.2 mg/kg/day (range 4.6-7.4) and 6.7 mg/kg/day (range 3.6-20.0) for the two groups, respectively). However, our findings are consistent with those of V. Hariraj, et al. (2023) [26], who reported that carbamazepine-induced severe cutaneous adverse drug reactions were 3.6 times more likely to occur in patients with a daily dose of 200 mg or less compared to those with a daily dose of 400 mg or more. Additionally, analytical results showed that haemoglobin (g/l) levels were increased in epileptic patients with high doses of CBZ. This contrasts with the results of O.S.E. Shimi, et al. (2021) [27], who suggested that haemoglobin content, platelet count, serum C4, and IgA levels were negatively correlated with CBZ use.

CBZ has been reported to cause serious adverse skin reactions in Vietnamese patients. In the HLA class I group, HLA-B*15:02 has been found to be associated with the incidence of severe cutaneous adverse reactions associated with CBZ treatment, while no association has been found between HLA-A and the reactions [28]. In our previous study (2023), we observed a higher prevalence of three alleles (HLA-B*46:01:01, HLA-DQA1*03:02:01, and HLA-DRB1*09:01:02) in the CBZ hypersensitivity group compared to the CBZ tolerant group when typing three loci of HLA class I (HLA-A, -B, and -C) and two loci of HLA class II (HLA-DQA1 and -DRB1). The

sensitivity and specificity of the HLA-B*46:01:01/HLA-DRB1*09:01:02 combination with respect to CBZ hypersensitivity diagnosis were 46.88 and 84.85%, respectively. These findings suggest that the presence of HLA-B*46:01:01/HLA-DRB1*09:01:02 may serve as a potential marker for CBZ-induced hypersensitivity reactions in Vietnamese patients [29]. In the present study, we found that the allele DQB1*03:03:02 was significantly associated with hypersensitivity symptoms in Vietnamese patients treated with CBZ (p=0.0431). However, this significance was lost after applying the Bonferroni correction. This is consistent with the findings of X.J. He, et al. (2013) [30] in the Han ethnic group of northeastern China (p=0.022, after applying Bonferroni correction p_c>0.05).

An association between the HLA allele and adverse drug reactions has been identified in several ethnicities. Several studies have evaluated the association between haplotype and drug-induced hypersensitivity reactions due to linkage disequilibrium in the HLA region. A previous study showed a relatively high risk (16.09) of haplotype HLA-A*24:02~B*59:01~C*01:02 carriers in 15 Japanese patients exhibiting CBZ-induced hypersensitivity reactions (10 patients with mild symptoms and 5 patients with SJS) [31]. In 21 Mexican Mestizo patients with epilepsy, haplotype A*02:01:01~B*35:01:01~C*04:01:01 was found to be associated with lamotrigine-induced macular rash (p=0.0048) [32]. In our study, the frequency of haplotype DQB1*03:03:02-DPB1*05:01:01 was 0.095 in the hypersensitivity group and 0.022 in the tolerance group; however, there was no statistically significant difference between the two groups (p=0.1881). Additionally, due to the lack of publicly available HLA-DPB1 data for the Vietnamese population, a comparison with the control group was not possible.

Currently, studies show that the hydrophobicity and electrical charge of amino acids greatly affect the strength of protein molecules, especially the hydrophobicity of the ligand-HLA complex. However, reports directly related to drug interactions of HLA class II molecules are still limited, necessitating additional research [33-35]. In general, the binding of CBZ to HLA is probably weaker than natural and drug-target interactions because the HLA binding site does not spontaneously evolve to recognise the drug [28]. This poses challenges for drug combinations as fewer interactions can be formed between the drug and the HLA recognition site. However, finding specific alleles and modelling the interactions between them are essential because of their application in disease screening and treatment.

Several limitations need to be rectified by future research. Firstly, because the sample size is relatively small, larger studies should be conducted to clarify the findings in this study more thoroughly. Secondly, there is not enough data to analyse the haplotype, more data is needed for analysis. Thirdly, the exclusion of patients without genotyping results is another limitation, and future endeavours with more financial support should aim to include more patients to obtain a more comprehensive understanding and provide data that can reflect the whole population.

5. Conclusions

Genetic susceptibility to the risk of carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy is not found in the HLA DPB1 region. The frequency of the HLA-DQB1*03:03:02 allele was higher in the hypersensitivity group than in the tolerant group (p=0.0431) although this difference was not statistically significant after applying the Bonferroni correction and the evidence of *in silico* interaction between CBZ and HLA-DQB1*03:03 at the peptide binding cleft suggested that HLA-DQB1*03:03:02 allele exhibited a tendency to behave as a susceptibility factor for developing carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy.

CRediT author statement

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] World Health Organization (2022), *Epilepsy*, https://www.who.int/en/news-room/fact-sheets/detail/epilepsy, accessed 19 September 2022.

[2] N.A. Tuan, L.Q. Cuong, P. Allebeck, et al. (2008), "The prevalence of epilepsy in a rural district of Vietnam: A population-based study from the EPIBAVI project", *Epilepsia*, **49(9)**, pp.1634-1637, DOI: 10.1111/j.1528-1167.2008.01663.x.

[3] H. Arif, R. Buchsbaum, D. Weintraub, et al. (2007), "Comparison and predictors of rash associated with 15 antiepileptic drugs", *Neurology*, **68(20)**, pp.1701-1709, DOI: 10.1212/01.wnl.0000261917.83337.db.

[4] F.J.F. Fernández, J.A. Garrido, M. Hauben, et al. (1995), "Severe adverse cutaneous reactions to drugs", *The New England Journal of Medicine*, **332(14)**, pp.959-960, DOI: 10.1056/NEJM199504063321414.

[5] J. Trowsdale (2011), "The MHC, disease and selection", *Immunology Letters*, **137(1-2)**, pp.1-8, DOI: 10.1016/j.imlet.2011.01.002.

[6] J.H. Karnes, L. Bastarache, C.M. Shaffer, et al. (2017), "Phenome-wide scanning identifies multiple diseases and disease severity phenotypes associated with HLA variants", *Science Translational Medicine*, **9(389)**, pp.139-148, DOI: 10.1126/scitranslmed.aai8708.

[7] W.G. Horta, E. Paradela, A. Figueiredo, et al. (2015), "Genetic association study of the HLA class II alleles DRB1, DQA1, and DQB1 in patients with pharmacoresistant temporal lobe epilepsy associated with mesial hippocampal sclerosis", *Seizure*, **31**, pp.7-11, DOI: 10.1016/j.seizure.2015.06.005.

[8] A. Fadda, M. Alsabbagh, D. Vasudeva, et al. (2022), "The complex etiology of Epilepsy: Genetic analysis and HLA association in patients in the Middle East", *Research Square*, pp.1-14, DOI: 10.21203/rs.3.rs-2358929/v1.

[9] T. Schwede, J. Kopp, N. Guex, et al. (2003), "SWISS-MODEL: An automated protein homology-modeling server", *Nucleic Acids Research*, **31(13)**, pp.3381-3385, DOI: 10.1093/nar/gkg520.

[10] A. Waterhouse, M. Bertoni, S. Bienert, et al. (2018), "SWISS-MODEL: Homology modelling of protein structures and complexes", *Nucleic Acids Research*, **46(Web Server Issue)**, pp.W296-W303, DOI: 10.1093/nar/gky427.

[11] M. Biasini, M. Bertoni, A. Waterhouse, et al. (2014), "SWISS-MODEL: Modelling protein tertiary and quaternary structure using evolutionary information", *Nucleic Acids Research*, **42(Web Server Issue)**, pp.W252-W258, DOI: 10.1093/nar/gku340.

[12] G. Studer, C. Rempfer, A.M. Waterhouse, et al. (2020), "QMEANDisCo-distance constraints applied on model quality estimation", *Bioinformatics*, **36(6)**, pp.1765-1771, DOI: 10.1093/bioinformatics/btz828.

[13] A. Grosdidier, V. Zoete, O. Michielin (2011), "SwissDock, a protein-small molecule docking web service based on EADock DSS", *Nucleic Acids Research*, **39**, Suppl. 2, pp.W270-W277, DOI: 10.1093/nar/gkr366.

[14] A. Grosdidier, V. Zoete, O. Michielin (2007), "EADock: Docking of small molecules into protein active sites with a multiobjective evolutionary optimization", *Proteins*, **67(4)**, pp.1010-1025, DOI: 10.1002/prot.21367.

[15] E.F. Pettersen, T.D. Goddard, C.C. Huang, et al. (2004), "UCSF Chimera - A visualization system for exploratory research and analysis", *Journal of Computational Chemistry*, **25(13)**, pp.1605-1612, DOI: 10.1002/jcc.20084.

[16] J.M. Nunes, S. Buhler, D. Roessli, et al. (2014), "The HLA-net GENE[RATE] pipeline for effective HLA data analysis and its application to 145 population samples from Europe and neighbouring areas", *Tissue Antigens*, **83(5)**, pp.307-323, DOI: 10.1111/tan.12356.

[17] F.F.G. Galarza, S. Christmas, D. Middleton, et al. (2011), "Allele frequency net: A database and online repository for immune gene frequencies in worldwide populations", *Nucleic Acids Research*, **39**, Suppl. 1, pp.913-919.

[18] T.N. Que, N.B. Khanh, P.D. Tung, et al. (2022), "Frequency and distribution of HLA-DQB1 alleles from 2076 cord blood samples of the Vietnamese cohort", *International Journal of Immunogenetics*, **49(5)**, pp.340-344, DOI: 10.1111/iji.12592.

[19] D.M. Duc, L.H. G. Linh, N.T. Vinh, et al. (2020), "High-resolution HLA typing of HLA-A, -B, -C, -DRB1, and -DQB1 in Kinh Vietnamese by using Next-Generation sequencing", *Frontiers in Genetics*, **11**, pp.1-10, DOI: 10.3389/fgene.2020.00383.

[20] H.M. Berman, J. Westbrook, Z. Feng, et al. (2000), "The protein data bank", *Nucleic Acids Research*, **28(1)**, pp.235-242, DOI: 10.1093/nar/28.1.235.

[21] P. Benkert, M. Biasini, T. Schwede (2011), "Toward the estimation of the absolute quality of individual protein structure models", *Bioinformatics*, **27(3)**, pp.343-350, DOI: 10.1093/bioinformatics/btq662.

[22] P. Benkert, S.C.E. Tosatto, D. Schomburg (2008), "QMEAN: A comprehensive scoring function for model quality assessment", *Proteins: Structure, Function, and Genetics*, **71(1)**, pp.261-277, DOI: 10.1002/prot.21715.

[23] V.B. Chen, W.B. Arendall, J.J. Headd, et al. (2010), "MolProbity: All-atom structure validation for macromolecular crystallography", *Acta Crystallographica. Section D, Biological Crystallography*, **66(1)**, pp.12-21, DOI: 10.1107/S0907444909042073.

[24] S. Liang, N.V. Grishin (2002), "Side-chain modelling with an optimized scoring function", *Protein Sci.*, **11(2)**, pp.322-331, DOI: 10.1110/ps.24902.

[25] K.W. Chong, D.W.S. Chan, Y.B. Cheung, et al. (2014), "Association of carbamazepine-induced severe cutaneous drug reactions and HLA-B*1502 allele status, and dose and treatment duration in paediatric neurology patients in Singapore", *Archives of Disease in Childhood*, **99(6)**, pp.581-584, DOI: 10.1136/archdischild-2013-304767.

[26] V. Hariraj, W.K. Wo, S.C. Lee, et al. (2023), "Carbamazepine-induced severe cutaneous adverse drug reactions: A 21-year comparison between children and adults in Malaysia", *The Journal of Clinical Pharmacology*, **63(10)**, pp.1126-1132, DOI: 10.1002/jcph.2289

[27] O.S.E. Shimi, A.A. Farag, A.M.E. Rebigi, et al. (2021), "Carbamazepine-induced hematological and immunological alterations in Egyptian children with idiopathic generalized seizures", *Journal of Child Science*, **11(1)**, pp.e265-e272, DOI: 10.1055/s-0041-1736156.

[28] N.V. Dinh, C. Vidal, H.C. Chu, et al. (2019), "Developing pharmacogenetic screening methods for an emergent country: Vietnam", *World Allergy Organization Journal*, **12(5)**, DOI: 10.1016/j.waojou.2019.100037.

[29] B.P. Thao, N.T.T. Linh, L.L. Phuong, et al. (2023), "Next-generation sequencing-based HLA typing reveals the association of HLA-B*46:01:01 and HLA-DRB1*09:01:02 alleles with carbamazepine-induced hypersensitivity reactions in Vietnamese patients with epilepsy", *Human Immunology*, **84(3)**, pp.186-195, DOI: 10.1016/j.humimm.2023.01.005.

[30] X.J. He, L.Y. Jian, X.L. He, et al. (2013), "Association between the HLA-B 15:02 allele and carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Han individuals of northeastern China", *Pharmacological Reports*, **65(5)**, pp.1256-1262, DOI: 10.1016/s1734-1140(13)71483-x.

[31] H. Ikeda, Y. Takahashi, E. Yamazaki, et al. (2010), "HLA class I markers in Japanese patients with carbamazepine-induced cutaneous adverse reactions", *Epilepsia*, **51(2)**, pp.297-300, DOI: 10.1111/j.1528-1167.2009.02269.x.

[32] I.F. Galindo, I.E.M. Juárez, N.M. Jaramillo, et al. (2014), "HLA-A*02:01:01/-B*35:01:01/-C*04:01:01 haplotype associated with lamotrigine-induced maculopapular exanthema in Mexican Mestizo patients", *Pharmacogenomics*, **15(15)**, pp.1881-1891, DOI: 10.2217/pgs.14.135.

[33] J. Kraml, A.S. Kamenik, F. Waibl, et al. (2019), "Solvation free energy as a measure of hydrophobicity: Application to serine protease binding interfaces", *Journal of Chemical Theory and Computation*, **15(11)**, pp.5872-5882, DOI: 10.2217/pgs.14.135.

[34] J. Kalayan, R.H. Henchman, J. Warwicker (2020), "Model for counterion binding and charge reversal on protein surfaces", *Molecular Pharmaceutics Journal*, **17(2)**, pp.595-603, DOI: 10.1021/acs.molpharmaceut.9b01047.

[35] D. Chowell, S. Krishna, P.D. Becker, et al. (2015), "TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes", *Proceedings of The National Academy of Sciences of The United States of America*, **112(14)**, pp.E1754-E1762, DOI: 10.1073/pnas.1500973112.