A European study of HLA-B in Stevens–Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs

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Background **Stevens–Johnson syndrome (SJS) and its severe form, toxic epidermal necrolysis (TEN), are rare but life-threatening cutaneous adverse reactions to drugs, especially to allopurinol, carbamazepine, lamotrigine, phenobarbital, phenytoine, sulfamethoxazole, oxicam and nevirapine. Recently, a strong association between carbamazepine and allopurinol induced SJS or TEN has been described with respectively, HLA-B*1502 and HLA-B*5801 in a Han Chinese population from Taiwan and other Asian countries.**

Objective **The objective is to further investigate the relationship between SJS/TEN and HLA-B in a large number of patients in a European population.**

Methods **HLA-B genotyping was performed on 150 patients included in a European study (RegiSCAR) of SJS and TEN. We focused on patients related to 'high-risk' drugs including: 31 cases related to allopurinol, 28 to sulfamethoxazole, 19 to lamotrigine and 14 to oxicam.**

Results **Sixty-one percent of 31 allopurinol-induced SJS/TEN patients carried the HLA-B*5801 allele and the figure was 55% for 27 patients of European ancestry [odds ratio = 80 (34–187)], ($P < 10^{-6}$) as previously observed in Han Chinese. For other drugs, two rare alleles showed a weaker association with SJS/TEN in a limited number of patients: B*38 for sulfamethoxazole or lamotrigine-related patients, and B*73 for oxicam.**

Conclusion **At variance with prior results in Asia, this study shows that even when HLA-B alleles behave as strong risk factors, as for allopurinol, they are neither sufficient nor necessary to explain the disease. Further investigations are necessary to delineate the exact role of the HLA region in SJS/TEN, and to look for other associations in other regions of the genome.**
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Introduction

Adverse reactions to drugs are important causes of morbidity and even of death in developed countries [1]. Cutaneous eruptions are the most frequently occurring adverse reaction to drugs. Among hospitalized patients, the incidence of these reactions ranges from 1 to 3%. The frequency of cutaneous reactions to some drugs may exceed 10% [2]. Severe cutaneous adverse reactions (SCAR) include Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug reactions with eosinophilia and systemic symptoms also called drug-induced hypersensitivity syndrome. All are probably delayed-type immune-mediated reactions [3]. SJS and TEN are considered to be two forms of the same disease, TEN being more severe. Patients develop an acute exanthema, which progresses towards limited (SJS) or widespread (TEN) blistering and erosion of the skin and mucous membranes, resulting from apoptosis of keratinocytes [4]. The incidence of SJS/TEN is estimated to be approximately one to two patients per million inhabitants per year [5]. These reactions have high morbidity and mortality. Despite the fact that more than 100 different drugs have been associated with the development of SJS/TEN in single case reports or

retrospective studies [6], half of the cases are caused by a limited number of drugs: antibacterial sulfonamides, especially sulfamethoxazole (SMX), allopurinol, carbamazepine, lamotrigine, phenobarbital, phenytoine, NSAID of the oxicam type and nevirapine [7–9].

Although the pathophysiology of SJS and TEN remains largely unknown, previous work suggests an immune mechanism involving a drug-dependent cytotoxic cell response against epidermal cells. Cytokines such as IFNγ, Fas ligand (FasL) and others are likely to play an important role in the extension of apoptosis of keratinocytes. The initial events triggering the immune reaction remain to be identified [10].

Genetic susceptibility to SCARs has been suspected for years because of a few reports of family cases [11,12], and ancient studies suggesting weak associations with serologically determined HLA [13].

It has also been proposed that drug-reactive metabolites, either produced in excess or not detoxified, played a key role in initiating an abnormal immune response. Such defects could be genetically determined, as suggested by several examples of individual pharmacogenetic variability associated with specific adverse drug reactions [14]. Several lines of evidence, however, have recently suggested that drug-specific immunological response is directed against the parent form of the drug more often than against a metabolite [15].

In the previous years growing interest focused on the role of HLA in severe drug reactions, which was initiated by the demonstration that HLA-B*5701, was strongly associated with hypersensitivity to the anti-HIV medication abacavir [16,17]. Subsequently, a Taiwanese study reported a 100% association with HLA-B*1502 in 44 Han Chinese patients affected by carbamazepine-induced SJS/TEN [18]. This was followed by another study from the same group showing again a 100% association between HLA-B*5801 and 51 allopurinol-induced severe cutaneous reactions, including 21 with SJS/TEN [17].

These observations led us to perform HLA-B typing in 150 European patients with SJS and TEN, focusing on subgroups defined by exposure to drugs highly suspected of inducing SJS/TEN.

Patients and methods

Patients

All 150 patients who were tested had a diagnosis of SJS or TEN validated as 'probable' or 'definite' by the international expert committee of the RegiSCAR study using standardized criteria for clinical case review. The first 70 patients had been approached retrospectively months or years after recovery from SJS or TEN with the help of a French patient association. Following publications suggesting that HLA-B associations might be drug specific we enlarged this original group by analyzing 80 prospectively ascertained patients of the RegiSCAR study, chosen for being associated with one 'highly suspected' drug. This resulted in subgroups of patients attributed to allopurinol ($n = 31$), SMX ($n = 28$), lamotrigine ($n = 19$), oxicam-NSAIDs ($n = 14$) and carbamazepine ($n = 12$). The last group of 46 patients consisted of patients exposed to miscellaneous drugs (less than 10 patients exposed for each) or with doubtful drug causes.

The above 'high-risk' drugs were considered responsible when (i) they were taken by the patient in the time period between 4 and 10 days before the index day (day of onset of the reaction), (ii) the treatment had begun within less than 42 days before the index day and (iii) there had been no exposure to another 'high-risk' drug in the same period.

Information related to ethnicity was limited to skin phenotype, place of birth of the patient and his/her parents (when available). From these criteria we established a probable ancestry as European, Asian or African. Tables 1–5 present clinical characteristics of the SJS/TEN patients according to the causal agent.

The indication for which the drug had been prescribed was mainly hyperuricemia for the allopurinol patients (23/26 prospective patients); anti-infectious SMX was taken for a variety of infectious conditions. For lamotrigine, the indication was mostly epilepsy (12/18), whereas this was the case for a minority of patients treated with carbamazepine, the other being treated for psychiatric disorders or chronic pain.

For each patient, written informed consent, approved by the ethical committee of each participating country, was obtained. A standardized clinical assessment was made and a blood sample was taken for DNA extraction. Genomic DNA extraction was carried out by the CEPH-Laboratoire Jean-Dausset (Paris, France).

Controls

As the frequency of HLA alleles has been determined in several large samples of European populations we considered that controls matched on age and sex, who had been enrolled in the RegiSCAR study at a ratio of 1/1, would provide no advantage in comparison with data of external literature. For comparison, we used allele frequencies from European populations available on dbMHC: *http://www.ncbi.nlm.nih.gov/projects/mhc/ihwg.cgi*

HLA-B genotyping

Low-resolution HLA-B genotyping was carried out with the OLERUP SSP HLA-B kit in a PCR with

sequence-specific primers, according to the protocol and recommendations of the manufacturer (PCR-SSP, GenoVision Inc., West Chester, Pennsylvania, USA). Each tube contains a dried primer solution consisting of a specific primer mix, that is, allele-specific and group-specific primers and a control primer pair matching nonallelic sequences. This internal positive control indicates that the PCR was successful.

After migration of PCR products on 2% agarose gels, the HLA-B allele types were determined with the aid of the HELMBERG-SCORE software (GenoVision).

For all HLA-B alleles that appeared to have an increased frequency among the patient population (B*15, B*35, B*38, B*51, B*58, B*73), high resolution HLA-B subtyping was carried out using sequence specific primers of the Lamda One subtype kits (PCR-SSP, InGen, Technopolis, Chilly Mazarin, France). The protocol recommended by the manufacturer was used for each subtyping assay. Gel migration results were analyzed with the Lamda One software to obtain the specific HLA-B subtype.

Sequencing

To analyze the sequence of the HLA-B*5801 gene in three allopurinolinduced SJS/TEN patients (018, 043, 053), two HLA-B*5801 specific primers were designed, the forwards in the 5′ NCR (5′-CCAGTTCTAAAGT CCCCACG-3′) the other in the 3′ NCR (5′-TTCTG GTTAGTCATGGTAAGC-3′). Using these primers in a PCR reaction amplified a gene fragment of 2903 bp, which was ligated into the pGEM-T easy vector (Promega Corporation, Madison, Wisconsin, USA). Clones from each patient-amplified sequence were sequenced using the Big Dye Teminator V3.1 cycle sequencing kit (Applied Biosystems, Foster City, California, USA) and a panel of 14 internal primers designed from the published HLA-B*5801 sequence to produce overlapping sequences in both the forwards and reverse directions. Products from sequencing reactions were migrated on the ABI model 3100 DNA sequencer (Applied Biosystems). As the sequence of HLA-B*5801 fragment for each patient was 100% identical to that of the published HLA-B*5801 gene, further sequencing of other clones was deemed unnecessary.

Statistical methods

Hardy–Weinberg equilibrium was tested by an exact test, with 100 simulations.

The allele frequencies between patients and controls from the reference population were compared using a χ^2 test. Fisher's exact test was used for alleles present in fewer than five patients or controls. Odds ratios (OR) and 95% confidence intervals (CI) were calculated according to Woolf's formula. All *P*-values were corrected using Bonferronni method, the correction factor being the number of alleles observed in the whole sample, that is, 27.

Results

No deviation from the Hardy–Weinberg equilibrium was observed in our sample of 150 patients. Most patients (133, 89%) were of European ancestry and could be qualified as 'Caucasians'. One patient was from North Africa, eight originated from sub-Saharan Africa, six from Asia and two from South America.

HLA-B allele distributions among all patients related to 'highly suspected' drugs are shown in Tables 1–5. In these tables, statistics compared all exposed patients with general population data. In Table 6 we present an overview of carrier prevalence and OR from analyses restricted to patients of European ancestry.

The results with carbamazepine have already been published [19]. As a group, the 12 patients (Table 1) had a highly significant increase in the allelic frequency of HLA-B*1502 (4/24 compared with 2/2580 in miscellaneous European populations, $P < 10^{-4}$). All four reactions occurred in patients of Asian ancestry.

Table 1 HLA-B genotyping in patients with Carbamazepine-related SJS or TEN

Patient	Age	Sex		Ancestry	HLA-B 1	HLA-B, 2
3	31	M	SJS	Asian (Vietnam)	**15**	38
25	46	M	SJS/TEN	European (D)	35	44
72	52	M	TEN	European (F)	50	51
118	40	M	SJS/TEN	Asian (China)	13	**15**
184	54	M	SJS	European (F)	07	44
186	39	F	SJS/TEN	Asian (Cambodia)	13	**15**
204	74	F	SJS/TEN	European (F)	37	73
289	46	M	SJS	European (D)	08	27
323	57	M	SJS/TEN	European (F)	18	57
350	42	F	TEN	European (F)	08	35
383	26	F	SJS	Asian (Reunion)	**15**	37
551	37	M	SJS	European (F)	08	35

A significant excess of HLAB*15, all being B*1502, restricted to patients of Asian ancestry was seen.
SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.
Bold values are statistically significant association.

Table 2 HLA-B genotyping in patients with Allopurinol-related SJS or TEN

Patient	Age	Sex		Ancestry	HLA1	HLA2
18	30	F	SJS	Asian (Pakistan)	07	**58**
43	49	M	SJS	South American (Cuba)	**58**	**58**
53	83	M	SJS/TEN	European (UK)	44	**58**
73	81	F	SJS	European (D)	27	35
91	42	M	SJS/TEN	European (D)	18	44
100	58	F	SJS/TEN	European (D)	08	44
104	75	F	SJS	European (D)	49	**58**
105	21	M	SJS	European (D)	13	44
106	74	M	SJS/TEN	European (D)	44	51
125	68	M	SJS/TEN	European (I)	18	**58**
141	66	F	SJS	European (I)	18	**58**
152	82	M	SJS	European (I)	08	**58**
153	60	F	SJS	European (D)	44	57
171	42	F	SJS	European (D)	07	**58**
177	46	M	TEN	European (F)	44	**58**
185	56	M	SJS/TEN	European (F)	35	52
224	53	M	SJS	Asian (India)	15	**58**
225	41	M	SJS/TEN	European (F)	53	**58**
233	35	M	SJS/TEN	European (D)	51	**58**
263	69	M	SJS/TEN	European (D)	35	35
354	64	F	SJS	European (D)	08	13
570	37	M	TEN	African (Senegal)	49	**58**
588	34	M	SJS/TEN	European (D)	08	51
593	77	F	SJS	European (D)	18	**58**
644	79	F	SJS	European (D)	51	**58**
721	62	M	SJS/TEN	European (D)	44	**58**
859	37	M	SJS	European (D)	**58**	81
907	39	M	SJS	European (D)	40	41
909	26	F	SJS	European (D)	35	44
980	56	F	TEN	European (D)	35	**58**
1059	74	F	TEN	European (D)	08	**58**

B*58 was present in 19/31 patients, with an allele frequency of 20/62 (0.32). This was significantly higher ($P<10^{-8}$) than the allele frequency in European populations (0.008). All B*58 alleles were B*5801 by high resolution genotyping. No other allele was significantly more frequent than expected.
SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.
Bold values are statistically significant association.

In the group of 31 allopurinol-associated patients we found a significant increase of B*58 allele (Table 2). The carrier frequency was 0.61 (19/31), and the allele frequency 0.32 (20/62) compared with a frequency of 0.008 among European populations in the dbMHC database [$P < 10^{-8}$, OR = 61 (32–118)]. All B*58 alleles were B*5801, as were all the 28 B*58 alleles in the European populations included in the dbMHC database. Among our 31 patients, four were of non-European ancestry (two from Asia, one from South America and one from Africa). All four had B*5801, but that did not decrease substantially the highly significant association with this allele in European patients (Table 6). In the 12 patients who did not have the B*5801allele, there was no other HLA-B allele that seemed to be more prevalent in the control population (Table 2).

In three patients, the B*5801 gene was cloned and analysis of its sequence showed no new mutation compared with the NCBI reference [20].

For other drugs, there was no single allele found in a large proportion of patients, but a few associations were anyhow significant after correction for multiple comparisons. For SMX (Table 3) B*38 was significantly associated [allele frequency 0.125 (7/56) compared with 0.022, P cor < 0.01, OR = 6.4 (2.8–14)].

Among patients related to lamotrigine (Table 4), there was also an increased allele frequency of B*38 [0.13 (5/38) versus 0.022, P cor < 0.02, OR = 6.8 (2.6–18)].

We also noticed that three European patients in our series expressed HLA B*73, a very rare allele found in only two of 3644 alleles in European populations according to the dbMHC database. One had reacted to carbamazepine (Table 1) and two to an oxicam, NSAID (Table 5). For the latter, despite an elevated OR [152 (20–1167)] the association was not significant ($P < 0.73$).

In the miscellaneous group, we observed no significant association (data not shown but available online).

High-resolution genotyping was performed for all 'low resolution' alleles that exhibited a significant association after correction for multiple tests. These analyses confirmed the associations of B*1502 with carbamazepine-related patients only when of Asian origin. In analyses restricted to European patients, as shown in Table 6, only the association of B*5801 with allopurinol-related

Table 3 HLA-B genotyping in patients with Sulfamethoxazole-related TEN or SJS

Patient	Age	Sex		Ancestry	HLA-B, 1	HLA-B, 2
4	45	M	TEN	European (F)	35	**38**
20	26	M	SJS	European (D)	35	44
68	39	F	SJS	South-American (Colombia)	42	44
164	24	F	SJS/TEN	European (D)	15	44
170	37	F	TEN	European (F)	07	51
183	43	F	SJS/TEN	European (F)	40	44
189	72	F	SJS/TEN	European (F)	**38**	44
191	14	F	SJS/TEN	European (F)	44	44
202	77	M	TEN	European (F)	18	**38**
214	77	F	SJS/TEN	European (F)	13	44
217	54	M	TEN	European (F)	18	35
221	33	F	TEN	European (F)	07	**38**
232	44	M	SJS	European (F)	35	51
268	73	F	TEN	European (F)	44	51
273	34	M	TEN	European (F)	44	44
276	91	F	SJS/TEN	European (D)	07	18
277	40	F	SJS/TEN	European (F)	35	58
285	62	M	SJS	European (F)	**38**	40
288	84	F	SJS	European (F)	35	41
316	48	F	TEN	European (IL)	18	49
320	26	M	SJS	European (D)	35	51
407	69	M	SJS	European (F)	44	44
529	46	F	SJS/TEN	European (F)	15	44
731	24	F	SJS/TEN	European (Ir)	15	27
893	21	M	TEN	African (Congo)	15	35
902	38	M	SJS	European (D)	**38**	57
920	20	F	SJS	European (D)	13	18
945	72	M	SJS	European (F)	35	**38**

7/28 patients were HLA-B*38 positive, the allele frequency was 0.125 (7/56) compared with 0.022 in European populations, P cor <0.01. By high resolution genotyping B*38 alleles were B*3801 (cases 189, 202, 902, 945), B*3802 (case 221) and B*3811 (cases 4, 285).
HLA-B*35 was slightly over-represented (9/56, 0.16 versus 0.087), but not significantly after Bonferronni correction. By high resolution genotyping B*35 alleles were B*3501 (4), B*3502 (2), B*3503 (2) and B*3534 (1).
HLA*B44 was slightly but not significantly, over-represented (14/56, 0.25 versus 0.156).
SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.
Bold values are statistically significant association.

Table 4 HLA-B genotyping in patients with Lamotrigine-related SJS or TEN

Patient	Age	Sex		Ancestry	HLA-B, 1	HLA-B, 2
2	66	M	SJS	European (D)	7	15
12	51	F	SJS/TEN	European (D)	**38**	44
41	10	F	TEN	African (Mali)	15	53
42	24	F	SJS	European (D)	15	44
63	42	F	SJS	European (D)	08	35
187	72	M	SJS	European (F)	07	**38**
210	62	F	SJS	European (D)	27	51
282	55	F	SJS	European (D)	44	51
530	10	F	TEN	European (UK)	07	51
660	66	F	SJS/TEN	European (D)	39	51
661	36	F	SJS	European (F)	**38**	44
761	64	M	SJS	European (D)	08	18
1156	28	F	SJS	European (F)	15	57
1167	26	F	TEN	European (D)	15	**38**
1183	25	F	SJS	European (D)	15	35
1219	26	F	SJS/TEN	European (IL)	07	**38**
1255	13	M	SJS	European (D)	18	51
1275	27	F	TEN	European (A)	18	57
1277	18	F	SJS/TEN	European (F)	13	44

B*38 was present in 5/19 patients, with an allele frequency of 0.13 (5/38), which was significantly higher than the 0.022 expected in European populations (P cor <0.003). By high resolution genotyping, 4 alleles were B*3801 (cases 12, 660, 1167, 1219) and 1 was B*3811 (187).
B*51 (always B*5101 by high resolution) was also over-expressed but the association was no more significant after Bonferronni correction.
The slightly increased prevalence of B*15 (6/38, 0.16 versus 0.058) was not significant after Bonferronni correction.
SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.
Bold values are statistically significant association.

patients was both strong [OR = 80 (34–187)] and statistically significant ($P < 10^{-6}$). The significant B*38 associations with patients towing to SMX or lamotrigine corresponded to three different alleles, none of which showed a significant link.

As we observed variability in the HLA-B alleles, we grouped them in supertypes or public epitopes. Supertypes did not differ from the general population, but our total sample showed a significant increase of the public epitope Bw4 (164/300 alleles, prevalence 0.55 versus an

Table 5 HLA-B genotyping in patients with Oxicam nsaids-related SJS or TEN

Patient	Age	Gender		Ancestry	HLA-B, 1	HLA-B, 2
173	30	F	SJS	European (F)	40	40
203	20	F	SJS	European (F)	40	44
208	31	F	SJS	European (F)	18	44
216	82	F	SJS/TEN	European (F)	08	35
220	58	F	SJS	European (F)	44	44
226	27	F	SJS	European (F)	38	**73**
242	47	F	TEN	European (F)	07	35
283	31	F	TEN	European (F)	51	57
284	71	F	SJS	European (F)	40	56
318	51	F	SJS	European (F)	35	**73**
358	26	F	SJS	European (F)	18	57
377	46	M	TEN	European (F)	07	44
378	30	F	TEN	European (F)	44	51
490	63	F	SJS	European (F)	44	51

The extremely rare allele B*73 (0.001 prevalence in European populations) was present in 2 cases, both B*7301 (P 0.027, P cor <0.16).
The slight excess of B*44, with an allele frequency of 0.25 (7/28) versus 0.156 in European populations was not significant. There was no over-representation of any subgroup by high resolution genotyping (4 B*4402, 3 B*4403).
SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.
Bold values are statistically significant association.

Table 6 Alleles associated to SJS or TEN in relation to high-risk drugs in patients of European ancestry

		Phenotype frequency no. (%)				
	Allele	Patients	Controls[a]	OR	P	P_{cor}[b]
(a) Low resolution genotyping						
Allopurinol	B*58	15/27 (55%)	28/1822 (1.5%)	80 (34–187)	$<10^{-8}$	$<10^{-6}$
Sulfamethoxazole	B*38	7/25 (28%)	79/1822 (4.3%)	8.6 (3.5–21)	$<10^{-4}$	<0.003
Lamotrigine	B*38	5/17 (24%)	79/1822 (4.3%)	6.8 (2.2–21)	0.0007	0.02
Oxicam NSAIDs	B*73	2/14 (14%)	2/1822 (0.1%)	152 (20–1167)	0.027	0.73
(b) High resolution genotying						
Allopurinol	B*5801	15/27 (55%)	28/1822(1.5%)	80 (34–187)	$<10^{-8}$	$<10^{-6}$
Sulfamethoxazole	B*3801	4/25 (16%)	78/1822 (4.3%)	4.3 (1.4–12.7)	0.022	0.59
	B*3802	1/25 (4%)	1/1822 (0.05%)	76 (4.6–1250)	0.027	0.73
	B*3811	2/25 (8%)	NA			
Lamotrigine	B*3801	4/17 (24%)	78/1822 (4.3%)	4.7 (1.3–16)	0.037	1.0
	B*3811	1/17 (5.9%)	NA			
Oxicam NSAIDs	B*7301	2/14 (14%)	2/1822 (0.1%)	152 (20–1167)	0.027	0.73

NSAID, nonsteroidal anti-inflammatory drugs; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis; OR, odds ratio.
[a]From a mixed European population of 1822 persons (*http://www.ncbi.nlm.nih.gov/projects/mhc/ihwg.cgi*).
[b]After Bonferronni correction (multiplication factor 27).

expected prevalence of 0.37, $P < 0.0001$). As this could have been related to the significant excess of B*58 and B*38 in our European patients, we recalculated the prevalence of Bw4 after excluding all patients with one of the significantly associated allele (B*5801, B*1502, B*38 and B*73). The prevalence of Bw4 decreased slightly to 0.50, but remained significantly higher than expected ($P = 0.004$).

Discussion

The data presented in this study were obtained on the largest cohort of patients affected by SJS or TEN that ever submitted samples for genetic analysis. Whether obtained prospectively or retrospectively, diagnosis and drug causality of each patient were determined by using the same standardized rules.

The focus on association with HLA-B has been prompted by the reports of 100% associations of SJS/TEN with HLA-B*1502 and HLA-B*5801 when related to carbamazepine and allopurinol, respectively, in the Han Chinese population of Taiwan [18,21].

On the basis of these reports we enlarged our series by specifically selecting among new enrolled patients those related to 'highly suspected' drugs, to evaluate the hypothesis of a drug-specific relationship with HLA-B.

Therefore, our sample is suitable for analyses of drug-related HLA associations, but is not appropriate for detecting more general disease-related association.

Our study suffers from several limitations. One is the lack of clear information on the ethnic background of controls. Actually, the database we used for controls was issued from defined geographical areas, here Western Europe, but without mention of ethnicity. As has been technically built from reports by many blood centers, we may assume that this database is representative of the mixed population living in Western Europe with a large majority

of so-called 'Caucasians', like our population of patients. To be conservative we presented two sets of analyses: one including all patients, the other restricted to patients of European ancestry.

The choice of population controls instead of drug-exposed controls is also debatable. Several theoretical advantages of using drug-exposed controls would have been present. That could prevent confusion with HLA-B associated with the underlying diseases rather than with the drug reaction. It could also help calculating the positive predictive values of associations. It, however, also has practical disadvantages. All drugs under scrutiny have several indications, for example, for anticonvulsants not only idiopathic epilepsy but also neuralgia, brain tumors, and psychiatric disorders, so that drug-exposed controls should be matched not only on medication, but also on indication for use and ethnicity. This was actually very difficult and would have resulted in low numbers of controls and considerable loss in statistical power. Furthermore, SCARs are so rare that we did not expect a measurable difference in the prevalence of 'associated alleles' between drug-exposed controls and the general population. This was confirmed by the results of the Taiwanese group who used both types of controls and observed that the ORs for B*1502 in carbamazepine-related SJS were largely overlapping with respective values of 895 (50–15 800) with population controls and 2504 (126–49 522) with exposed controls [18]. We, therefore, consider that the use of population controls may result in underestimates of the OR of associations but cannot create major bias.

The results we obtained for carbamazepine-induced patients of SJS/TEN were published previously [19]. They showed that HLA-B*1502 was not a strong marker for SJS/TEN in Europe, in contrast with the observations in Taiwan. Interestingly, all four patients of our series who expressed B*1502 had Asian ancestry.

The fact that B*1502 is a marker of SJS/TEN in Asia has now been confirmed in patients from countries other than Taiwan [22]. Another report from the UK also suggested that this specific allele is not a marker of cutaneous reactions to carbamazepine in European patients [23]. The latter paper included a variety of mild and severe cutaneous reactions, whereas the Taiwanese group had shown that the association with carbamazepine was specific to the phenotype of the reaction, that is, restricted to patients of SJS or TEN [18,22].

By contrast, the association of B*5801 with allopurinol-induced patients of SJS/TEN was replicated in our European sample of SJS and TEN patients. It was weaker than in Han Chinese, as only 61% of our patients expressed the marker instead of 100%. No other allele was significantly associated with patients who were negative for B*5801, but the statistical power for detecting another association was low, because the remaining population included only 12 patients. As at this stage we have only tested a population of patients with SJS and TEN, we do not know whether the association also applies to other phenotypes of SCAR related to allopurinol, as was observed in Han Chinese.

For the other medications investigated we also observed significant associations with B*38 for patients related to SMX or to lamotrigine and with B*73 for patients induced by oxicam NSAIDs. In each group the associated allele was infrequent; however, the ORs as well as the statistical power of borderline efficacy to assure a true relationship were low. When we looked at subgroups defined by high-resolution genotyping there was no significant association with any subtype of B*38.

Taking together the present results and those of prior studies, it appears that the relationship between HLA-B and severe drug reactions is not as straightforward as suggested by the initial reports from Taiwan.

As the RegiSCAR group and the Taiwanese investigators used the same clinical and pathological criteria, we are confident that the differences cannot be explained by discrepancies in phenotype characterization.

An interesting point is the high prevalence of gout, hyperuricemia and exposure to allopurinol in Han Chinese in Taiwan despite a lack of both obesity and high alcohol consumption [24]. This could explain a higher number of reactions in the population, but probably not a higher rate among allopurinol users. We suggest that the most probable explanation for the different prevalence of B*5801 among SJS/TEN patients in Western Europe (61%) and Taiwan (100%) is linked to the highly different allelic frequency in these populations. Actually, the ORs for SJS/TEN related to B*5801 were strongly elevated in both populations of patients, that is, 61 (32–118) in European patients versus 393 (23–6665) in Taiwanese patients, the difference being not significant. By contrast, the allelic frequency of B*5801 is significantly higher in Southeast Asia than in Western Europe, 0.055 versus 0.008 according to the dbMHC database. In Taiwan the carrier prevalence of B*5801 was 20% in a control group [21]. As many patients, who also carried the allele, were tolerant to allopurinol, Hung *et al.* [21] suggested that HLA-B*5801 was necessary but not sufficient for the occurrence of allopurinol-induced SCAR. From our own results we can add that it is a strong risk factor but neither sufficient nor necessary.

Haplotype analyses made the hypothesis very unlikely that the HLA-B association in Han Chinese patients only resulted from linkage disequilibrium with another gene in the MCH region [21]. We, therefore, raise the hypothesis

that B*5801 has a specific role in the initiation of an immune response to allopurinol. From our results we have to speculate that other HLA alleles must play a similar role. This could be true not only for allopurinol but for all other 'highly suspected' drugs evaluated in this study. In this respect, the increased prevalence of Bw4 in our patients may indicate that several other HLA-B alleles sharing a Bw4 'public epitope' were associated with epidermal necrolysis, but not detected because of insufficient statistical power. Interestingly, Bw4 is known as a target for killer cell immunoglobulin-like receptors that control positively or negatively the functions of 'natural killer' cells and NK-T (Natural Killer T) cells [25]. Such cells had been implicated in the mechanisms of keratinocyte apoptosis in TEN [26]. HLA-B*1502, a strong marker of SJS/TEN to carbamazepine, in Asia is associated with the Bw6 instead of Bw4.

From an immunological point of view it would make sense that recognition of drugs should be restricted by the MHC, especially class I antigen given the role of cytotoxic T-cells in cutaneous drug reactions [3,15], but it is likely that other factors would also be implicated to explain the diversity in the final phenotype of drug hypersensitivity. Severe cutaneous adverse reactions probably have a complex determinism, the specific environmental factor being the causing drug, and the 'drug-specific' genes in combination with 'phenotype-specific' genes could be involved. In patients of SJS/TEN, the rarity of the disease could very well be caused by a combination of variants of genes, in addition to genes in the HLA region. If this hypothesis is correct, new methods allowing the study of epistasis should give an insight into the genetic susceptibility of this severe disease.

These results also have some practical implications. Obviously, B*1502 cannot be considered as a useful predictive marker in the European population. Whether B*5801 can be considered as a good marker for reducing the risk of SJS or TEN related to allopurinol does need further analyses. As a result of the very low frequency of this allele in Europe, and because the disease could be linked to other more prevalent and yet undetermined alleles, the positive and negative predictive values of HLA testing before prescribing are probably not good enough.

Supplementary data

Supplementary table are available at *The Pharmacogenetics and Genomics Journal* Online (*www.pharmacogeneticsandgenomics.com*).

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