Abstract
Allopurinol, a commonly prescribed medication for gout and hyperuricemia, is a frequent cause of severe cutaneous adverse reactions (SCAR), which include the drug hypersensitivity syndrome, Stevens-Johnson syndrome, and toxic epidermal necrolysis. The adverse events are unpredictable and carry significant morbidity and mortality. To identify genetic markers for allopurinol-SCAR, we carried out a case-control association study. We enrolled 51 patients with allopurinol-SCAR and 228 control individuals (135 allopurinol tolerant subjects and 93 healthy subjects from the general population), and genotyped for 823 single nucleotide polymorphisms in genes related to drug metabolism and immune response. The initial screen revealed strong association between allopurinol-SCAR and SNPs in the MHC region, including BAT3 (encoding HLA-B associated transcript 3), MSH5 (mutS homolog 5), and MICB (MHC class I polypeptide-related sequence B) (P<10^{-7}). We then determined the alleles of HLA loci A, B, C, and DRB1. The HLA-B*5801 allele was present in all (100%) of all 51 patients with allopurinol-SCAR, but only in 20(15%) of 135 tolerant patients (odds ratio 580.3 [95% CI, 34.4-9780.9]; Pc=4.7×10^{-24}) and in 19 (20%) of 93 of healthy subjects (393.51 [23.23-6665.26]; Pc=8.1×10^{-18}). HLA alleles A*3303, Cw*0302, and DRB1*0301 were in linkage disequilibrium and formed an extended haplotype with HLA-B*5801. Our results indicated that allopurinol-SCAR is strongly associated with a genetic predisposition in Han Chinese. In particular, HLA-B*5801 allele is an important genetic risk factor for this life-threatening condition.

Introduction
Allopurinol is widely used for hyperuricemia-related diseases, such as gout, Lesch-Nyhan syndrome, and recurrent urate kidney stones (Figure 1) (1). Although alternative uric-acid lowering agents, such as

Fig 1. Allopurinol is a structural analog of hypoxanthine. It can inhibit xanthine oxidase to produce uric acid.
probencid and sulfinpyrazone, are available at the present time, allopurinol is still the most frequently used antihyperuricemic agent because of its convenient once-daily regimen and its advantages to treat both urate over-production and urate under-excretion (2). However, allopurinol is also one of the most frequent causes of adverse drug reactions, accounting for 5% of all cases of severe cutaneous adverse reactions (SCAR) (3).

Allopurinol-SCAR includes drug hypersensitivity syndrome (HSS), Stevens-Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN). Even though rare, the mortality rate can be as high as 26% (4-5). SJS/TEN is characterized by a rapidly developing blistering exanthema of macules and target-like lesions accompanied by mucosal involvement and skin detachment. Hypersensitivity syndrome (HSS) has systemic manifestations with multiorgan involvement, in addition to the exanthema (5). Currently, there are no tests that can be used to predict who will develop SCAR on allopurinol.

Susceptibility to such idiosyncratic reactions is thought to be genetically determined, and familial predisposition to allopurinol-hypersensitivity has been reported (6). The responsible genetic factors for allopurinol-SCAR, however, have yet to be identified. As genetic factors influencing immune response and drug metabolism may be involved in the pathogenesis of the severe cutaneous adverse reactions, we hypothesize that the genetic polymorphisms of these genes might confer the susceptibility of adverse events. We aimed in this study to identify genetic markers for allopurinol-SCAR.

Characteristics of Patients and Controls

In the study, we recruited 51 patients with allopurinol-SCAR, which included SJS (13 cases), SJS/TEN (5 cases), TEN (3 cases) and HSS (30 cases). The onset of symptoms for all patients was within the first 3 months of allopurinol exposure and 3 patients had a second attack within 2 days of re-exposure. Twenty-one patients received multiple drugs in addition to the allopurinol, but their medical records revealed no adverse drug reactions when these concomitant medications were taken without allopurinol. All patients received allopurinol because of hyperuricemia with or without gouty arthritis.

Two control groups were used in this study. The first control group was the 135 consecutive patients who received...
allopurinol for at least 6 months (median=22 months, range=6-107 months) without evidence of adverse reactions. The second control group consisted of 93 healthy subjects randomly selected from a biobank under a nation-wide population study, in which 3312 Han Chinese descendants were recruited based on the geographic distribution across Taiwan. All participants were unrelated Han Chinese residing in Taiwan.

**Association Screen for Candidate Gene SNPs**

We initially screened 30 patients with allopurinol-SCAR and 60 tolerant subjects for SNP association. A total of 823 SNPs were selected from the National Center for Biotechnology Information's SNP database for genotyping (dbSNP: build 118). These included 197 SNPs from 4 Mb of the MHC region on chromosome 6p21.3 (7) and 626 SNPs selected from genes encoding immune-related molecules and drug metabolizing enzymes. Of the total of 823 SNPs screened, 29 SNPs in the MHC region were found to have significant association (p<0.01) with the allopurinol-SCAR patients when compared to the tolerant group (Figure 2). In particular, 3 SNPs in the MHC class III regions have p values less than 10^{-7} (-log10 p value >7, Figure 2). These SNPs were rs3117583 of BAT3 (encoding for HLA-B associated transcript 3), rs1150793 of MSH5 (encoding mutS homolog), and rs2855804 of MICB (encoding MHC class I polypeptide-related sequence).

Outside the MHC region, only 3 SNPs showed potential associations (p<0.01). Two SNPs (rs2268791, rs1594) were located in the CFLAR (CASP8 and FADD like apoptosis regulator) gene on chromosome 2, and one SNP (rs2304224) belongs to KIR2DL1 (a killer cell immunoglobulin-like receptor) gene on chromosome 19. No other significant associations were found in the other SNPs, including the SNPs of allopurinol drug metabolizing enzymes.

**HLA Allele Frequency**

Since the most significant association was seen with the SNPs in the MHC region, we genotyped the individual HLA alleles. As shown in Table 1, alleles HLA-A*3303, B*5801, Cw*0302 and DRB1*0301 occurred at significantly increased frequencies among the allopurinol-SCAR patients compared to the two control groups. In particular, the HLA-B*5801 was present in all 51 (100%) patients with allopurinol-SCAR but in only 15% (20/135) of the allopurinol-tolerant group (odds ratio 580.3 [95% CI, 34.4-9780.9], Pc=4.7×10^{-24}), and 20% (19/93) of the general population (odds ratio 393.5 [95% CI 23.2-6665.26], Pc=8.1×10^{-18}). This association was only seen with allopurinol-SCAR and not with the patients’ underlying diseases, such as gout, renal insufficiency, or autoimmune disease, etc. (data not shown).

Guided by 5 patients (no. 5, 14, 18, 19 and 35) who were homozygous for the HLA-B*5801 alleles, we analyzed the allele distribution of the combined HLA loci...
and defined the extended haplotype. The HLA-B*5801 extended haplotype was formed by conserved alleles at closely linked loci as HLA-A*3303-Cw*0302-B*5801-DRB1*0301. This extended haplotype was present in 21(41%) of the 51 patients with allopurinol-SCAR (Table 1), 7% of the tolerant patients, and 10% of the healthy subjects.

**Conclusion**

To our knowledge, this is the largest pharmacogenetic study of allopurinol-SCAR. In this study, we identified a strong association of the allele HLA-B*5801 with the susceptibility of allopurinol-induced HSS, SJS, and TEN in Han Chinese. In fact the association is 100% in that the HLA-B allele B*5801 was present in all 51 patients with allopurinol-induced SCAR, with odds ratio exceeding that reported for the association between HLA-B27 and ankylosing spondylitis (8). Although other ethnic allopurinol-SCAR patients were not available for our study, the fact that HLA-B*5801 allele is also present in other populations (7% in Africa, 2~7% in Caucasian, and 8% in Asian Indian) (9) suggests that this association may also exist in other ethnic groups. However, as pharmacogenetic results can vary by the study populations, it remains to be seen to what extent this study applies to other populations.

We have recently reported a strong association of a specific HLA-B allele (B*1502) in carbamazepine induced Stevens-Johnson syndrome (10). This study, together with our previous report, suggests that HLA-B alleles might play a major role in the pathogenesis of immune-mediated SCAR. A specific HLA-B molecule may function as antigenic presentation of certain drug or its metabolite for HLA restricted T-cell activation. Further studies along these lines may increase our understanding of the pathogenesis of these potentially life-threatening clinical conditions.

In summary, we have shown that allopurinol-induced severe cutaneous adverse reactions are associated with a strong genetic predisposition. Genetic polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allopurinol-SCAR (n = 51)</th>
<th>Tolerant control (n = 135)</th>
<th>Odds ratio</th>
<th>P-value*</th>
<th>General population control (n = 93)</th>
<th>Odds ratio</th>
<th>P-value*</th>
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</thead>
<tbody>
<tr>
<td>B*5801</td>
<td>51 (100)</td>
<td>20 (15)</td>
<td>580.3</td>
<td>4.7 × 10⁻²⁴</td>
<td>19 (20)</td>
<td>393.5</td>
<td>8.1 × 10⁻¹⁰</td>
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<td>Cw*0302</td>
<td>48 (94)</td>
<td>19 (14)</td>
<td>97.7</td>
<td>1.4 × 10⁻¹⁸</td>
<td>19 (20)</td>
<td>62.3</td>
<td>2.5 × 10⁻¹³</td>
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<tr>
<td>A*3303</td>
<td>54 (67)</td>
<td>24 (18)</td>
<td>9.3</td>
<td>2.2 × 10⁻⁶</td>
<td>20 (22)</td>
<td>7.3</td>
<td>4.7 × 10⁻²</td>
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<tr>
<td>DRB1*0301</td>
<td>33 (65)</td>
<td>17 (13)</td>
<td>12.7</td>
<td>2.8 × 10⁻⁶</td>
<td>14 (15)</td>
<td>10.3</td>
<td>8.5 × 10⁻⁴</td>
</tr>
<tr>
<td>B<em>5801, Cw</em>0302</td>
<td>48 (94)</td>
<td>19 (14)</td>
<td>97.7</td>
<td>1.4 × 10⁻¹⁸</td>
<td>19 (20)</td>
<td>62.3</td>
<td>2.6 × 10⁻¹³</td>
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<tr>
<td>B<em>5801, Cw</em>0302, A*3303</td>
<td>34 (67)</td>
<td>17 (13)</td>
<td>13.9</td>
<td>5.4 × 10⁻⁷</td>
<td>16 (17)</td>
<td>9.6</td>
<td>1.7 × 10⁻⁵</td>
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<tr>
<td>B<em>5801, Cw</em>0302, DRB1*0301</td>
<td>30 (59)</td>
<td>11 (8)</td>
<td>16.1</td>
<td>7.4 × 10⁻⁷</td>
<td>10 (11)</td>
<td>11.9</td>
<td>7.8 × 10⁻⁴</td>
</tr>
<tr>
<td>B<em>5801, Cw</em>0302, A<em>3303, DRB1</em>0301</td>
<td>21 (41)</td>
<td>9 (7)</td>
<td>9.8</td>
<td>0.039</td>
<td>9 (10)</td>
<td>6.5</td>
<td>&gt;0.05</td>
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</tbody>
</table>

*Numbers in parentheses indicate percentage.

*The P values were adjusted by using Bonferroni’s correction for multiple comparisons to account for the observed alleles.
in the MHC region, particularly the HLA-B*5801 allele, is highly associated with individuals who are at risk for allopurinol-induced HSS, SJS or TEN.


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References