

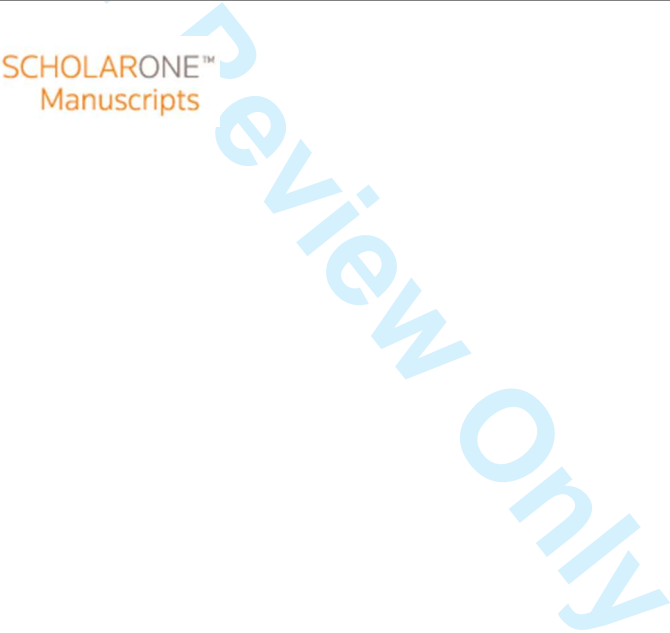


HLA-B*58:01 genotyping to prevent allopurinol-induced severe cutaneous adverse reactions: national prospective study

Journal:	<i>BMJ</i>
Manuscript ID:	BMJ.2015.025516.R1
Article Type:	Research
BMJ Journal:	BMJ
Date Submitted by the Author:	22-Jun-2015
Complete List of Authors:	<p>Ko, Tai-Ming; Institute of Biomedical Sciences, Academia Sinica, Tsai, Chang-Youh; Taipei Veterans General Hospital, Taipei, Taiwan, Division of Allergy, Immunology & Rheumatology Chen, Shih-Yang; Country Hospital, Taipei, Taiwan, Chen, Kuo-Shu; Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan, Yu, Kuang-Hui; Chang Gung Memorial Hospital, Rheumatology, Allergy and Immunology Chu, Chih-Sheng; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Huang, Chung-Ming; China Medical University Hospital, Taichung, Taiwan, Wang, Chrong-Reen; National Cheng Kung University Hospital, Tainan, Taiwan, Internal Medicine Weng, Chia-Tse; National Cheng Kung University Hospital, Tainan, Taiwan, Yu, Chia-Li; National Taiwan University Hospital, Taipei, Taiwan, Hsieh, Song-Chou; National Taiwan University Hospital, Taipei, Taiwan, Tsai, Jer-Chia; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Lai, Wen-Ter; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Tsai, Wen-Chan; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Yin, Guang-Dar; Far Eastern Polyclinic, Taipei, Taiwan, Ou, Tsan-Teng; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Cheng, Kai-Hung; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Yen, Jeng-Hsien; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Liu, De-Ling; Taipei Veterans General Hospital, Taipei, Taiwan, Lin, Tsung-Hsien; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Chen, Der-Yuan; Taichung Veterans General Hospital, Taichung, Taiwan, Hsiao, Pi-Jung; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan, Weng, Meng-Yu; National Cheng Kung University Hospital, Tainan, Taiwan,</p>

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

	Chen, Yi-Ming; Taichung Veterans General Hospital, Taichung, Taiwan, Chen, Chen-Hung; The Tri-Service General Hospital, Taipei, Taiwan, Liu, Ming-Fei; National Cheng Kung University Hospital, Yen, Hsueh-Wei; Kaohsiung Medical University Chung-Ho Memorial Hospital, Lee, Jia-Jung; Kaohsiung Medical University Chung-Ho Memorial Hospital, Kuo, Mei-Chuan; Kaohsiung Medical University Chung-Ho Memorial Hospital, Wu, Chen-Ching; Kaohsiung Medical University Chung-Ho Memorial Hospital, Hung, Shih-Yuan; E-Da Hospital, Kaohsiung, Taiwan, Luo, Shue-Fen; Chang Gung Memorial Hospital, Yang, Ya-Hui; Fooyin University, Chuang, Hui-Ping; Institute of Biomedical Sciences, Academia Sinica, Chou, Yi-Chun; Institute of Biomedical Sciences, Academia Sinica, Liao, Hung-Ting; Institute of Biomedical Sciences, Academia Sinica, Wang, Chia-Wen; Institute of Biomedical Sciences, Academia Sinica, Huang, Chun-Lin; Institute of Biomedical Sciences, Academia Sinica, Chang, Chia-Shuo; Institute of Biomedical Sciences, Academia Sinica, Lee, Ming-Ta Michael; Institute of Biomedical Sciences, Academia Sinica, Chen, Pei; Institute of Biomedical Sciences, Academia Sinica, Wong, Chih-Shung; Cathay General Hospital, Anesthesiology; Pharmigene, Inc, Chen, Chien-Hsiun; Institute of Biomedical Sciences, Academia Sinica, Wu, Jer-Yuarn; Institute of Biomedical Sciences, Academia Sinica, Chen, Yuan-Tsong; Institute of Biomedical Sciences, Academia Sinica, Shen, Chen-Yang; Institute of Biomedical Sciences, Academia Sinica,
Keywords:	Pharmacogenomics, Adverse Drug Reactions, Prospective Study, Severe Cutaneous Adverse Reactions, Gout and Hyperuricemia, Allopurinol, Personal and Preventive medicine



**HLA-B*58:01 genotyping to prevent
allopurinol-induced severe cutaneous adverse
reactions: national prospective study**

Tai-Ming Ko¹, Chang-Youh Tsai², Shih-Yang Chen³, Kuo-Shu Chen⁴, Kuang-Hui
Yu⁵, Chih-Sheng Chu^{6,7,8}, Chung-Ming Huang⁹, Chrong-Reen Wang¹⁰, Chia-Tse
Weng¹⁰, Chia-Li Yu¹¹, Song-Chou Hsieh¹¹, Jer-Chia Tsai^{6,7,8}, Wen-Ter Lai^{6,7,8},
Wen-Chan Tsai^{6,7,8}, Guang-Dar Yin¹², Tsan-Teng Ou^{6,7,8}, Kai-Hung Cheng^{6,7,8},
Jeng-Hsien Yen^{6,7,8}, De-Ling Liu², Tsung-Hsien Lin^{6,7,8}, Der-Yuan Chen¹³, Pi-Jung
Hsiao^{6,7,8}, Meng-Yu Weng¹⁰, Yi-Ming Chen¹³, Chen-Hung Chen¹⁴, Ming-Fei Liu¹⁰,
Hsueh-Wei Yen^{6,7,8}, Jia-Jung Lee^{6,7,8}, Mei-Chuan Kuo^{6,7,8}, Chen-Ching Wu^{6,7,8},
Shih-Yuan Hung¹⁵, Shue-Fen Luo⁵, Ya-Hui Yang¹⁶, Hui-Ping Chuang¹, Yi-Chun
Chou¹, Hung-Ting Liao¹, Chia-Wen Wang¹, Chun-Lin Huang¹, Chia-Shuo Chang¹,
Ming-Ta Michael Lee^{1,17}, Pei Chen¹, Chih-Shung Wong¹⁸, Chien-Hsiun Chen¹,
Jer-Yuarn Wu¹, Yuan-Tsong Chen^{1,19}, and Chen-Yang Shen^{1,20}, for the Taiwan
Allopurinol-SCAR Consortium*

1 ¹ Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan,
2
3
4
5
6
7 ² Taipei Veterans General Hospital, Taipei, Taiwan,
8
9
10 ³ Country Hospital, Taipei, Taiwan,
11
12
13 ⁴ Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan,
14
15
16 ⁵ Chang Gung Memorial Hospital, Taoyuan, Taiwan,
17
18
19 ⁶ Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan,
20
21
22 ⁷ Kaohsiung Municipal Hsiaokang Hospital, Kaohsiung, Taiwan,
23
24
25 ⁸ Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan,
26
27
28 ⁹ China Medical University Hospital, Taichung, Taiwan,
29
30
31 ¹⁰ National Cheng Kung University Hospital, Tainan, Taiwan,
32
33
34 ¹¹ National Taiwan University Hospital, Taipei, Taiwan,
35
36
37 ¹² Far Eastern Polyclinic, Taipei, Taiwan,
38
39
40 ¹³ Taichung Veterans General Hospital, Taichung, Taiwan,
41
42
43 ¹⁴ The Tri-Service General Hospital, Taipei, Taiwan,
44
45
46 ¹⁵ E-Da Hospital, Kaohsiung, Taiwan,
47
48
49 ¹⁶ Fooyin University, Kaohsiung, Taiwan,
50
51
52 ¹⁷ Laboratory for International Alliance on Genomic Research, Core for Genomic
53
54 Medicine, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
55
56
57
58
59
60

1¹⁸ PharmiGene, Inc and Department of Anesthesiology, Cathay General Hospital,

2 Taipei, Taiwan,

3¹⁹ Department of Pediatrics, Duke University Medical Center, Durham, NC, USA.

4²⁰ College of Public Health, China Medical University Hospital, Taichung, Taiwan.

5 *Other members of the Taiwan Allopurinol-SCAR Consortium are listed in the

6 Supplementary Appendix.

7

8 Drs. T.-M. Ko, C.-Y. Tsai, S.-Y. Chen, K.-S. Chen, and K.-H. Yu contributed equally

9 to this article.

10

11 **Keywords:** Pharmacogenomics, Adverse Drug Reactions, Prospective Study, Severe

12 Cutaneous Adverse Reactions, Gout and Hyperuricemia, Allopurinol, Personal and

13 Preventive medicine

14

15

16

17

18

19

Correspondence should be addressed to:

Chen-Yang Shen, Ph.D., Institute of Biomedical Sciences, Academia Sinica, 128,
Academia Road, Section 2. Nankang, Taipei 11529, Taiwan; e-mail:
bmcys@ibms.sinica.edu.tw; Phone: +886-2-27899036

Yuan-Tsong Chen, M.D., Ph.D., Institute of Biomedical Sciences, Academia Sinica,
128, Academia Road, Section 2. Nankang, Taipei 11529, Taiwan; e-mail:
chen0010@ibms.sinica.edu.tw; Phone: +886-2-27899081; Fax: +886-2-27899085

Jer-Yuarn Wu, Ph.D., Institute of Biomedical Sciences, Academia Sinica, 128,
Academia Road, Section 2. Nankang, Taipei 11529, Taiwan; e-mail:
jywu@ibms.sinica.edu.tw; Phone: +886-2-27899075

1 ABSTRACT

2 **OBJECTIVE:** To evaluate the impact of using prospective HLA-B*58:01 screening
3 to identify at-risk subjects for preventing life-threatening severe cutaneous adverse
4 reactions (SCARs) induced by allopurinol, which is one of the common causes of
5 SCARs.

6 **DESIGN:** Prospective cohort study.

7 **SETTING:** 15 medical centers in different geographic regions of Taiwan, from July
8 2009 through August 2014.

9 **PARTICIPANTS:** We recruited 2926 subjects who had an indication for allopurinol
10 treatment but had not taken allopurinol previously.

11 **MAIN OUTCOME MEASURES:** The incidence of allopurinol-induced SCARs
12 with and without screening.

13 **RESULTS:** DNA purified from each subject's peripheral blood was used to assess
14 the presence of allele HLA-B*58:01. Subjects who tested positive (19.6% of the total)
15 were advised to avoid allopurinol and were referred to an alternate medication or
16 advised to continue with their pre-study medication; those testing negative (80.4%)
17 were given allopurinol. Subjects were interviewed once a week for 2 months to
18 monitor symptoms. The estimated historical incidence of allopurinol-induced SCARs
19 was used for comparison. Mild, transient rash without blisters developed in 3.3% of

1 subjects during follow-up. None of the subjects were hospitalized owing to adverse
2 drug reactions. SCARs did not develop in any of the HLA-B*58:01-negative subjects
3 receiving allopurinol; this is in contrast to the 7 expected cases of SCARs based on
4 the estimated historical nationwide incidence of allopurinol-induced SCARs (0.30%;
5 $P = 0.0026$, the two-side one-sample binomial test; 95% confidence interval 0% to
6 0.17%).

7 **CONCLUSIONS:** Identification of subjects carrying allele HLA-B*58:01 and the
8 absence of allopurinol therapy for these subjects were strongly associated with
9 decreased incidence of allopurinol-induced SCARs.

1 INTRODUCTION

2 Developing a reliable pharmacogenomics-based approach to prevent adverse
3 reactions with severe complications is a major goal of personalized medicine¹⁻³.
4 Severe cutaneous adverse reactions (SCARs) constitute a set of life-threatening
5 conditions that include drug rash with eosinophilia and systemic symptoms (DRESS),
6 Stevens–Johnson syndrome (SJS), and toxic epidermal necrolysis (TEN)⁴ with the
7 lethality rate of TEN up to 35%. SCARs are often caused by drugs but may not be
8 accurately predicted based on the pharmacological action of a particular drug⁵.
9 SCARs are associated with chemotoxic and T cell–mediated inflammatory injuries
10 and can be characterized by a severe idiosyncratic reaction in skin, blistering
11 exanthema of macular papules, or mucosal involvement⁶.

12 Allopurinol, a first-line prescription medication for gout and hyperuricemia⁷⁻¹⁰, is
13 one of the most common causes of SCARs in Asia and Europe¹¹⁻¹³. Through 2012, the
14 literature reported approximately 1000 subjects who had allopurinol-induced SCARs;
15 these patients represented multiple ethnicities and geographic regions¹². Although
16 allopurinol has SCARs-related risks and other anti-gout medicines are available,
17 allopurinol is still a common treatment for gout and hyperuricemia owing to its
18 relative low cost, efficacy, and convenience.

1 We have reported that allopurinol-induced SCARs correlate strongly with allele
2 human leukocyte antigen (HLA)-B*58:01 in Han Chinese populations¹⁴, as confirmed
3 in Han Chinese from Hong Kong and mainland China and in Japanese, Korean, Thai,
4 and other Asian populations as well as European populations¹⁵⁻²¹. Among subjects of
5 Han Chinese descent, allopurinol-induced SCARs almost never occur in non-carriers
6 of HLA-B*58:01, strongly suggesting that this allele is involved directly in the
7 pathogenesis of SCARs. In addition, it is notable that HLA-B*58:01 can present the
8 allopurinol metabolite, oxypurinol, directly to cytotoxic T cells without antigen
9 processing²²⁻²⁴. More importantly, allopurinol/oxypurinol-specific T cell-mediated
10 cytotoxicity is restricted to carriers of HLA-B*58:01^{23,24}.

11 Based on our previous findings¹⁴, an extremely high risk (odds ratio, 580.3; 95%
12 confidence interval, 34.4–9780.9; $P = 4.7 \times 10^{-24}$) to develop allopurinol-induced
13 SCARs was found in Han Chinese who carry HLA-B*58:01 compared with those
14 who do not carry this allele. Hence, if HLA-B*58:01 was to be used as a marker to
15 predict allopurinol-induced SCARs, the test would have high sensitivity (100.0%) and
16 specificity (85.2%)¹⁴. Based on a predicted incidence of allopurinol-induced SCARs
17 of 0.30%, HLA-B*58:01 would have a negative predictive value of 100.0% and a
18 positive predictive value of 2.0%. Thus, due to the 100% of negative predictive value,
19 the use of HLA-B*58:01 genotyping to prevent allopurinol-induced SCARs in routine

1 clinical practice appears warranted. We therefore sought to determine whether
2 prospective screening via HLA-B*58:01 genotyping prior to allopurinol treatment
3 could reduce the incidence of allopurinol-induced SCARs.

4 5 **METHODS**

6 **Study Design**

7 Because of tight association of HLA-B*58:01 and the life-threatening
8 allopurinol-induced SCARs, this study was approved by our Institutional Review
9 Board as a nonrandomized study, using historical incidence as a control. We recruited
10 subjects from 15 participating hospitals throughout Taiwan (see author affiliation and
11 **Supplementary Appendix**). There were 9 points of interaction with
12 HLA-B*58:01–negative subjects and 10 points of interaction with HLA-B*58:01
13 carriers, namely the initial screening visit, a second clinic visit for HLA-B*58:01
14 carriers, and telephone interviews for both groups weekly during the 2-month
15 follow-up. Subjects aged 6 months to 99 years who had not previously taken
16 allopurinol within 3 months were recruited. In accordance with clinical indications at
17 the time of screening, these subjects would have received allopurinol and thus were
18 invited to participate in the study. The efficacy of all the medicines for reducing uric
19 acid level was evaluated based on the guideline for the management of gout⁸⁻¹⁰.

1 We excluded subjects who had undergone a bone marrow transplant, who were
2
3
4
5
6
7 not of Han Chinese descent, and those who had a history of allopurinol-induced
8
9
10 hypersensitivity. Han Chinese descent was confirmed via a multiple-choice
11
12
13 questionnaire that asked subjects to report the ethnicity of both parents and
14
15
16 grandparents.

17
18
19 We prescribed and dispensed allopurinol to all subjects at the initial screen, but
20
21
22 we asked that each subject defer taking allopurinol until the HLA-B*58:01
23
24
25 genotyping results were finalized. Blood samples were collected and transferred to
26
27
28 our central laboratory for HLA-B*58:01 genotyping. We reported the genotyping
29
30
31 results to the participating physicians within 3 days.

32
33 HLA-B*58:01–positive subjects were asked to return to their respective hospitals
34
35
36 within 3 days. We then explained their risk of allopurinol-induced SCARs and
37
38
39 recommended that they take alternative medicine. HLA-B*58:01–negative subjects
40
41
42 (who also were counseled about SCARs risk) were started on allopurinol. In our
43
44
45 previous large-scale retrospective study¹⁴, all patients developed SCARs during the
46
47
48 study period within 2 months of allopurinol treatment commencement, which was in
49
50
51 agreement with what has consistently been reported in the literature²⁵. We therefore
52
53
54 interviewed all subjects by telephone during the 2-month period following initial
55
56
57 screening (for HLA-B*58:01–negative subjects) or after the second clinic visit (for
58
59
60

HLA-B*58:01-positive subjects) to monitor for symptoms of adverse drug reactions (ADRs), including SCARs. If early symptoms of SCARs developed, a subject was asked to return to the clinic immediately for dermatological evaluation. We monitored all subjects throughout the study's duration, with the exception of those who had a protocol violation or were lost during follow-up.

The study was performed in accordance with Good Clinical Practice Standards and the provisions of the Declaration of Helsinki. The research ethics committee at Academia Sinica and the institutional review board at each participating clinic approved the study. We obtained written informed consent from all subjects or from parents or guardians for subjects who were ≤ 21 years of age.

Genotyping of HLA-B*58:01

Whole blood (2 ml) was collected from each subject in a Monovette tube that was stored at 4–12°C, and each sample was sent to the central lab on the day obtained. We isolated genomic DNA with the QIAamp DNA purification system (Qiagen). The presence or absence of HLA-B*58:01 was determined with the PG5801 DNA detection kit (Pharmigene). The kits are based on a real-time PCR with sequence-specific primers for HLA-B*58:01. To confirm the genotyping results, the first 900 samples were also examined in parallel with an HLA sequence-specific

oligonucleotide reverse line blot (Dynal Biotech); the results were consistent in each sample.

Annual Incidence

The cases of SCARs were based on diagnostic code 695.1 in both the International Classification of Diseases, 9th Revision, and Clinical Modification (ICD-9-CM), which are commonly used in studies of ADRs^{26,27}. The ICD-9-CM 695.1 code covers all SCARs, including DRESS, SJS, and TEN. The number of subjects having this code was determined from the National Health Insurance Research Database (NHIRD), as provided by the National Health Insurance Administration of Taiwan. NHIRD is very reliable and applicable for nationwide studies in Taiwan²⁸⁻³⁰. The Taiwanese government established NHIRD when the National Health Insurance system was launched in 1995. NHIRD is single-payer health insurance plan managed by the Taiwanese government, and it provides healthcare for nearly all Taiwanese (enrolment was 99.5% in 2008). More than 92% of Taiwanese healthcare facilities have been contracted by the National Health Insurance system. Data obtained from NHIRD are therefore comprehensive. We estimated the annual incidence of allopurinol-induced SCARs in Taiwan as the annual

number of SCARs cases caused by allopurinol divided by the annual number of new allopurinol users

In 2005, we published an article stating the potential of HLA-B*58:01 as a biomarker for preventing allopurinol-induced SCARs¹⁴. After this, some physicians began to genotype HLA-B region before allopurinol treatment. This measure could confound our analysis. Therefore, to obtain a suitable control group, we adopted the most recent, non-confounded data (i.e. the 2001-2004 data) from NHIRD.

Statistical Analysis

Based on the prevalence of allele HLA-B*58:01 (20%) in the Han Chinese population residing in Taiwan³¹, we calculated that 2169 subjects would provide a power of 86% (the two-side one-sample binomial test) to detect a reduction in the incidence of allopurinol-induced SCARs from 0.30% (i.e., 30 cases per 10,000 new recipients) to 0.03%. The two-side one-sample binomial test was used to compare the rate of allopurinol-induced SCARs in the prospective screening population with historical incidence. All *P* values are two-tailed, and a *P* < 0.05 was considered statistically significant.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

1 **RESULTS**

2 **Subjects**

3 From July 2009 through August 2014, we enrolled 2926 subjects, 2910 of which
4 underwent genotyping and were included in the 2-month follow-up (**Figure 1**). Male
5 and female subjects accounted for 82.8% and 17.2%, respectively, with mean age 54.9
6 years (range, 14–99) (**Table 1**). Indications for allopurinol treatment included chronic
7 tophaceous gout (35.2% of subjects), hyperuricaemia (23.9%), chronic tophaceous
8 gout plus hyperuricaemia (16.1%), chronic tophaceous gout plus other conditions
9 (7.4%), and other conditions (17.4%) (**Table 1**).

10

11 **Screening for HLA-B*58:01**

12 Among the 2910 enrolled subjects, 571 (19.6%) were identified as having allele
13 HLA-B*58:01 and were counseled not to take allopurinol; these subjects were
14 prescribed alternative drugs or counseled to continue taking their pre-study
15 medication. Of these subjects, we monitored for adverse events and found that 2 were
16 lost during follow-up, 354 took an alternative medication, and 215 took their
17 pre-study medication (**Figure 1**). Alternative medications were benzbromarone,
18 bisoprolol fumarate, bromhexine hydrochloride, brompheniramine, colchicine,
19 febuxostat, hydroxychloroquine, sulfasalazine, sulfonylurea, and sulfipyrazone

(**Supplementary Table 1**). The remaining 2339 subjects (80.4%) were negative for HLA-B*58:01. Among them, 155 did not take allopurinol and 11 were lost during follow-up, leaving 2173 HLA-B*58:01–negative subjects who took allopurinol and were monitored (**Figure 1**).

Adverse Events Monitoring

Of all 2910 subjects, mild and transient rash and itching developed in 97 (3.3%), but none had a combination of rash, itching, and localized blisters (**Table 2**). Among the 97 subjects with rash or itching, 3 were found to carry HLA-B*58:01 and presented with symptoms after taking alternative medicine (benzbromarone) (**Table 2**). None of the subjects was diagnosed with SCARs as defined by the RegiSCAR Group (main characteristics including multi-systemic involvement and frequent eosinophilia). Other adverse events were fever, sore throat, fatigue, dizziness, insomnia, and gastrointestinal symptoms. These adverse events were found in both HLA-B*58:01–positive and –negative subjects. There was no significant correlation between specific symptoms and whether a patient was HLA-B*58:01–positive or –negative (**Table 2**).

Estimating the Expected Historical Incidence of SCARs

NHIRD data revealed that allopurinol was prescribed for at least 3 months for 137,380 persons in 2001, 117,896 persons in 2002, 107,873 in 2003, and 102,060 in 2004 who had not previously taken allopurinol—at least dating back to the beginning of the previous calendar year (**Table 3**). Historical incidence of allopurinol-induced SCARs in 2001, 2002, 2003, and 2004 was then compared with the incidence seen in study subjects. Our estimated incidence of SCARs among allopurinol users in 2001, 2002, 2003, and 2004 in Taiwan was thus 0.32%, 0.30%, 0.28%, and 0.29%, respectively. The mean (0.30%) was used as the historical incidence for further analysis.

Incidence of SCARs after Genetic Screening

Based on the estimated historical incidence of 0.30%, 7 cases of DRESS, SJS, or TEN were to be expected among our 2173 subjects who took allopurinol. However, no case of DRESS, SJS, or TEN was found for any of the subjects, which differed significantly from the historical incidence by combined the data from 2001 to 2004 ($P = 0.0026$, the two-side one-sample binomial test; 95% confidence interval 0% to 0.17%) (**Table 3**).

DISCUSSION

Principal findings

Our results indicate that screening Han Chinese patients for allele HLA-B*58:01 prior to initiating allopurinol therapy and subsequently withholding allopurinol from HLA-B*58:01-positive patients would likely reduce the incidence of allopurinol-induced SCARs. In the present study, adverse cutaneous reactions, including oral lesions and rash, that occurred in the subjects were mild, transient, and localized. In addition, under continuous and systematic monitoring of dermatological symptoms, many HLA-B*58:01-negative subjects with transient and mild skin lesions resumed taking allopurinol without a recurrence of symptoms. Notably, we did not identify any subject with SCARs, which indicates that the incidence of allopurinol-induced SCARs in HLA-B*58:01-negative persons is quite low. Thus far, all study participants have been followed-up for at least 9 months and no cases of SCARs has been reported, hence, in this cohort, the incidence of SCARs at 2 months is the same as that at 9 months. Moreover, we attempted to identify SCARs in our prospective cohort by searching the NHIRD using the unique identification numbers of individual Taiwanese patients; no SCARs were identified by this approach. Therefore, allopurinol-SCARs can be successfully prevented by implementing a genetic screening protocol.

1 Our results support HLA-B*58:01 screening to prevent allopurinol-induced
2 SCARs³². As for any new pharmacogenomic test, however, the use and safety of the
3 alternative medication(s) must be documented. Of the 569 HLA-B*58:01 carriers, 354
4 (62.2%) were given alternative treatment, whereas the other carriers continued to take
5 their pre-study medication such as colchicine and nonsteroidal anti-inflammatory
6 drugs. Among the 354 HLA-B*58:01 carriers treated with an alternate therapy, the
7 only symptom documented during the 2-month follow-up was mild, transient rash in 3
8 subjects (0.8%).

9
10 **Implications for clinical practice**

11 In addition to the obvious patient safety benefit, HLA-B*58:01 screening could also
12 be considered a potentially cost-effective intervention. As the first line treatment for
13 hyperuricemia, many medical societies worldwide, including the American College of
14 Rheumatology, currently recommends the use of a xanthine oxidase inhibitor (XOI)
15 with either allopurinol or febuxostat²⁹. Benzbromarone is a uricosuric agent that has
16 been used to control hyperuricemia. It is effective in lowering serum uric acid levels,
17 especially in patients with urate under-excretion. However, benzbromarone has a risk
18 of severe hepatotoxicity as well as acute renal colic, and it has been withdrawn from
19 the market or not available in some countries, including US and some European

1 countries³³. These are the major reasons why benzbromarone or other uricosuric
2 agents are not used in all of the gouty patients³⁴.
3
4 With regard to gout patients, there are two potential treatment strategies with identical
5 therapeutic efficacy but different costs for government and society. One strategy is
6 global substitution of allopurinol with the new xanthine oxidase inhibitor, febuxostat;
7 the other is to use allopurinol for patients who are HLA-B*58:01-negative and to
8 substitute allopurinol with febuxostat in patients who are HLA-B*58:01-positive.
9 Recently, cost-effectiveness analyses carried out in Thai and Korean populations
10 suggested that HLA-B*58:01 testing is a better cost-effective measure than global
11 substitution of febuxostat for allopurinol^{35,36}. Because the negative predictive value of
12 HLA-B*58:01 for allopurinol-induced SCARs is 100%, the risk of developing
13 allopurinol-induced SCARs among HLA-B*58:01-negative patients would be
14 extremely low. Considering the cost-effectiveness or efficacy of other medications for
15 similar indications, avoiding prescription of allopurinol for HLA-B*58:01-positive
16 patients is likely prudent, despite the low estimated positive predictive value (2%) of
17 the test.

19 **Potential impact of this study**

1 In the present study, prospective screening by HLA-B*58:01 genotyping prior to
2
3
4
5
6
7 allopurinol treatment in 2926 subjects who had an indication for allopurinol treatment
8
9
10 could successfully reduce the incidence of allopurinol-induced SCARs (from 7
11
12
13 expected cases of SCARs to none in the 2173 patients who took allopurinol). The
14
15
16 results of this study suggest that HLA-B*15:02 screening of approximately 110,000
17
18
19 new users of allopurinol in Taiwan each year may prevent about 330 cases of
20
21
22 allopurinol-induced SCARs every year. Based on our previous experience, this
23
24
25 expectation of impact is reasonable. Carbamazepine, which formerly was the leading
26
27
28 drug causing SJS/TEN in Taiwan, is now down to the number eight in the list of drugs
29
30
31 causing these life-threatening conditions. This is attributable to our previous
32
33
34 prospective study showing that HLA-B*15:02 screening could reduce the incidence of
35
36 carbamazepine-induced SJS/ TEN³⁰ with subsequent Taiwan's National Health
37
38
39 Insurance coverage of the genotyping, which led to wide screening for HLA-B*15:02
40
41
42 by the medical community.
43
44
45
46
47 **Strengths and limitations of study**

48
49
50 The development of a reliable pharmacogenomics-based approach to preventing
51
52
53 adverse reactions with severe complications is one of best examples to demonstrate
54
55
56 that the concept of personalised medicine can be a clinical reality. To date, there have
57
58
59
60

1 been 3 critical findings involving ADRs, which include HLA-B*15:02 for
2
3
4
5
6
7 carbamazepine-induced SJS/TEN, HLA-B*57:01 for abacavir-induced drug
8
9
10 hypersensitivity, and HLA-B*58:01 for allopurinol-induced SCARs. These findings
11
12
13 reveal the immense potential benefits of applying this concept of genetic testing to
14
15 prevent ADRs in the clinical setting due to the extremely high negative predictive
16
17 values. Therefore, to achieve this goal, solid evidence collected from different clinics
18
19 based on reliable laboratory tests as well as the development of effective strategies to
20
21
22 incorporate these tests into routine practice is essential. More importantly, a
23
24
25 prospective study to demonstrate that all of these relevant processes can be performed
26
27
28 in clinical settings is critically essential. Therefore, the PREDICT-1 Study Team and
29
30
31 our group provided the required crucial and strong evidences by using a
32
33
34 “prospective-screening” approach to prevent abacavir-induced drug hypersensitivity
35
36
37 in 2008³⁷ and carbamazepine-induced SJS/TEN in 2011³⁰. This present study we
38
39
40 reported here is the third case, i.e., involving the use of HLA-B*58:01 genotyping to
41
42
43 prevent allopurinol-induced SCARs. Compared with the use of other HLA alleles as
44
45
46 biomarkers for preventing drug hypersensitivity, HLA-B*58:01 has the potential for
47
48
49 application in a broader spectrum of ethnicities. Specifically, the strong association
50
51
52 between HLA-B*58:01 and allopurinol-induced SCARs has been found in ethnicities
53
54
55 other than the Han Chinese including Thai, Japanese, Korean, and European^{16-18,20,21,38}.

1 Studies in Taiwan, Japan, Europe, and Israel have shown that allopurinol is now the
2
3
4
5
6
7 major cause of drug-induced SCARs¹¹⁻¹³. Our results suggest that in countries where
8
9
10 the HLA-B*58:01 is relatively prevalent (e.g., the allele frequency of HLA-B*58:01
11
12
13 in the Taiwanese population is 10% and the carrier prevalence among subjects with
14
15 HLA-B*58:01 is 20%), screening for this allele could be beneficial for preventing
16
17
18 allopurinol-induced SCARs. Furthermore, in countries where the allele frequency of
19
20
21 HLA-B*58:01 is relatively low (~ 1%), restricting the screening for this allele to a
22
23
24 more high-risk group of patients (e.g., chronic renal failure) could also be a potential
25
26
27 strategy for preventing SCARs. For countries or populations in which the prevalence
28
29
30 is ill-defined, further studies to estimate the prevalence are suggested for possible
31
32
33 application of this screening. In addition, because the association between the
34
35
36 HLA-B*58:01 allele and mild cutaneous adverse reaction induced by allopurinol has
37
38
39 been found in mainland China¹⁹, future investigations may be needed to examine
40
41
42 whether screening for the HLA-B*58:01 allele could reduce the prevalence of
43
44
45 allopurinol-induced maculopapular eruption (MPE).
46
47
48
49
50

51 **CONCLUSION**

52
53 Because the contribution of HLA-B*58:01 to allopurinol-induced SCARs is
54
55
56 causal^{14,23,24}, the present prospective study with a large number of study subjects
57
58
59
60

1 provides a strong basis for routine testing for this allele as well as for general
2 implementation of personalized medicine testing.

4 **Sources of Funding**

5 Supported by grants from the Academia Sinica Genomic Medicine Multicenter Study
6 (40-05-GMM) and Taiwan Biobank, Academia Sinica Taiwan.

8 **Acknowledgments**

9 We thank the subjects who participated in this study and the research and nursing staff
10 for their meticulous data collection. We gratefully acknowledge the members of the
11 Translational Resource Center (TRC) and the National Center for Genome Medicine
12 (NCGM) at Academia Sinica for their support in subject recruitment, genotyping, and
13 statistical analysis.

1 **REFERENCES**

2 1. Mini E, Nobili S. Pharmacogenetics: implementing personalized medicine.
3 Clinical cases in mineral and bone metabolism : the official journal of the Italian
4 Society of Osteoporosis, Mineral Metabolism, and Skeletal Diseases 2009;6:17-24.
5 2. Hamburg MA, Collins FS. The path to personalized medicine. The New England
6 journal of medicine 2010;363:301-4.
7 3. Ng PC, Murray SS, Levy S, Venter JC. An agenda for personalized medicine.
8 Nature 2009;461:724-6.
9 4. Ghislain PD, Roujeau JC. Treatment of severe drug reactions: Stevens-Johnson
10 syndrome, toxic epidermal necrolysis and hypersensitivity syndrome. Dermatology
11 online journal 2002;8:5.
12 5. Greenberger PA. 8. Drug allergy. The Journal of allergy and clinical
13 immunology 2006;117:S464-70.
14 6. Fritsch PO, Sidoroff A. Drug-induced Stevens-Johnson syndrome/toxic
15 epidermal necrolysis. American journal of clinical dermatology 2000;1:349-60.
16 7. Chao J, Terkeltaub R. A critical reappraisal of allopurinol dosing, safety, and
17 efficacy for hyperuricemia in gout. Curr Rheumatol Rep 2009;11:135-40.
18 8. Emmerson BT. The management of gout. The New England journal of medicine
19 1996;334:445-51.
20 9. Neogi T. Clinical practice. Gout. The New England journal of medicine
21 2011;364:443-52.
22 10. Nuki G. An appraisal of the 2012 American College of Rheumatology
23 Guidelines for the Management of Gout. Curr Opin Rheumatol 2014;26:152-61.
24 11. Markel A. Allopurinol-induced DRESS syndrome. The Israel Medical
25 Association journal : IMAJ 2005;7:656-60.
26 12. Ramasamy SN, Korb-Wells CS, Kannangara DR, et al. Allopurinol
27 hypersensitivity: a systematic review of all published cases, 1950-2012. Drug safety :
28 an international journal of medical toxicology and drug experience 2013;36:953-80.
29 13. Halevy S, Ghislain PD, Mockenhaupt M, et al. Allopurinol is the most common
30 cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and
31 Israel. Journal of the American Academy of Dermatology 2008;58:25-32.
32 14. Hung SI, Chung WH, Liou LB, et al. HLA-B*5801 allele as a genetic marker for
33 severe cutaneous adverse reactions caused by allopurinol. Proceedings of the National
34 Academy of Sciences of the United States of America 2005;102:4134-9.
35 15. Chiu ML, Hu M, Ng MH, et al. Association between HLA-B*58:01 allele and
36 severe cutaneous adverse reactions with allopurinol in Han Chinese in Hong Kong.
37 The British journal of dermatology 2012;167:44-9.

- 1 16. Tassaneeyakul W, Jantararoungtong T, Chen P, et al. Strong association between
2 HLA-B*5801 and allopurinol-induced Stevens-Johnson syndrome and toxic
3 epidermal necrolysis in a Thai population. *Pharmacogenetics and genomics*
4 2009;19:704-9.
- 5 17. Kang HR, Jee YK, Kim YS, et al. Positive and negative associations of HLA
6 class I alleles with allopurinol-induced SCARs in Koreans. *Pharmacogenetics and*
7 *genomics* 2011;21:303-7.
- 8 18. Kaniwa N, Saito Y, Aihara M, et al. HLA-B locus in Japanese patients with
9 anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal
10 necrolysis. *Pharmacogenomics* 2008;9:1617-22.
- 11 19. Cao ZH, Wei ZY, Zhu QY, et al. HLA-B*58:01 allele is associated with
12 augmented risk for both mild and severe cutaneous adverse reactions induced by
13 allopurinol in Han Chinese. *Pharmacogenomics* 2012;13:1193-201.
- 14 20. Goncalo M, Coutinho I, Teixeira V, et al. HLA-B*58:01 is a risk factor for
15 allopurinol-induced DRESS and Stevens-Johnson syndrome/toxic epidermal
16 necrolysis in a Portuguese population. *The British journal of dermatology*
17 2013;169:660-5.
- 18 21. Lonjou C, Borot N, Sekula P, et al. A European study of HLA-B in
19 Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk
20 drugs. *Pharmacogenetics and genomics* 2008;18:99-107.
- 21 22. Yun J, Mattsson J, Schnyder K, et al. Allopurinol hypersensitivity is primarily
22 mediated by dose-dependent oxypurinol-specific T cell response. *Clinical and*
23 *experimental allergy : journal of the British Society for Allergy and Clinical*
24 *Immunology* 2013;43:1246-55.
- 25 23. Yun J, Marcaida MJ, Eriksson KK, et al. Oxypurinol directly and immediately
26 activates the drug-specific T cells via the preferential use of HLA-B*58:01. *Journal of*
27 *immunology* 2014;192:2984-93.
- 28 24. Lin CH, Chen JK, Ko TM, et al. Immunologic basis for allopurinol-induced
29 severe cutaneous adverse reactions: HLA-B*58:01-restricted activation of
30 drug-specific T cells and molecular interaction. *The Journal of allergy and clinical*
31 *immunology* 2014.
- 32 25. Fam AG, Dunne SM, Iazzetta J, Paton TW. Efficacy and safety of
33 desensitization to allopurinol following cutaneous reactions. *Arthritis and rheumatism*
34 2001;44:231-8.
- 35 26. Kim SC, Newcomb C, Margolis D, Roy J, Hennessy S. Severe cutaneous
36 reactions requiring hospitalization in allopurinol initiators: a population-based cohort
37 study. *Arthritis care & research* 2013;65:578-84.

1 27. Schneider G, Kachroo S, Jones N, et al. A systematic review of validated
2 methods for identifying erythema multiforme major/minor/not otherwise specified,
3 Stevens-Johnson Syndrome, or toxic epidermal necrolysis using administrative and
4 claims data. *Pharmacoepidemiology and drug safety* 2012;21 Suppl 1:236-9.
5 28. Lin HW, Tu YY, Lin SY, et al. Risk of ovarian cancer in women with pelvic
6 inflammatory disease: a population-based study. *The Lancet Oncology*
7 2011;12:900-4.
8 29. Wu CY, Chen YJ, Ho HJ, et al. Association between nucleoside analogues and
9 risk of hepatitis B virus-related hepatocellular carcinoma recurrence following liver
10 resection. *JAMA : the journal of the American Medical Association*
11 2012;308:1906-14.
12 30. Chen P, Lin JJ, Lu CS, et al. Carbamazepine-induced toxic effects and
13 HLA-B*1502 screening in Taiwan. *The New England journal of medicine*
14 2011;364:1126-33.
15 31. Chung WH, Hung SI, Chen YT. Human leukocyte antigens and drug
16 hypersensitivity. *Current opinion in allergy and clinical immunology* 2007;7:317-23.
17 32. Hershfield MS, Callaghan JT, Tassaneeyakul W, et al. Clinical
18 Pharmacogenetics Implementation Consortium guidelines for human leukocyte
19 antigen-B genotype and allopurinol dosing. *Clinical pharmacology and therapeutics*
20 2013;93:153-8.
21 33. Lee MH, Graham GG, Williams KM, Day RO. A benefit-risk assessment of
22 benzbromarone in the treatment of gout. Was its withdrawal from the market in the
23 best interest of patients? *Drug safety : an international journal of medical toxicology*
24 and drug experience 2008;31:643-65.
25 34. Sivera F, Andres M, Carmona L, et al. Multinational evidence-based
26 recommendations for the diagnosis and management of gout: integrating systematic
27 literature review and expert opinion of a broad panel of rheumatologists in the 3e
28 initiative. *Annals of the rheumatic diseases* 2014;73:328-35.
29 35. Saokaew S, Tassaneeyakul W, Maenthaisong R, Chaiyakunapruk N.
30 Cost-effectiveness analysis of HLA-B*5801 testing in preventing allopurinol-induced
31 SJS/TEN in Thai population. *PloS one* 2014;9:e94294.
32 36. Park DJ, Kang JH, Lee JW, et al. Cost-effectiveness analysis of HLA-B5801
33 genotyping in the treatment of gout patients with chronic renal insufficiency in Korea.
34 *Arthritis care & research* 2014.
35 37. Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity
36 to abacavir. *The New England journal of medicine* 2008;358:568-79.

- 1 38. Tohkin M, Kaniwa N, Saito Y, et al. A whole-genome association study of major
2 determinants for allopurinol-related Stevens-Johnson syndrome and toxic epidermal
3 necrolysis in Japanese patients. The pharmacogenomics journal 2013;13:60-9.
4

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1. Enrollment and Outcomes.
Allopurinol was prescribed and provided for all subjects at the time of the screening visit, but patients were asked to defer taking the drug until the results of genetic testing were available. All subjects, regardless of HLA-B status, were followed for 2 months, with weekly telephone interviews.

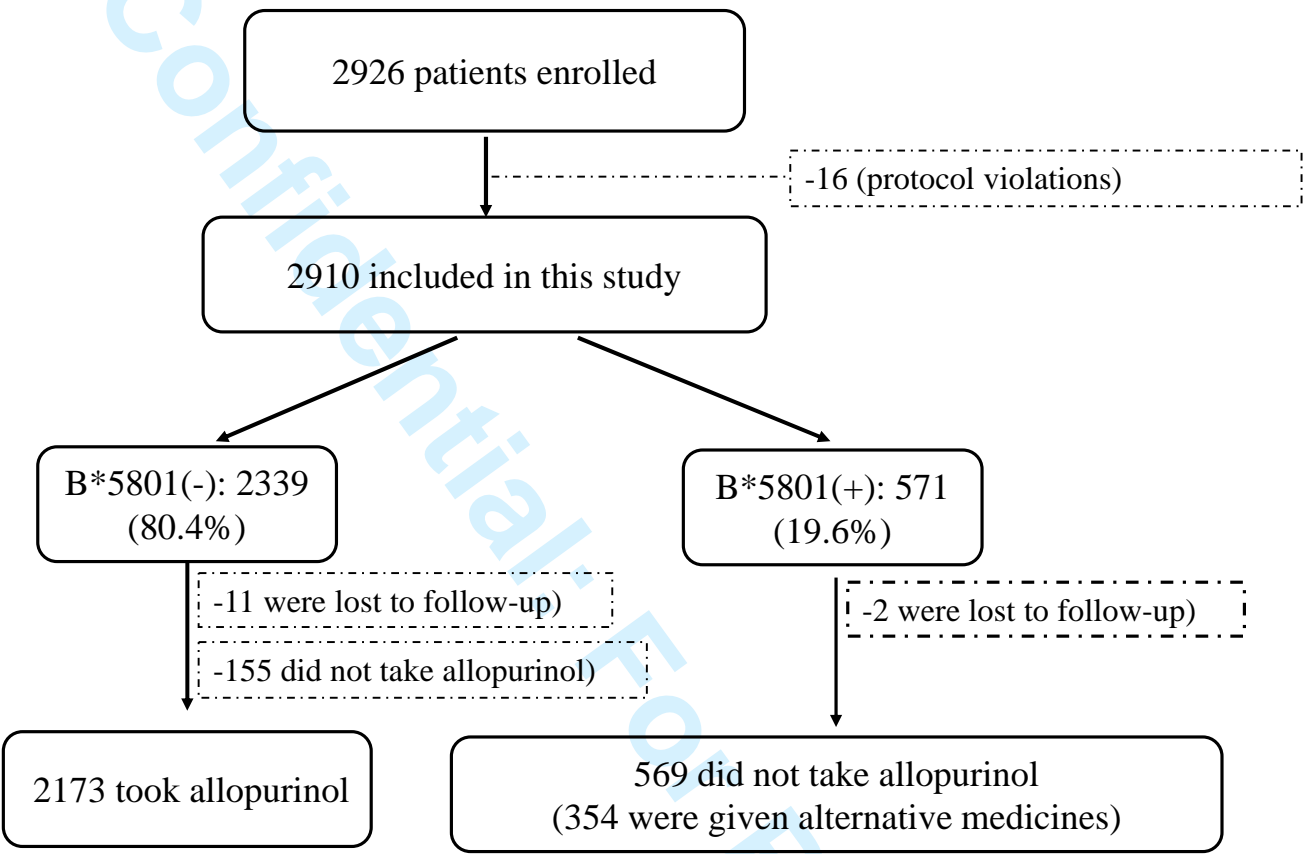


Table 1. Subject description

Characteristic	HLA-B*58:01- Positive (N=571)	HLA-B*58:01- Negative (N=2339)	<i>P</i> value [†]	Total (N=2910)
Gender—no. (%)				
Male	460 (80.6)	1950 (80.4)		2410 (82.8)
Female	111 (19.4)	389 (16.6)	0.1107	500 (17.2)
Age—yr				
Mean	54.8	54.9	0.8602	54.9
Range	19–99	14–95		14–99
Kidney function				
Renal insufficiency	120	444	0.2705	564
Indication for allopurinol—no. (%)				
Chronic tophaceous gout	204 (35.7)	820 (35.0)	0.7641	1024 (35.2)
Hyperuricaemia	141 (24.7)	555 (23.7)	0.6278	696 (23.9)
Chronic tophaceous gout; hyperuricaemia	97 (16.9)	371 (15.9)	0.5113	468 (16.1)
Chronic tophaceous gout; other	43 (7.5)	173 (7.4)	0.9126	216 (7.4)
Other conditions*	86 (15.1)	420 (17.9)	0.1017	506 (17.4)

* These conditions include urate nephropathy, prevention of recurrent nephrolithiasis, and prevention of recurrent calcium oxalate stones.

[†] The comparison between positive and negative cases for the clinical characteristics.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 2. Adverse events during the 2-month follow-up

Adverse Event	HLA-B*58:01- Positive with Alternative Medication (N=354)	HLA-B*58:01- Negative with Allopurinol (N=2173)	Total
Mild cutaneous events			
Rash and itching	3*	94	97
Blisters	0	0	0
Oral ulcers	0	2	2
Rash, itching, oral ulcers, and fever	0	1	1
Rash, itching, and other adverse events	0	22	22
Severe cutaneous events			
Drug reaction with eosinophilia and systemic symptoms	0	0	0
Urticaria	0	0	0
Stevens-Johnson syndrome or toxic epidermal necrolysis	0	0	0
Other adverse events†			
Fever	0	1	1
Sore throat	0	2	2
Fatigue	0	5	5
Other	20	117	137

*Among these three subjects, the alternative drug was benzbromarone.
†Subjects may have had more than one adverse event. Adverse events with a low frequency are not listed.

Table 3. Historical incidence of allopurinol-induced SCARs in 2001, 2002, 2003, and 2004, as compared with the incidence among study subjects

Variable	2001	2002	2003	2004
New recipients of allopurinol (no.)	137380	117896	107873	102060
Allopurinol-induced SCARs* (no.)	438	348	307	295
Incidence of allopurinol-induced SCARs (%)	0.32%	0.30%	0.28%	0.29%
<i>P</i> value for comparison between historical incidence and incidence among study subjects†	0.0018	0.0026	0.0038	0.0040

*SCARs: Severe cutaneous adverse reactions.

†All *P* values were calculated with the use of the two-side one-sample binomial test.

Supplementary Appendix

The following institutions and investigators, in addition to the authors, participated in the Taiwan Allopurinol-SCAR consortium are as follows:

Kaohsiung Medical University Chung-Ho Memorial Hospital: Wen-Chol Voon, Kun-Tai Lee, Hsiang-Chun Lee, Po-Chao Hsu, Hung-Chun Chen, Jin-Yuh Guh, Shang-Jyh Hwang, Shin Shyi Jang, Kun Der Lin, Hsuan-Fu Kuo, Sheng-Wen Niu.

National Taiwan University Hospital: Chih-Chao Yang.

Chang Gung Memorial Hospital: Yung-Chiao Wu Chan, Chung Lee, Yao-Fan Fang, Chang-Fu Kuo, Chen-Hung Lai.

The Tri-Service General Hospital: Hsiang-Cheng Chen, San-Yuan Kuo, Tsung-Yun Hou, Feng-Cheng Liu.

E-Da Hospital: Li-Chun Ho, Yi-Jer Lee, Min-Yu Chang, Yi-Ting Chen, Ho-Ching Chen.

Institute of Medicine, Chung Shan Medical University: Cheng-Chung Wei, Gregory-Jiazer Tsay, Pei-Ying Liang.

Supplementary Table 1.

Alternative medicines for HLA-B*58:01-positive individuals

Alternative medicines	N *	%
ANSRON	1	0.27
BENZBROMARONE	256	69.19
BENZBROMARONE+COLCHICINE	6	1.62
BENZBROMARONE+SULFIN	1	0.27
BISOPROLOL FUMARATE	1	0.27
BROMHEXINE HCL	3	0.81
BROMPHEIRAMINE	16	4.32
COLCHICINE	21	5.68
COLCHICINE+BROMPHEIRAMINE	1	0.27
COLCHICINE+EURICON	1	0.27
FEBURIC	27	7.30
FEBURIC FC	1	0.27
FEBURIC+COLCHICINE	1	0.27
FEBUXOSTAT	3	0.81
FEBUXOSTAT F.C.	1	0.27
NOGOUT	4	0.81
SALAZINE EC+PLAQUENIL	1	0.27
SULFANILYLUREA	1	0.27
SULFIN	1	0.27
SULFINPYRAZONE	17	4.59
URINORM	5	1.35
URISUE	1	0.27
Total	370	100.00

*Some patients took multiple alternative medicines.