ภาวะพหุสัณฐานของยีนที่ตำแหน่งเอชแอลเอ-บีและเอชแอลเอ-ซีที่ใช้เป็นตัวบ่งชี้การเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอล

นางสาวศุณิชา ลิ้มกอปรไพบูลย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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HLA-B AND HLA-C LOCI GENETIC POLYMORPHISM AS A MARKER OF SEVERE CUTANEOUS ADVERSE REACTIONS IN THAI PATIENTS ON ALLOPURINOL

Miss Sunicha Limkobpaiboon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Clinical Pharmacy
Department of Pharmacy Practice
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Thesis Title	HLA-B AND HLA-C LOCI GENETIC POLYMORPHISM AS A MARKER OF SEVERE CUTANEOUS ADVERSE REACTIONS IN THAI PATIENTS ON ALLOPURINOL
By	Miss Sunicha Limkobpaiboon
Field of Study	Clinical Pharmacy
Thesis Advisor	Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.
Thesis Co-Advisor	Assistant Professor Ajchara Koolvisoot, M.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

..... Dean of the Faculty of
(Associate Professor Pintip Pongpech, Ph.D.) Pharmaceutical Sciences

THESIS COMMITTEE

..... Chairman
(Assistant Professor Narat Kasettratat)

..... Thesis Advisor
(Associate Professor Duangchit Panomvana Na Ayudhya, Ph.D.)

..... Thesis Co-Advisor
(Assistant Professor Ajchara Koolvisoot, M.D.)

..... External Examiner
(Associate Professor Sasijit Vejbaesya, M.D.)

..... External Examiner
(Naruemon Dhana)

ศุณิชา ลิ้มกอปรไพบูลย์ : ภาวะพหุสัณฐานของยีนที่ตำแหน่งเอชแอลเอ-บีและเอชแอลเอ-ซีที่ใช้เป็นตัวบ่งชี้การเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอล. (HLA-B AND HLA-C LOCI GENETIC POLYMORPHISM AS A MARKER OF SEVERE CUTANEOUS ADVERSE REACTIONS IN THAI PATIENTS ON ALLOPURINOL) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ภญ.ดร.ดวงจิตต์ พนมวัน ณ อยุธยา, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ.พญ.อัจฉรา กุลวิสุทธิ์, 101 หน้า.

งานวิจัยนี้มีสองวัตถุประสงค์หลัก วัตถุประสงค์ที่ 1 ศึกษาถึงรายชื่อยาที่ก่อให้เกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงสูงสุดในช่วงระหว่างปี 2546-2550 รวมถึงความชุกและอัตราการเสียชีวิตที่เกิดขึ้นโดยใช้วิธีการศึกษาแบบเก็บข้อมูลย้อนหลังจากฐานข้อมูลของศูนย์ติดตามอาการไม่พึงประสงค์จากยาโรงพยาบาลศิริราชในช่วงเวลาดังกล่าว วัตถุประสงค์ที่ 2 ศึกษาถึงความสัมพันธ์ระหว่าง *HLA-B*5801* และ *HLA-Cw*0302* อัลลีลส์ ต่อการเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอลโดยการตรวจภาวะพหุสัณฐานของยีนที่ตำแหน่งข้างต้นเปรียบเทียบข้อมูลในผู้ป่วยที่เกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงจากการใช้ยาอัลโลพูรินอล จำนวน 25 ราย ผู้ป่วยที่เกิดผื่นประเภทอื่นจากการใช้ยาอัลโลพูรินอล จำนวน 9 ราย และผู้ป่วยที่สามารถใช้ยาอัลโลพูรินอลได้โดยไม่เกิดอาการไม่พึงประสงค์ จำนวน 48 ราย

ผลการวิจัยพบว่า ในช่วงปี 2546-2550 พบผู้ป่วยที่มีประวัติเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงทั้งหมด 136 ราย ยาที่ก่อให้เกิดความชุกสูงสุดของการเกิด SJS, TEN และ HSS ได้แก่ คาร์บามาซีปีน (3.26 ต่อ 1,000 ราย), อัลโลพูรินอล (0.21 ต่อ 1,000 ราย) และ ฟีนายโทอิน (2.64 ต่อ 1,000 ราย) ตามลำดับ อัตราการเสียชีวิตจาก SJS, TEN และ HSS พบ 6.90%, 50.0% และ 12.82% ตามลำดับ อัลโลพูรินอลเป็นสาเหตุของการเสียชีวิตมากที่สุด ผู้ป่วยผื่นผิวหนังชนิดรุนแรงจากการใช้ยาอัลโลพูรินอลทั้ง 25 ราย ที่เข้าร่วมการศึกษาพบ *HLA-B*5801* และ *HLA-Cw*0302* อัลลีลส์ทุกราย (100%) ขณะที่ในผู้ป่วยกลุ่มควบคุมที่เข้าร่วมการศึกษา 48 รายพบภาวะพหุสัณฐานของยีนที่ตำแหน่งข้างต้นเพียง 7 ราย (14.58%) ความเสี่ยงในการเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงจากการใช้ยาอัลโลพูรินอลในผู้ป่วยที่มี *HLA-B*5801* และ *HLA-Cw*0302* อัลลีลส์สูงเป็น 282 เท่าของผู้ป่วยที่ไม่พบสารพันธุกรรมนี้ (95% CI 15.45-5153.83, P-value < 0.001) รายงานนี้เป็นรายงานแรกที่รายงานความสัมพันธ์ของ *HLA-B*5801* และ *HLA-Cw*0302* อัลลีลส์ กับการเกิดผื่น exfoliative dermatitis สมการทำนายโอกาสเกิดผื่นทางผิวหนังจากยาอัลโลพูรินอลโดยการวิเคราะห์แบบถดถอยโลจิสติกแสดงให้เห็นว่าปัจจัยหลักสามประการที่มีส่วนต่อการเกิดผื่นผิวหนังจากยาอัลโลพูรินอล ได้แก่ ปัจจัยทางด้านพันธุกรรม (*HLA-B*5801*) เพศหญิง และโรคเบาหวานที่เป็นร่วมด้วย

ภาควิชา.....เภสัชกรรมปฏิบัติ.....ลายมือชื่อนิสิต.....ศุณิชา.....

สาขาวิชา.....เภสัชกรรมคลินิก.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2552.....ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

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KEYWORDS :ALLOPURINOL / MORTALITY RATE / SCAR / HLA-B*5801

SUNICHA LIMKOBPAIBOON : HLA-B AND HLA-C LOCI GENETIC POLYMORPHISM AS A MARKER OF SEVERE CUTANEOUS ADVERSE REACTIONS IN THAI PATIENTS ON ALLOPURINOL. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D., THESIS CO-ADVISOR : ASST. PROF. AJCHARA KOOLVISOOT, M.D., 101 pp.

There were two main purposes in this present study; first, to investigate the causative drugs, the prevalence and mortality rates related to severe cutaneous adverse reactions (SCAR) during 2003-2007 using retrospective data collected from electronic database of Adverse Drug Reaction Monitoring Center and Siriraj Computer Center, Siriraj Hospital, Bangkok; second, to determine the association between *HLA-B*5801* and *HLA-Cw*0302* alleles to SCAR induced by allopurinol in Thai patients using case-control study. There were 25 case patients who experienced allopurinol induced SCAR, 9 case patients who experienced other cutaneous adverse reaction from allopurinol and 48 allopurinol tolerant controls participate in the study.

SCAR was found in 136 patients. The prevalence of SJS, TEN and HSS were most often found in patients treated with carbamazepine (3.26 per 1,000 patients), allopurinol (0.21 per 1,000 patients) and phenytoin (2.64 per 1,000 patient), respectively. Mortality rates of SJS, TEN and HSS were 6.90%, 50.0% and 12.82% respectively. Allopurinol revealed the highest mortality rate. *HLA-B*5801* and *HLA-Cw*0302* alleles were found in all 25 cases (100%) of patient with SCAR, only 7 controls (14.58%) of allopurinol tolerant patients have *HLA-B*5801* and *HLA-Cw*0302* alleles. Risk of SCAR in patients with *HLA-B*5801* and *HLA-Cw*0302* alleles was 282 times higher (95%CI 15.45 to 5153.83, P-value < 0.001). This study was the first that reported the presence of *HLA-B*5801* and *HLA-Cw*0302* alleles in all patients who experienced exfoliative dermatitis from allopurinol. Model to predict the adverse drug reactions from allopurinol using logistic regression demonstrated its association to three main factors; *HLA-B*5801* allele, female gender and underlying of diabetes mellitus.

Department : Pharmacy Practice ..... Student's Signature SUNICHA .....
Field of Study : Clinical Pharmacy ..... Advisor's Signature Duangchit Panomvana .....
Academic Year : 2009 ..... Co-Advisor's Signature .....

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LIST OF ABBREVIATIONS

ADR	=	Adverse Drug Reaction
ddH_2O	=	Double distilled water
DNA	=	Deoxyribonucleic acid
dNTPs	=	Deoxynucleotide triphosphate
DRESS	=	Drug Rash with Eosinophilia and Systemic Symptoms
EDTA	=	Ethylenediaminetetraacetic acid
HLA-B*1502	=	Human Leukocyte Antigen-B*1502
HLA-B*5801	=	Human Leukocyte Antigen-B*5801
HLA-Cw*0302	=	Human Leukocyte Antigen-Cw*0302
HSS	=	Drug Hypersensitivity Syndrome
MHC	=	Major Histocompatibility Complex
ml	=	Milliliter
µl	=	Microliter
nm	=	Nanometer
OD	=	Optical Density
PCR	=	Polymerase Chain Reaction
PCR-SSP	=	Polymerase Chain Reaction Sequence Specific Primer
PCR-SSOP	=	Polymerase Chain Reaction Sequence-Specific Oligonucleotide Probe
SBT	=	Sequence Base Typing
SCAR	=	Severe Cutaneous Adverse Reaction
SDS	=	Sodium dodecyl sulfate
SJS	=	Stevens - Johnson syndrome
TEN	=	Toxic Epidermal Necrolysis

CHAPTER I
INTRODUCTION

Background and Rationale

Hyperuricemia is fairly common, with prevalence ranging between 2.6% and 47.2% in various populations.[1] Most patients present asymptomatic whereas complications such as renal calculi, uric acid nephropathy and gout might be found in some patients. Gout is one of rheumatic disease which its pathogenesis of disease is well understood. Current treatment guidelines are to control uric acid level to be below saturation point for prevention of recurrent gouty arthritis and to reduce risk of renal complication. Standard treatment can prevent the disease from become chronic phase. However, there are two levels of problem of the treatment. First, inappropriate treatment in general practice caused by overuse, under use or inappropriate choosing of medication. Second, in tertiary care setting, problem caused by complicated gouty arthritis uncontrolled symptom of disease despite fully prescription. Besides, other problem is the severe adverse drug reactions to antigout.[2]

Severe adverse drug reactions are idiosyncratic, uncommon and not related to dosage. Two types of adverse drug reactions are observed; type A reaction can be predicted from the pharmacological effect. While type B reaction cannot be predicted.[3] Severe adverse drug reaction is caused by drug hypersensitivity which drug allergy is included; the reaction can cause disability or death that may lead to medical litigation.[4] Losses incurred not only the cost of treatment but include the loss of the soul that inestimable. Rather than non-steroidal anti-inflammatory drugs (NSAIDs), allopurinol is widely used for gouty arthritis treatment. This is because of its high efficacy to lower uric acid level and can be used in patient with renal insufficiency whereas drug allergy that related to allopurinol is frequently reported.[5-8] Non-serious allergic rash was found approximately 2% to the use of allopurinol.[9-10] Moreover, severe adverse drug reactions that include Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug hypersensitivity syndrome (HSS), also called DRESS (drug rash with eosinophillia and systemic symptoms), are frequently reported in conjunction with allopurinol. These events are severe cutaneous

adverse reactions (SCAR)[8] which incidences are rare but has significant impact on patient's well being because of high morbidity and mortality rate. These three types of SCAR are delayed type immune-mediated reaction.[8] The incidence of SJS and TEN in European is about 1-6 and 0.4-1.2 cases per million person years respectively.[11] In Thailand, Spontaneous Reporting System (SRS) for adverse drug reaction monitoring shows incidences of SJS and TEN to be 0.3 and 0.06 case per million person years respectively.[12] Incidence of HSS is unknown because of unspecific symptoms associated with multi-organ involvement.[12] Mortality rate of SJS, TEN and HSS were found up to 5%, 30-50% and 10% respectively.[9, 13]

The adverse drug reaction monitoring of Food and Drug Administration, Ministry of Health, Thailand, reported that top 5 causative drugs of Stevens-Johnson syndrome during 2006-2008 that categorized by adverse event's name are cotrimoxazole, allopurinol, carbamazepine, phenytoin and fluconazole. If categorized by products in the same period of time, allopurinol is at number 17 of 1,344 most common reported products. Up to 1,068 adverse drug reactions were reported with causality assessment related to allopurinol; SJS was the most frequently reported events.[14]

This is the reason why "adverse drug reaction" is an interesting question as it is one of the dangers arising from drug use. In the past, adverse drug reactions cannot be prevented but several recent studies revealed that one of risk factors of adverse drug reactions is genetic predisposition. It is the fact that genetic variations are important and relevant to both of treatment response and the occurrence of adverse drug reaction, the scientific knowledge that study about this relationship is known as pharmacogenomics.[15] There was a pharmacogenomic study concerning the relationship between serious adverse events or drug hypersensitivity and Human leukocyte antigens (HLAs).[16] Most of the events are skin or mucosal reactions, hepatitis or renal failure might be found in the worse cases.

HLAs are group of genes (approximately 200 genes) reside on multi-locus on shot arm of 6 chromosome that have an important role in responding of the immune system in human body. HLAs control antigen production on cell surface which will differentiate normal cells from infected cells or alien cells by T-cell receptors (TCRs) on T-cell lymphocytes. HLAs can be divided into three groups including (1) HLA class I molecules e.g. HLA-A, HLA-B and HLA-C (Alphabet following HLA such as

A, B, C shows location of the gene) that can be found on the surface of the nucleus cell, HLA class I act as intracellular antigen. (2) HLA class II molecules e.g. HLA-DP, HLA-DQ and HLA-DR are found on the surface of immune cells, these genes represent extracellular antigen. (3) HLA class III molecules which is group of complement genes e.g. C2, C4, factor B and cytokines (such as tumor necrosis factor (TNF)). There are highly genetic variations among HLAs genes especially HLA-B genes which show the highest genetic variability.[17]

Several recent studies have shown the association between genetic variations of HLA genes and drug hypersensitivity in many drugs including abacavir, nevirapine, carbamazepine and allopurinol. Nowadays, the type of genetic variation that relevant to adverse drug reaction is identifiable. Pharmacogenomics are found as part of the information in many drug package inserts such as abacavir, an antiviral drug which reports of serious drug hypersensitivity are commonly found in white people. United States Food and Drug Administration, USFDA, recommends that genetic testing of *HLA-B*5701* allele is needed for screening patients before using of abacavir.[18] Genetic testing of *HLA-B* 5701* allele is very useful for HIV patient to avoid drug hypersensitivity in white people whereas benefit for Asian and African ethnics group is unclear.[17] USFDA also recommends genetic testing of *HLA-B*1502* allele for screening prior to exposure to carbamazepine.[19] Chaichon et al.[20] found that patients with *HLA-B*1502* have 25.5 times higher risk of SJS and TEN than patients who do not have this allele in using of carbamazepine.

Some recent studies show strong association between *HLA-B*5801* allele in Han Chinese ethnicity and allopurinol-induced SJS, TEN and HSS from case-control study by Hung et al.[21] *HLA-B*5801* allele was found in all 51 cases (100%) of SCAR, while only 20 cases from 135 cases (14.81%) of patient who used this medication without SCAR have *HLA-B*5801*allele. Risk of SJS and TEN in patients with *HLA-B*5801* allele was 580 times higher. Likewise, *HLA-Cw*0302* allele was found in 48 from 51 cases (94%) of SCAR. This allele was found only 19 from 135 cases (14%) of patient who used this medication without SCAR. Patients with *HLA-Cw*0302* allele have 97.7 times higher risk of SJS and TEN from allopurinol than patients who do not have this allele. Teruki et al.[22] reported 3 case studies that experienced SCAR from allopurinol. HLA genotyping was done in the first patient who experienced SJS and *A31*, *A33*, *B51* and *B58* were found. The second patient

with HSS, HLA genotyping revealed *A31*, *A33*, *B39* and *B58*. The last patient SJS overlap TEN, *A24*, *A33*, *B52* and *B58* were found. This result confirms that *HLA-B58* alleles are more likely found in patients with SJS, TEN and HSS from allopurinol. Another study which investigated European patients who experienced SCAR, SJS or TEN from allopurinol, Lonjou et al. [8], they found that only 61% of patients (19 from 31 cases) with SJS and TEN had *HLA-B*5801* allele. Kaniwa et al.[23], they found that the presence of *HLA-B*5801* allele only 40% of patients with SJS and TEN in Japanese patients. Wichittra et al.[24] found that the presence of *HLA-B*5801* allele showed 348 times higher risk of SJS and TEN from allopurinol as compared to patients without this allele in Thai people. *HLA-B*5801* allele is found in different percentages among different ethnics population, 2-4% in Africans, 1-6% in whites, 3-15% in Asian Indians and up to 8.8-10.9% in Chinese population.[16] This allele is also frequently found (up to 8.4 %) in Thais.[25]

Data from the study mentioned above is an important beginning to create research interests which focus on Thai patients who had severe cutaneous adverse reactions, whether or not there are any association between HLA-*B*5801* and *HLA-Cw*0302* alleles to severe cutaneous adverse reactions from allopurinol. In the future this information could be useful for physician; to increase vigilance when prescribing this drug in order to reduce the incidence of severe cutaneous adverse reactions from allopurinol which quite often are life-threatening. Researches regarding association of pharmacogenomics to severe adverse drug reaction can be directly beneficial to the patient since the information obtained could enhance higher safety of drug use and enhance better quality of drug therapy in the future.

Hypothesis

Patients, who had severe cutaneous adverse reaction from allopurinol when test HLA genotyping by sequence specific primer (SSP), they will be found the genetic marker *HLA-B*5801* and *HLA-Cw*0302* alleles.

Objective

1. To determine the causative drugs, prevalence and mortality rates related to SCAR, which were from drug exposure during 2003-2007 of patients at Siriraj Hospital

2. To determine association between *HLA-B*5801* and *HLA-Cw*0302* alleles as a genetic marker of severe cutaneous adverse reaction from allopurinol in Thai patient.

Expected Outcome

1. Information about association between *HLA-B*5801* and/or *HLA-Cw*0302* alleles in Thai patients with severe cutaneous adverse reaction from allopurinol.

2. Model equation for predicting adverse drug reactions from allopurinol.

Operational Definitions

1. **Adverse Drug Reaction (ADR)** [26] is a response to a medical product which is noxious and unintended and with occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease or for the modification of physiologic function.

2. **Drug hypersensitivity syndrome (HSS) or Allopurinol hypersensitivity syndrome (AHS)** suggested criteria for the diagnosis of AHS, according to Zinger and Wallace SL[9]

2.1 A documented intake allopurinol.

2.2 Lack of exposure to a different drug causing a similar clinical picture.

2.3 Presence of at least 2 major criteria or 1 major and 1 minor criteria.

a) Major criteria include
   I. Worsening renal function
   II. Acute hepatocellular injury and,
   III. Rash, manifesting by toxic epidermal necrolysis, erythema multiforme, diffuse maculopapular rash or exfoliative dermatitis.

b) Minor criteria include fever, leukocytosis, and eosinophilia

3. ***HLA-B*5801*** [21] is Human leukocyte antigen locus B* 5801 associated with severe cutaneous adverse reaction from allopurinol.

4. ***HLA-Cw*0302*** [21] is Human leukocyte antigen locus C* 0302 associated with severe cutaneous adverse reaction from allopurinol.

5. **Severe cutaneous adverse reaction (SCAR)**[8] includes Stevens-Johnson Syndrome (SJS), Toxic Epidermal Necrolysis and Drug Reactions with Eosinophilia and Systemic Symptoms also called Drug Hypersensitivity Syndrome.

6. **Stevens-Johnson syndrome (SJS)**[27] is severe adverse drug reactions characterized by high fever, wide-spread blistering exanthema of macules and atypical target-like lesions, mucosal involvement. SJS will be considered if less than 10% of the body surface area (BSA) of skin is detached.

7. **Stevens-Johnson syndrome overlap toxic epidermal necrolysis**[27] is severe adverse drug reactions characterized by high fever, wide-spread blistering exanthema of macules and atypical target-like lesions and mucosal involvement. SJS overlap TEN will be considered by epidermal detachment ranging 10-30% of BSA.

8. **Toxic epidermal necrolysis**[27] is severe adverse drug reactions characterized by high fever, wide-spread blistering exanthema of macules and atypical target-like lesions, and mucosal involvement. TEN will be considered if epidermal detachment more than 30% of BSA detached**.**

CHARPTER II
LITERATURE REVIEWS

Gouty Arthritis

Gout is a metabolic disease in which hyperuricemia and arthritis are variably expressed. Gout is related to deposition of monosodium urate crystals in the synovial fluid. It occurs primarily in men, with onset usually in the fourth through sixth decades; in woman, it is more likely to follow menopause.[28]

Genetic of Gout[1]

Since the distant past, gout has been recognized as a familial disorder. The familial incidences reported range from 11% to 80%. Available data are considered, they suggest that serum urate concentrations are controlled by polygenic traits. Several rare forms of hyperuricemia and gout, such as hypoxanthine phosphor-ribosyltransferase deficiency, phosphoribosyl-1-pyrophosphate synthetase over-activity, and familial hyperuricemia nephropathy, have a genetic basis.

Etiology[1, 28]

Gout is liked to high levels of uric acid; however, there is not an absolute association between hyperuricemia and gout. Hyperuricemia is a biochemical abnormality defined solely by the serum urate concentration that results from urate overproduction, decrease excretion, or combination data shown in table 2.1. Although hyperuricemia is not a requirement for the diagnosis of gout the risk of gout increases with the degree and duration of hyperuricemia data shown in table 2.2.

Table 2.1 Conditions associated with hyperuricemia [28-29]

Urate overproduction	Urate renal under excretion
Idiopathic (primary) gout	Idiopathic (primary) gout
Inherited enzymatic defects	Clinical disorders
Polycythemia vera	Hypertension
Paget's disease	Dehydration
Hemolytic disease	Obesity
Psoriasis	Sarcoidosis
Obesity	Renal insufficiency
Myelo-and lymphoproliferative disease	Lead toxicity
Drugs	Drugs
- Cytotoxic agent	- Ethanol
- High-dose salicylate	- Diuretic e.g. thiazide diuretic
- Ethanol	- Low-dose salicylates
- Warfarin	- Cyclosporin
	- Levodopa
	Starvation
	- Acidosis
	- Toxemia of pregnancy
	- Salt restriction

Table 2.2 Annual incidence of gouty arthritis according to the serum urate concentration [1, 29]

Serum urate concentration (mg/dL)	Annual incidence of gout (%)
< 7.0	0.1-0.5
7.0-8.9	0.5-1.2
≥ 9.0	4.9-5.7

Clinical Feature [1, 28]

Stages of gout have three possible exist: acute gouty arthritis, intercritical gout and tophaceous gout. Acute gouty arthritis, the first attack usually occurs between age 40 to 60 years in mens and after age 60 in woman. Onset before age 25 should raise the possibility of an unusual form of gout, perhaps one related to a specific enzymatic defect that causes marked purine overproduction, an inherited renal disorder, or the use of cyclosporine. A single joint is involved in about 85% to 90% of first attacks, with the first metatar-sophalangeal joint being the most commonly affected site. The initial attack is polyarticular in 3% to 14%. Acute gout is predominantly a disease of the lower extremities, but eventually, any joint of any extremity may be involved. Fever may be present.

As the acute gouty attack subsides, the patient becomes asymptomatic and enters the intercritical period. Some patients never have a second attack. However, most patients suffer a second attack within 6 months to 2 years. If recurrent gout is untreated, nodules (tophi) can develop on the extensor surfaces of the elbows, in joints and in surrounding tissues, especially the interphalangeal joints of the hands or feet and the helix of the ear. The diagnosis of gout in a hyperuricemic patient with a history of acute attacks of monarthritis may be difficult or inconclusive during the intercritical period.

Eventually, the patient may enter a phase of chronic polyarticular gout with no pain-free intercritical periods. At this stage, gout may be easily confused with other types of arthritis or other conditions. The time from the initial attack to the beginning of chronic symptoms or visible tophaceous involvement is highly variable in studies of untreated patients. The rate of formation of tophaceous deposits correlates with both the degree and the duration of hyperuricemia. Tophaceous gout is the consequence of the chronic inability to eliminate urate as rapidly as it is produced. As the urate pool expands, deposits of urate crystals appear in cartilage, synovial membranes, tendons, soft tissues, and elsewhere. Tophi are rarely present at the time of an initial attack of primary gout; they are more likely to be present in gout secondary to myeloproliferative diseases, in juvenile gout-complicating glycogen storage diseases (GSDs), in Lesch-Nyhan syndrome, or after allograft trans-plantation in patients treated with cyclosporine.

Diagnosis

The definitive diagnosis of gout is best established by aspiration of the joint and identification of intracellular needle-shaped crystals that have negative birefringence with compensated polarized light microscopy. However, criteria have been proposed for a presumptive diagnosis. These include the triad of acute monarticular arthritis, hyperuricemia, and a dramatic response to colchicine therapy, and a set of criteria proposed by the American College of Rheumatology shown in table 2.3.

Table 2.3 American College of Rheumatology preliminary criteria for gout [30]

Gout may be diagnosed if one of the following criteria is present
Monosodium urate crystals in synovial fluid Tophi confirmed with crystals examination At least six of the following findings: - Asymptomatic swelling within a joint on a radiograph - First metatarsophalangeal joint is tender or swollen (i.e. podagra) - Hyperuricemia - Maximum inflammation developed within one day - Monoarthritis attack - More than one acute attack - Redness observed over joints - Subcortical cyts without erosions on a radiograph - Suspected tophi - Synovial fluid culture negative for organisms during an acute attack - Unilateral first metatarsophalangeal joint attack - Unilateral tarsal joint attack

Treatment

The therapeutic aims in gout are as follows: [28]

1. To terminate the acute attack as promptly and gently as possible.
2. To prevent recurrences of acute gouty arthritis.
3. To prevent or reverse complications of the disease resulting from the deposition of sodium urate or uric acid crystals in joints, kidneys, or other sites.

4. To prevent or reverse associated features of the illness that are deleterious, such as obesity, hypertriglyceridemia, and hypertension.

Acute Gouty Arthritis

Currently available treatment options for acute gout include colchicines, nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Colchicine and NSAIDs are usual first lines of treatment for acute gout [28] Colchicine, a plant derivative, inhibits leukocyte activation and migration is most effective when given in the first 24-48 hours of the attack. [31] Colchicine should be used with caution in patients with renal impairment because of the increased risk of bone marrow suppression.[28] Most potent NSAIDs effective in relieving pain and reducing inflammation in patient with acute gout. Indomethacin is classically described as being effective in gout. Recent studies suggest that the cyclooxygenase-2 inhibitors are equally effective as indomethacin in the treatment of acute gout.[31] Corticosteroids are effective in acute gout and treatment of choice for patient with renal impairment or gastrointestinal bleeding. Adrenocorticotropic hormone has also been shown to be effective in acute gout and is thought to be effective even in patients who are adrenally impairment. Urate lowering agents such as allopurinol or probenecid should not be started or discontinued during an acute attack.[31]

Chronic Gouty Arthritis

The long term management of gout consists of urate lowering agents and low dose of colchicine or other NSAIDs may be prescribed as prophylaxis against recurrent attack. [28, 31] In general, indications for urate lowering agents include two or more gout attack per year, tophaceous gout, erosive arthritis on radiographs, and uric acid kidney disease, including urate nephropathy, uric acid nephropathy, and uric acid nephrolithiasis. A serum uric acid level less than 6.0 mg/dl is generally recommended, because a serum uric acid below this level has been associated with reduced frequency or prevention of gout attack. Urate lowering agents using xanthine oxidase inhibitor or uricosuric agent is recommend and Medication management of gout summarize in table 2.4.

Table 2.4 Medication used in management of gouty arthritis [28-31]

Medication	Usual Dose	Cautions
Acute gouty arthritis		
- Colchicine	0.6 mg every 1 to 2 hours until pain and inflammation are alleviated, GI side effect develop, or a maximum of 10 tablet/24 hours is reached.	Avoid in patients with severe renal or hepatic impairment GI side effect; nausea, abdominal pain, diarrhea occur in up to 80% of patients administered small repeat dose.
- NSAIDs	Indomethacin, 200 mg/day in divided dose on the first day followed by 150 mg/day in dived dose, until the attack subsides, then taper. Naproxen, 500 mg bid for 4 to 10 days. Sulindac, 200 mg bid for 4 to 10 days.	Use with caution in older patients and in patients with renal disease. Maximum dosage of indomethacin often produce central nervous system.
- Corticosteroids	Oral; starting dose of 40-60 mg of prednisolone or equivalent daily with subsequent taper over 7-10 days especially useful in patient in whom NSAIDs and colchicines are contraindicate. IA; 40 mg IA with lidocaine for large joints, 10-20 mg for small joint or bursae. IV; such as methylprednisolone 100 mg IV daily with taper or IM such as triamcinolone 40 mg. IM, repeat in 12 hours if needed.	Avoid in patient with joint sepsis Body fluid retention, hypertension, acne, hyperglycemia, osteoporosis.
- ACTH	40-80 USP unit IM Q8-12 hours (usually 2-3 injections required).	
To prevent acute attacks		
- Colchicine	Small dose of colchicines (0.6 mg once or twice daily).	See above.
- NSAIDs	Useful if colchicines alone is insufficient and acute attacks recur frequently; usual dose is 150 to 300 mg of indomethacin per day or its equivalent.	See above.

Medication	Usual Dose	Cautions
To lower serum urate concentration		
- Probenecid	250 mg twice daily for one week; increase to 250-500 mg/day; may increase by 500 mg/month, if need, to maximum of 2-3g/day (dosage may be increased by 500 mg every 6 months if serum urate concentration are controlled).	Renal colic or deterioration of renal function and increase risk of nephrolithiasis high dose carry a risk of central nervous system and respiratory arrest.
- Sulfinpyrazone	Initial dose 200-400 mg/day in two divided doses with meals or at bedtime with milk. Increase dose gradually over 1-week period titrating to desired urate blood levels to 400-800 mg/day.	GI side effect; GI disturbances such as stomach pains, nausea, vomiting and exacerbation of ulcers increase risk of nephrolithiasis.
- Benzbromarone	Potent long-acting uricosuric drug 50-200 mg daily.	Cytolytic liver damage and fulminant liver failure.
- Allopurinol	100-300 mg/day in patients with normal renal function adjust dose for patients with renal insufficiency CrCl 60; 200 mg/day CrCl 40; 150 mg/day CrCl 20; 100 mg/day	GI side effect; nausea, vomiting. Dermatologic; rash develops in approximate 2 % the most serious side effect of allopurinol, which occurs in less than 1 in 1000 cases is SJS, TEN, HSS.

Allopurinol [9, 32]

Allopurinol, or 4-hydroxypirazolo pyrimidine, is an analog of hypoxanthine is the only inhibitor of xanthine oxidase in clinical use for treatment of gout and hyperuricemia. Allopurinol, developed in 1956, was initially tested as an adjuvant to increase the therapeutic effectiveness of 6-mercaptopurine in the treatment of leukemia. Incidentally, it was found to reduce serum uric acids level by inhibiting xanthine oxidase, allopurinol and its metabolite oxypurinol prevent the conversion of hypoxanthine to xanthine and to uric acid. Allopurinol is a molecular weight of 136.11 and its sodium salt has a weight of 158.09. Its pK_a is 10.2. The chemical structure of allopurinol is shown in Figure 2.1.

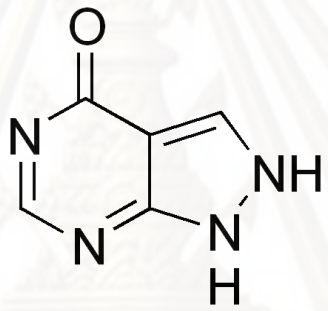

Figure 2.1 Chemical structure of allopurinol

Pharmacodynamic Properties[32]

Allopurinol acts upon purine catabolism without disruption of the biosynthesis of vital purines. The drug reduces the production of uric acid by inhibiting xanthine oxidase, the enzyme responsible for conversion of hypoxanthine to xanthine and of xanthine to uric acid, resulting in reduction in plasma and urinary concentrations of uric acid. The drug also inhibits de novo purine synthesis through a feedback mechanism, an effect which requires the presence of the enzyme hypoxanthine guanine phosphoribosyltransferase. However, patients with Lesch-Nyhan syndrome and a small percentage of adults with deficiency in this enzyme do not benefit from these effects of allopurinol. Allopurinol is metabolized primarily to oxypurinol (alloxanthine), which is also an inhibitor of xanthine oxidase.

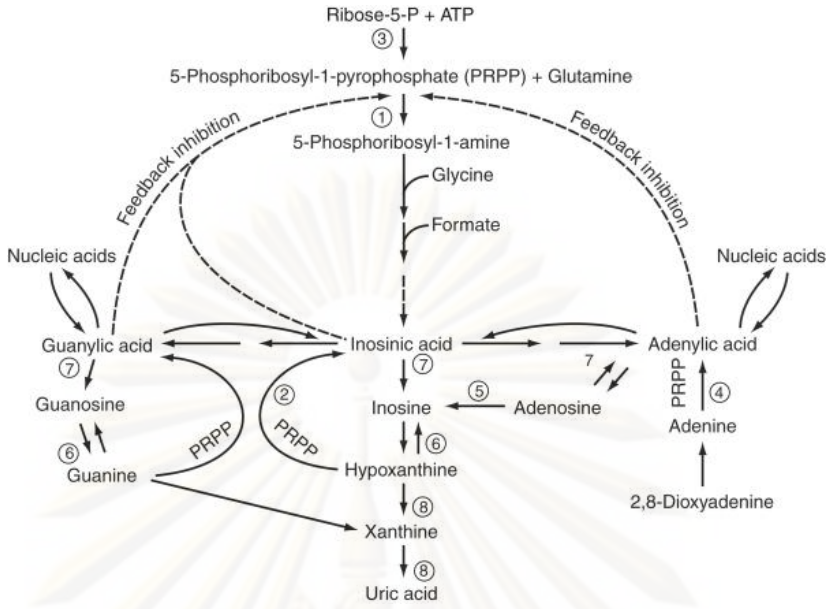

Figure 2.2 Outline of purine metabolism: (1) amidophosphoribosyltransferase, (2) hypoxanthine-guanine phosphoribosyltransferase, (3) phosphoribosylpyrophosphate (PRPP) synthetase, (4) adenine phosphoribosyltransferase, (5) adenosine deaminase, (6) purine nucleoside phosphorylase, (7) 5′-nucleotidase, (8) xanthine oxidase.[(1)]

Pharmacokinetic Properties[(32)]

Absorption

The absorption of allopurinol is approximately 80-90% from the gastrointestinal tract. Peak plasma concentration usually appears within 90 minutes (range 30 to 120 minutes) from oral administration.

Distribution

Following oral administration, the volume of distribution (Vd) of allopurinol ranges from 1.6 to 2.43 L/kg. Following intravenous administration of 100 mg and 300 mg, the Vd at steady-state of allopurinol is 0.84 L/kg and 0.87 L/kg, respectively. following a single 200 mg oral dose the Vd of allopurinol was similar in elderly (age range 71 to 93 years) and young (age range 24 to 35 years) subjects; however, the Vd of oxypurinol was significantly reduced in elderly subject (0.6 L/kg) compared with young subject (0.84L/kg).

Metabolism and Elimination

Allopurinol is metabolized in the liver approximately 70% and rapidly oxidized to oxypurinol. Approximately 10% of an administration dose is metabolized to allopurinol riboside. Oxypurinol is slightly less potent than allopurinol in its ability to inhibit xanthine oxidase.

Renal excretion is the major route of elimination. Approximately 80% of a dose is recovered in the urine within 24 hours after oral administration; 8% to 12% of dose is excreted in the urine as unchanged drug; 45% to 76% of a dose is excreted as oxypurinol. Elimination of oxypurinol may be reduced in elderly patients because of an age-dependent decline in renal function.

Adult Dosing[32]

- Calcium renal calculus, recurrent: 200 to 300 mg orally as a single or divided dose (2-3 times daily); maximum dose: 300 mg/dose; 800 mg/day.
- Cancer-hyperuricemia: optimal dosing and timing not yet defined.
- Gout: (mild) 100-300 mg/day orally as a single or divided dose (2-3 times daily).
- Gout: (moderate to severe) 400-600 mg/day orally as a single or divided dose (2-3 times daily); maximum dose 800 mg/day.
- Hyperuricemia - Tumor lysis syndrome: 600-800 mg/day orally, 12 hours to 3 days prior to initiation of chemotherapy.
- Hyperuricemia - Tumor lysis syndrome: 200-400 mg/$m^{(2)}$/day IV, 24-48 hours prior to initiation of chemotherapy as a single infusion OR in equally divided infusions at 6, 8, or 12 hour intervals; maximum dose 600 mg/day.

Pediatric dosing [32]

- Cancer - hyperuricemia: (under 6 y) 150 mg PO daily, evaluate response after 48 h and dose adjust accordingly.
- Cancer - hyperuricemia: (6 to 10 y) 300 mg PO daily, evaluate response after 48 h and dose adjust accordingly.
- Hyperuricemia - Tumor lysis syndrome: (under 6 yrs) 50 mg orally 3 times daily.

- Hyperuricemia - Tumor lysis syndrome: (6-10 yrs) 100 mg orally 3 times daily OR 300 mg orally once daily.

- Hyperuricemia - Tumor lysis syndrome: 200 mg/$m^{(2)}$/day IV starting 24-48 hours prior to initiation of chemotherapy as a single infusion or in equally divided infusions at 6, 8, or 12 hour intervals.

Table 2.5 Maintenance dose of allopurinol base on creatinine clearance[33]

Creatinine clearance (ml/min)	Allopurinol dose (mg)
0	100 mg every 3 days
10	100 mg every 2 days
20	100 mg daily
40	150 mg daily
60	200 mg daily
80	250 mg daily
100	300 mg daily
120	350 mg daily
140	400 mg daily

Drug interactions [32-33]

In regard to drug interactions of allopurinol some of these drug interactions are clinical significance. Agent such as didanosine concomitant use of allopurinol is contraindicated due to increased systemic exposure of didanosine and increased the potential for didanosine associated toxicity. Allopurinol may increase the level or effect of amoxicillin, ampicillin, mercaptopurine, azathioprine, carbamazepine and cyclophosphamide. Concurrence use of allopurinol and mercaptopurine may result in mercatopurine toxicity (bone marrow suppression, nausea, vomiting). The dose of oral mercaptopurine should be reduced to 1/3 (33%) to 1/4 (25%) of the usual dose. Probable mechanism is inhibiting of first pass oxidative metabolism of mercaptopurine by xanthine oxidase. On the other hand, the level or effect of allopurinol may be increased by ACE inhibitor e.g. enalapril, captopril, loop diuretic and thiazide diuretic. If allopurinol is used with ACEI concurrently, monitor carefully for hypersensitivity reaction. (Stevens-Johnson syndrome, skin eruptions anaphylactic coronary spasm) However, aluminium hydroxide can decrease ability of allopurinol to

reduce uric acid level by decrease absorption recommend taking aluminium hydroxide at least three hours after taking allopurinol. In addition to the pharmacokinetic drug interactions, pharmacodynamic interactions may also occur when allopurinol is administered with certain therapeutic agents.

Adverse Effect [1, 9, 34]

Although allopurinol is generally well tolerated, about 20% of patient who take allopurinol report side effect and 5% discontinuing this medication. Skin rash are more common about 2% of patients experience pruritus or rash, usually 3 week after initiation. The occurrence of a rash does not necessarily mean the drug should be discontinued. If rash is not severe. However, allopurinol is high risk drugs to caused severe cutaneuos adverse reaction. The most severe reaction is the allopurinol hypersensitivity syndrome, which may include fever, skin rash (SJS/TEN, diffuse maculopapular rash, erythema multiforme or exfoliative dermatitis) and single or multiple internal organ involvement (especially acute hepatocellular injury, worsening renal function or hematological abnormalities). Patient with preexisting renal impairment and use of thiazide diuretic are at greatest risk for developing allopurinol toxicity. By impairing allopurinol clearance, they increase allopurinol levels and may impair, as has been suggested, oxypurinol clearance. Futhermore, thiazides seem to potentiate the effect of allopurinol on pyrimidine metabolism and may predispose the patient to an “antigenic overload” and, consequently, to an immunologic reaction. Other severe reactions include agranulocytosis, aplastic anemia, myelosuppression, thrombocytopenia, granulomatous hepatitis and renal failure.

Adverse Drug Reaction (ADR) [26]

World Health Organization (WHO) has defined the meaning of ADR from 1972 that mean an adverse drug reaction is a response to a drug which is noxious and unintended and which occurs at dose normally used in man for prophylaxis, diagnosis or therapy of disease or for the modification of physiologic function.

Type of ADR

Classifications of ADR generally can be divided base on various ideas.

1. **Pharmacological classification**[35]

1.1 Type A ADR (Augmented) is a result of the pharmacological effect of drug or metabolite. Severity of symptom associated with dose, the incidence rates is high but, mortality rate is low. Type A ADR can generally be reproduced and studied in clinical trial and already often identified before being marketed. Characteristic of type A ADR as follow; toxicity of overdose e.g. liver failure from paracetamol overdose, side effect e.g. urinary retention or sedation during the use of anticholinergic drug, secondary effect e.g. diarrhea from use of broad spectrum antibiotic and drug interaction.

1.2 Type B ADR (Bizarre) is a reaction response to only some people and occurs in patient with sensitivity to medication. Type B ADR is opposite to type A ADR. They have little or no dose-response relationship, acute, unexpected, unpredictable and mortality rate is high. They may be both difficult to study experimental and to detect. Characteristic of type B ADR as follow; hypersensitivity immunological reaction e.g. anaphylaxis from allergy to penicillin and idiosyncratic reaction e.g. aplastic anemia from chloramphenicol.

2. **Immunological classification**

Gell and Coombs classification of clinical hypersensitivity is especially useful for allergic drug reactions.[36-37]

2.1 Type I immediate hypersensitivity reactions are IgE-mediated includes acute urticaria, allergic bronchospasm, angioedema, or anaphylaxis. In the previously sensitized patient, symptoms develop within minutes after drug exposure If IgE antibodies are synthesized de novo during the course of drug treatment, the onset of clinical symptoms is delayed by days to weeks.

2.2 Type II Cytotoxic The most common clinical manifestations of type II hypersensitivity reactions to drug are agranulocytosis, thrombocytopenia and immunoallergic hemolytic anemia. Type II reactions are antibody-mediated. They are caused by cytotoxic antibodies, which are primarily IgM or IgG.

2.3 Type III immune complex type III hypersensitivity reactions e.g. serum sickness Clinical manifestation, which typical appears 10-21 days after administration

of the culpable drug involve tissue injury by immune complexes. This response occurs when the antigen reacts in the tissue spaces with potential precipitating antibodies (mostly IgM), forming microprecipitates in and around small vessels, causing secondary damage to cells. When the antigen is in excess, soluble immune complexes are formed and further deposited in the endothelial lining of blood vessel walls, fixing complement and causing local inflammation. Immune complexes are primarily deposited in the lung, joints, kidneys and the skin.

2.4 Type IV delayed hypersensitivity reaction typically manifest as skin eruptions (contact dermatitis) in response to drugs, cosmetics and environmental chemicals, to which the skin is often exposed. The symptoms usually develop within 2-14 days after exposure to the (drug) allergen depending whether the patient is already sensitized or not. Type-IV reactions are triggered when the drug encounters T-lymphocytes, and is presented to T-lymphocytes by antigen-presenting cell (APCs), which results in lymphocyte stimulation and cytokine release.

Severe Cutaneous Adverse Reaction[8]

Severe cutaneous adverse reactions include Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis and drug reactions with eosinophilia and systemic symptoms also called drug hypersensitivity syndrome. All are probably delayed type immune-mediated reaction.

Stevens-Johnson syndrome and Toxic Epidermal Necrolysis[7, 27]

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe adverse drug reactions. Incidence is rare but they have significant impacts on patient's well being because of high mortality and morbidity rates.[7] SJS was first described in 1922 by two American physicians named Stevens and Johnson. They described an acute mucocutaneous syndrome in two young boys characterized by severe purulent conjunctivitis, severe stomatitis with extensive mucosal necrosis, and 'Erythema multiforme-like' cutaneous lesion. It became known as Stevens-Johnson syndrome and was recognized as a severe mucocutaneous disease with a prolong course and occasional fatalities, and is now known to be an adverse drug reaction and clinically distinct from erythema multiforme. [27] Toxic epidermal necrolysis (TEN) also called Lyell's syndrome was first described by the Scottish dermatologist Alan

Lyell in 1956. He reported four patients with an eruption ‘resembling scalding of the skin objectively and subjectively’ which he called toxic epidermal necrolysis or TEN.[27]

Clinical Features

SJS and TEN are characterized by high fever, wide-spread blistering exanthema of macules and atypical target-like lesions, mucosal involvement is also found especially affecting the mouth, the lips, the conjunctiva and the genitalia.[27, 38] SJS will be considered if less than 10% of the body surface area (BSA) of skin is detached. But if 10-30% of BSA of skin is detached, SJS overlap TEN is likely. While TEN is characterized by more than 30% of BSA detached.[27, 38] Initial symptom of both SJS and TEN begin with a prodromal phase of fever, sore throat and stinging eyes for 1-3 days. Mucosal lesions subsequently appear, follow by cutaneous lesions.[38] The morphology of the skin lesions appear as erythematous, dusky-red macular lesions take on a characteristic gray hue. This process can be very rapid or take several days.[27]

Epidemiology

The incidence of SJS and TEN in European is about 1-6 and 0.4-1.2 cases per million person years respectively.[11] In Thailand, Spontaneous Reporting System (SRS) for adverse drug reaction monitoring shows incidences of SJS and TEN as 0.3 and 0.06 case per million person years respectively.[12] TEN affects woman more frequently than men with a proportion of 1.5:1 and the incidence increase with age.[27]

Treatment

Optimal medical management of SJS and TEN requires early diagnosis, immediate discontinuation of the causative drug, supportive care and specific therapy. In such studies, several treatments, including cyclosporine (3-4mg/kg/day), cyclophosphamide (100-300 mg/day), plasmapheresis, and N-acetylcysteine (2g/6hr) have shown promising results. The use of systemic corticosteroids remains controversial, and it may even increase mortality. In 1998, we reported that commercial preparations of intravenous immunoglobulins (IVIG) contain antibodies against Fas that are able to block the binding of FasL to Fas.[27] Aseptic solutions are

recommended for topical treatment but sulfonamide-based topical remedies are not recommended, because sulfonamides are a known risk factor in the development of severe skin reaction. Antiseptic oral rinses are recommended for treating erosive lesions affecting the oral mucosa.[38]

Drug Hypersensitivity Syndrome

Drug hypersensitivity syndrome (HSS) was described in 1950 by Chaiken et al, as the triad of fever, rash, and multiorgan failure occurring 1-8 weeks after drug had been started. Roujeau and colleague rename the syndrome DRESS; drug rash with eosinophilia and systemic symptoms. Organ failure differentiates HSS from other drug associated eruptions. The incidence of HSS is unknown 1 in 1,000 to 1 in 10,000 for antiepileptic drugs.[39]

Clinical Features

This syndrome commonly begins with a fever shortly followed by a maculopapular rash, which is usually pruritic, and variable degrees of lymphadenopathy. The temperature range from 38^0C and 40^0C with spikes that usually generate a concern of an underlying infection the spiking fever often persists even for weeks despite discontinuation of the culpable drugs. The rash often generalizes into a severe exfoliative dermatitis or erythroderma. There is usually no mucocutaneous involvement, which helps distinguish HSS or DRESS from other forms of severe drug eruptions, such as SJS and TEN.[40] The clinical heterogeneity of HSS makes diagnosis difficult.[39] Involvement of other organs varies, depending on the drug: allopurinol-induced HSS/DRESS has more frequent renal involvement, where as there appears to be a higher risk of hepatic involvement in phenytoin or dapsone-induced disease.[40] Leukocytosis with atypical lymphocyte and eosinophilia of various degrees is also a prominent feature of this symptom. The eosinophilia may often be delayed for 1 to 2 weeks and occur even after elevation in the liver enzyme return to baseline.

Treatment

Early recognition of this syndrome is the most important step in treatment and is essential in improving patient outcomes, because many physicians are not familiar

with this syndrome. The mainstay of treatment is systemic corticosteroid: the usual dosage is prednisolone 40-60 mg/day. Systemic corticosteroids need to be tapered over 6-8 weeks to prevent the relapse of various symptoms of this syndrome.

Erythroderma or Exfoliative Dermatitis

Erythroderma or generalized exfoliative dermatitis defines any inflammatory dermatosis that involves all or nearly all the skin surface (sometimes started as more than 90%). It is a secondary process and represents the generalized spread of dermatosis or systemic disease throughout the skin.[41] Although the disease affect both men and woman, it is more common in men with an average male to female ratio of 2.3:1. The average age at onset is 55 years, although exfoliative dermatitis may occur at any time. The most common cause of exfoliative dermatitis is preexisting dermatoses, drug reaction malignancies and other miscellaneous or idiopathic disorder.[42]

Clinical Features [41]

Exfoliative dermatitis is an uncommon but important dermatological emergency, as the systemic effects are potentially fatal. The condition often develops suddenly, particularly when associated with leukaemia or eczema. A patchy erythema may rapidly spread to be universal within 12-48 hour and be accompanied by pyrexia, malaise and Shivering. Scaling appears 2-6 days later and, at this stage, the skin is hot, red, dry and obviously thickened. The patient experiences irritation and tightness of the skin and feels cold. The exfoliation of scales may be copious and continuous. Scalp and body hair is lost when erythroderma has been present for some weeks. The nail become thickened and may be shed. Pigmentary changes occur and, in those with a dark skin, hypopigmentation is seen.

Treatment [41]

Inpatient treatment and skill nursing care is mandatory. The patient is nursed in a comfortably warm room at a steady temperature (preferably 30-32 ^{0}C) vital sign and fluid electrolyte are regularly monitor. A pressure-relieving mattress is sometimes used. Soothing emollient creams and topical steroids are a mainstay of local treatment and are often adequate. Systemic steroids are life saving in severe cases. The

maintenance of normal haemodynamics, attention to electrolyte equilibrium and adequate nutritional support (particularly with regard to minimizing protein losses) are vital for severely ill patients. Cardiac failure and intercurrent infections are treated as necessary.

Maculopapular Eruption[43]

Maculopapular eruptions, the most frequent of all cutaneous drug reactions, are often indistinguishable from viral exanthems. They are the classic ampicillin and amoxicillin drug rashes, but practically any drug can trigger a maculopapular eruption. Red macules and papules become confluent in a symmetric, generalized distribution that often spares the face. Itching is common. Mucous membranes, palms, and soles may be involved. Fever may be present from the onset. These eruptions are identical in appearance to a viral exanthem and routine laboratory tests usually fail to differentiate the two diseases. Onset is 7 to 10 days after starting the drug but may not occur until after the drug is stopped. The rash lasts for 1 to 2 weeks and fades in some cases even if the drug is continued. Lesions clear rapidly following withdrawal of the implicated agent and may progress to a generalized exfoliative dermatitis if use of the drug is not discontinued. The pathogenesis is unknown.

Clinical Features[43]

The rash begins 5 to 10 days (range, 1 day to 4 weeks) after starting the drug and may occur after the drug is terminated. Latent periods of 2 to 3 weeks are seen with allopurinol, nitrofurantoin, and phenytoin. Eruptions may subside with continued use of the drug and may not recur on repeat exposure. The rash starts on the trunk as a mildly pruritic, red, maculopapular, sometimes confluent eruption and spreads in hours in a symmetric fashion to the face and extremities. The palms, soles, and mucous membranes are spared. Lesions appear confluent in intertriginous areas (axilla, groin, and inflammation skin). Pruritus occurs frequently, and the intensity varies.

Treatment[43]

Stop the offending drug and provide symptomatic relief. Topical corticosteroid creams and cool compresses are soothing and control itching. Treat severe itching or

an extensive eruption with prednisone (0.5 to 1.0 mg/kg/day) for 7 to 10 days. Antihistamines provide sedation but are usually not effective at controlling itching because histamine does not cause maculopapular lesions. Stop treatment of any drug causing a generalized, symmetric, maculopapular rash, and do not retreat with the same drug. Skin-test patients who require ampicillin if the nature of a previous reaction is unknown and there is no adequate substitute drug.

Fix Drug Eruption[43]

Fixed drug eruptions are a unique form of drug allergy that produce red plaques or blisters that recur at the same cutaneous or mucosal site each time the drug is ingested. The clinical pattern and distribution of lesions may be influenced by the drug in question, and the study of the pattern may provide useful information in selecting the most likely causative drug. Tetracycline and co-trimoxazole commonly cause lesions limited to the glans penis. Cases of familial occurrence suggest that a genetic predisposition might be an important causal factor.

Clinical Feature[43]

Single or multiple, round, sharply demarcated, dusky red plaques appear soon after drug exposure and reappear in exactly the same site each time the drug is taken. Lesions may be generalized but typically only a single lesion is present. The lesions are generally preceded or accompanied by itching and burning, the intensity of which is usually proportionate to the severity of the inflammatory changes. Pruritus and burning may be the only manifestations of reactivation in an old patch. The area often blisters and then erodes; desquamation or crusting (after bullous lesions) follows, and brown pigmentation forms with healing. Lesions can occur on any part of the skin or mucous membrane. Lips, hands, genitalia (especially male genitalia), and occasionally oral mucosa are favored sites. Regional lymphadenopathy is absent.

Treatment[43]

Stop the offending drug and provide symptomatic relief. Topical steroid are effective. Erosive lesions can be treated with wet compresses. Drug avoidance prevents recurrent.

Major Histocompatibility Complex

The major histocompatibility complex (MHC) is a region of DNA that encodes a group of molecules that recognize antigen.[44] MHC molecules play an important role in immunity, recognition of tumors and transplantation rejection. [16, 45-46] The MHCs of different organisms have specific names. In humans, the MHC is known as human leukocyte antigen (HLA).[16, 44-46] The MHC in humans (HLA) was subsequently discovered in the early 1950s. Several investigators independently noted that blood from multiparous women or from previously transfused individuals contain antibodies that agglutinated leukocytes.[47-48]

HLAs are group of genes (approximately 200 genes) located on the short arm of chromosome 6. (Figure 1) There are three classes of HLA including HLA classes I, II and III molecule. The HLA class I antigens (HLA-A, HLA-B and HLA-C) are expressed on all nucleated cells and are recognized by CD8+ T cells.[48-49] their structure comprises a large α-chain, and small β-chain known as β_2- microglobulin. The latter is encoded on a different chromosome and shows no sequence variability.[50] However, the HLA classes II antigen (HLA-DR, HLA-DQ and HLA-DP) have a selected tissue distribution, are expressed on the cell surface of the antigen presenting cells, and are recognized by CD4+ T cells.[48-49] Class II HLA molecules are two-chain (α and β) structure; both chains are encode in the HLA locus and are polymorphic.[50] HLA class III molecules which is group of complement genes e.g. C2, C4, factor B and cytokines (such as tumor necrosis factor (TNF).[17] The entire set of HLA-A, -B, -C, -DR, -DQ and -DP antigens encoded on chromosome 6 are called a haplotype.[48] A condition where two allele on other locus are trend to found together in a population at a greater frequency than that predicted simply by the product of their individual gene frequencies are call linkage disequilibrium.[51]

HLA Nomenclature[49]

Nomenclature for both accepted and novel HLA alleles is regulated by the WHO Nomenclature Committee for factors of HLA systems. HLA sequences are officially recorded on the IMGT/HLA Sequence Database (www.ebi.ac.uk/imgt). HLA genes are highly polymorphic. A guide to the most recent nomenclature for HLA antigens and alleles is summarized in table 1, where resolution of HLA alleles to the four-digit level is shown.

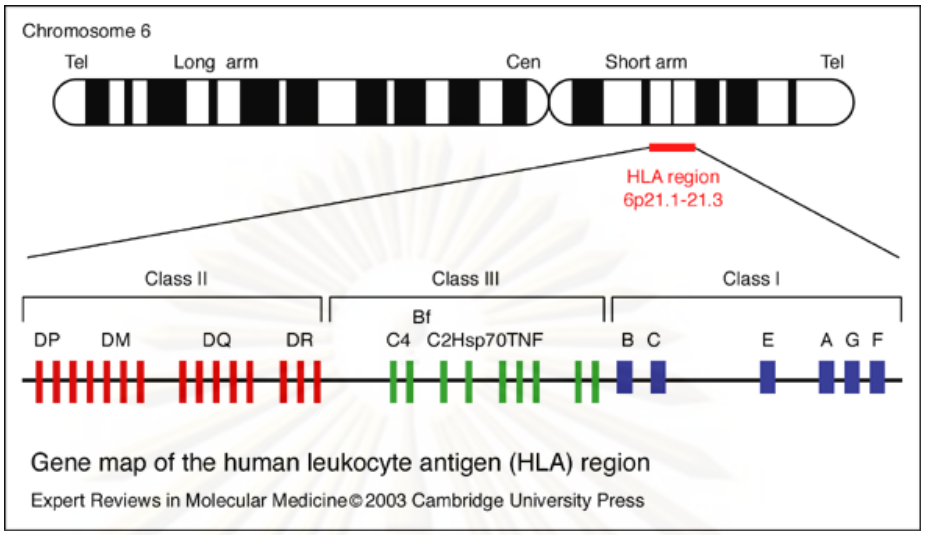

Figure 2.3 The HLA gene complex on the short arm of chromosome 6

Table 2.6 HLA nomenclature basic overview of the level of HLA typing performed in the histocompatibility laboratory.[49]

WHO nomenclature	Interpretation
HLA-B	Identification of HLA locus.
HLA-B58	HLA antigen defined by serology based technique.
*HLA-B*58*	Asterisk denotes HLA alleles defined by analysis of DNA.
*HLA-B*58*	Denotes the allele family.
2-digit resolution	Corresponds where possible to the serological group often term low resolution level used for matching in solid organ transplant.
*HLA-B*5801*	Allele sequence variation results in amino acid
4-digit resolution	substitutions, coding variation, or non-synonymous changes level of matching used in haemopoietic stem cell transplantation.

Codominant Expression [45]

Both HLA classes I and II molecule are codominantly expressed that is, every cell that expresses HLA molecule expresses proteins transcribed from both the maternal and the paternal chromosome. (Figure 2.4) The offspring will also inherit a set of HLA class I plus class II genes from their other parent. Because of the diversity of HLA molecules in the population, it can be almost guarantee that the HLA haplotype contributed by the second parent will differ from haplotype of the first parent. The figure 2 shows that the HLA haplotypes of any offspring will differ from the haplotypes of the parents and generally will differ from the haplotype of the offspring (MHC identity dose occur in monozygotic twins and can occur in family with a large number of children.

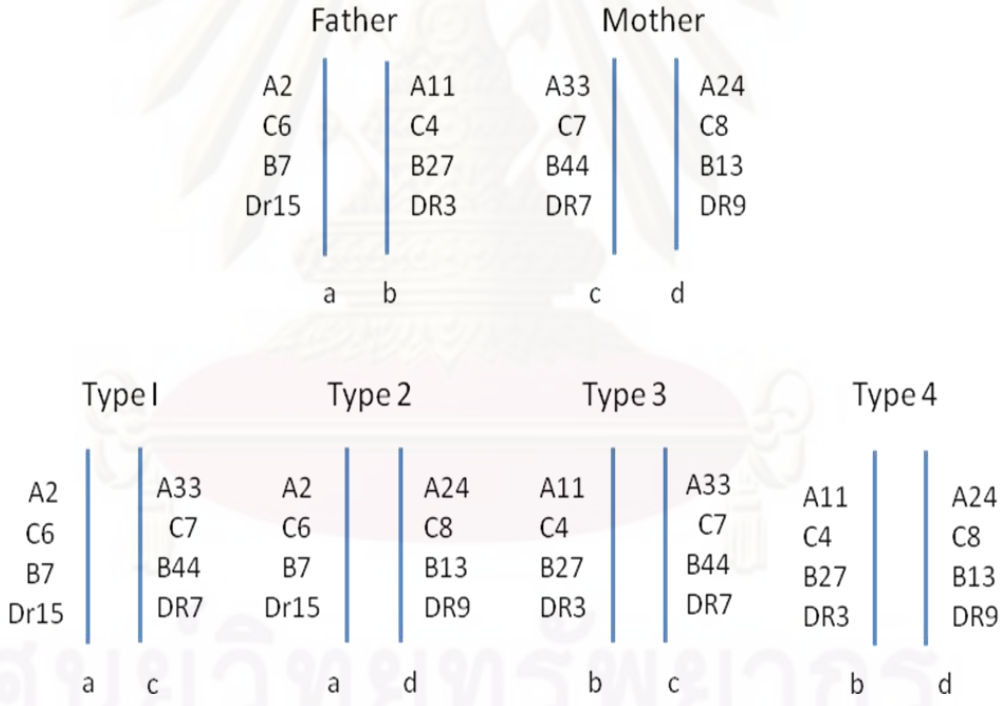

Figure 2.4 A set of HLA genes the HLA haplotype is passes on as a unit from parent to child; because HLA genes are so diverse in the population, the HLA haplotypes of children differ from those of their parents.[52]

Human Leukocyte Antigen Typing

Serological typing [48]

As mentioned earlier, HLA typing for organ transplantation has traditionally been performed serologically using alloantibodies of known HLA specificity to identify unknown cellular antigens. Although serological testing yields only low-resolution typing results, there are some advantages to this method. Serological typing is a relatively rapid method and reveals immunologically relevant epitopes. In addition, serological typing can be used to resolve some ambiguities or to confirm null alleles detect by molecular methods. Serological test include HLA phenotype determination where patient cells are tested with known alloantisera.

Molecular typing [48-49]

Molecular techniques for HLA typing of DNA sequence polymorphisms have largely replaced serology since they offer flexibility of resolution, much improved reproducibility and greater accuracy. The ability to amplify DNA segments by polymerase chain reaction (PCR) has facilitated the application of these techniques. The PCR – base methods can be broadly classified into three categories according to the readout used. First, those that generates PCR products containing internally located polymorphisms that can be identified by a secondary technique, such as PCR-sequence-specific oligonucleotide probes (PCR-SSOP), Sequence-based typing (SBT), or by other techniques involving digestion with restriction enzymes that yield characteristic restriction fragment length polymorphism (RFLP). Second, those in which the poly-morphisms are identified directly by the PCR process, without further steps, such as PCR-Sequence-specific primers (PCR-SSP) The techniques involve three general steps: (1) The extraction of genomic DNA, (2) The amplification of segments of the gene of interest (PCR technique) and (3) The detection of the sequence polymorphisms that define the alleles or allow the distinction of the allele differences.

1. DNA Extraction[48]

Genomic DNA is extracted from nucleated cells, typically using whole blood as the source of nucleated cells. Only a few micrograms of genomic DNA are sufficient to complete molecular typing. DNA purity is an important factor to achieve successful typing results. To amplify short DNA fragments, a salting out method is

adequate. However, to amplify longer fragments, other DNA extraction methods that yield higher purity are usually required.

2. DNA Amplification or Polymerase chain reaction technique [48-49]

The polymerase chain reaction (PCR) is a technique to amplify a specific region of a DNA strand. This technique can increase the number of DNA more than ever, millions of times. A PCR setup requires several component and reagents the components includes: DNA template, primer, Taq DNA polymerase, Photo mix; PCR buffer, deoxynucleotide triphosphate (dNTPs), $MgCl_2$, Glycerol and cresol red. DNA amount will amplify by continuous cycling. Each cycling consist of 3 main step as following.

2.1 Denaturation step: This step is the first regular cycling the reactions temperature approximates 96 ^{0}C. At this temperature, the double strand of DNA is denatured the molecule are separate to two single strand of DNA.

2.2 Primer annealing step: The reactions temperature is lowered to 50–70 ^{0}C allowing annealing of the primers to the single-stranded DNA template Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.

2.3 Primer extension step: The temperature is increased to 72 ^{0}C. At this temperature, Taq DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. This results in duplication of both original DNA strands.

3. Detection of sequence polymorphisms that define alleles [48]

3.1 PCR-Sequence-specific primers (PCR-SSP) [48-49]

PCR-SSP is currently the HLA typing system of choice in most histocompatibility and immunogenetics laboratories. SSP is a rapid method for typing that uses set of primer pairs to amplify specific region of genomic DNA. The efficiency of the amplification reaction is controlled by the primers that amplify conserved sequence of a selected gene. PCR-SSP reactions could be set up in a 96 well plate format with different allele-specific primer sets in each well. Each PCR reaction mixture contains the sequence-specific primers and a set of amplification

control primers. The amplification control primers should yield products for every specimen (except the negative control). The amplification control primers are designed to yield a PCR product of distinct size from the product of the specific allele-specific primers. The PCR-SSP product is visualized by size differences using agarose gel electrophoresis. Electrophoresis through agarose relies on the movement of negatively charge DNA (due to the phosphate backbone) toward s the anode. Fragments of DNA differentially migrate and thus can be identified according to their size. DNA is visualized on the gel by staining with ethidium bromide, which intercollates between the strands of DNA and fluoresces under ultraviolet light.

3.2 PCR-sequence-specific oligonucleotide probes (PCR-SSOP) [49]

PCR-SSOP was the first PCR-based technique used for detecting HLA polymorphism. The technique has advantage over PCR-SSP, in particular a large sample throughput can be achieved; however, the methodology and interpretation of results is complex. In PCR-SSOP, genetic amplification of the target DNA is performed in a PCR. The amplified DNA is next bound to a solid support membrane. Sequence-specific oligonucleotides (SSO) are used to probe the amplified DNA by hybridization to complementary regions on the amplified DNA (A to T and G to C). The probes are labeled with a radioactive biotinylated marker for detection. The resulting pattern is used to interpret the HLA type. There are modifications to the basic PCR-SSOP technique, such as reverse dot-blot and PCR oligocapture assays, but these are not applicable in routine HLA typing.

3.3 Sequence-based typing (SBT) [48-49]

SBT allows a greater resolution of HLA typing than both PCR-SSP and PCR-SSOP techniques. The most accurate procedure and the gold standard for HLA typing is the direct identification of the complete nucleotide sequence of the HLA allele caries by a DNA sample. The most widely used approach to detect the sequencing fragments is the dideoxy chain termination method. The performance of electrophoresis is usually assisted by the use of multiple dyes.

Human Leukocyte Antigen and Drug Hypersensitivity

More than 7% of people have experienced drug hypersensitivity that has significant impact to their lives.[16] Although the incidence of severe cutaneous adverse reactions (SCAR) includes Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug hypersensitivity syndrome (HSS) that are delayed type immune-mediated reaction are rare but they have significant impacts on patient's well being because of high mortality and morbidity rates.[8]

Familial occurrences of severe drug hypersensitivity and cases occurring in identical twins have been reported and suggest that the susceptibility to these idiosyncratic reactions is generally determined. The pathogenesis of drug hypersensitivity reactions is believed to be immune mediated. Eariler in-vitro studies suggest that the drug presentation is MHC class I restricted and there is clonal expansion of CD8+ cytotoxic T cells and these cells induce effector cytotoxic response. This concept is now supported by recent findings of strong genetic association between HLA class I alleles and hypersensitivity reactions to certain specific drugs. Several recent studies present evidence supporting HLA genotype as the major susceptible factor predisposing an individual to develop drug hypersensitivity.[16] Data shown in table 2.7.

Table 2.7 Prior studies of HLA association with drug hypersensitivity.

Culprit drug	Drug hypersensitivity	HLA association
Abacavir	Hypersensitivity reaction	*B*5701* [18]
Allopurinol	Eruption	*Aw33, B17/Bw58* [16]
	SCAR	*B*5801* [8, 21, 24]
Carbamazepine	SJS/TEN	*B*1502* [8, 20, 53-54]
Clozapine	Agranulocytosis	*B38, DR4, DQw3* [16]
Dipyrone	Agranulocytosis	*A24,B7,DQw1* [16]
Gold	Proteinuria, cutaneous reactions, thrombocytopenia	*B8, DR3, DR5* [16]
Hydralazine	SLE	*DR4* [16]
Levamisole	Agranulocytosis	*B27* [16]
Lamotrigene	SJS/TEN	*B*38* [8]
Oxicam NSAIDs	SJS/TEN	*A2,B12* [16] *B*73* [8]
Methazolamide	SJS with ocular involvement	*B59* [16]

Culprit drug	Drug hypersensitivity	HLA association
Nevirapine	Hypersensitivity reaction	*DRB1*0101,Cw8-B14* [16]
		*B*3505* [55]
Penicillamine	Penicillamine toxicity	*DR3* [16]
Sulfonamides	SJS/TEN	*A29, B12, DR7* [16]
		*B*38* [8]

SCAR, Severe cutaneous adverse reaction, SLE, Systemic lupus erythematosus

CHAPTER III

MATERIAL AND METHODS

Materials

1. Apparatus

1.1 Centrifuge (Nanofuge Hoefer)	Hoefer	USA
1.2 Centrifuge (Sorvall GLC-2)	Sorvall	Germany
1.3 Combimix-x3 Baxter	Baxter	USA
1.4 DC Power Supply (PS 500XT)	Hoefer	USA
1.5 DNA Thermal Cycler(GeneAmp 9600)	Perkin Elmer	USA
1.6 Dri-Bath (Thermolyne 16500)	Barnstead/Thermolyne	USA
1.7 Kubota 3700 Refrigerate centrifuge	Kubota	Japan
1.8 Gel Electrophoresis System	BRL	USA
1.9 Microwave	National	Japan
1.10 Multichannel Finnpipette	Labsystems	Finland
1.11 Pipetman (P10, 20, 200, 1000)	Gilson	France
1.12 Plastic Sealer (Krups Vacupack 2)	Krups	Sweden.
1.13 Plate Form Rotator (TPM-2)	Sarstedt	Germany
1.14 Shaking Water Bath (Haake SWB 20)	Haake	Germany
1.15 Spectrophotometer (UV 160A)	Shimadzu Corporation	Japan
1.16 Transluminator (FD-33002, UV300)	Fotodyne	USA
1.17 Vortex Mixer (Genie 2)	Scientific Industries INC	USA

2. Supplies

2.1 Disposables Nitrite Groves	TNT™ Blue	Malasia
2.2 Glass test tube 12 × 75 mm.	Pylex	USA
2.3 Micro tube (1.5 ml)	Treff AG.	Switzerland
2.4 Pipette tip (Blue and Yellow)	Scientific Plastics	USA
2.5 Plastic plate	TM Medipak	Thailand
2.6 Polaroid Film	Polaroid	UK
2.7 Para film	American National Can™	USA
2.8 Sterile Plastic Pipette	TM Medipak	Thailand

3. Reagent and Chemical

3.1 Absolute ethanol	Merck	Germany
3.2 Agarose (ultra PURE)	Gibco BRL	USA
3.3 Bromphenol blue	Sigma	USA
3.4 Ethidium bromide	Sigma	USA
3.5 Guanidine HCl	Boehringer Mannheim	Germany
3.6 dATP, dTTP, dGTP, dCTP	Boehringer Mannheim	Germany
3.7 Magnesium chloride ($MgCl_2$)	Sigma	USA
3.8 Phi X 174 DNA RF Hae III Digest	Biolabs	USA
3.9 Proteinase K	Boehringer Mannheim	Germany
3.10 10 X PCR buffer	Invitrogen	Japan
3.11 Sodium Dodecyl Sulfate (SDS)	Sigma	USA
3.12 Taq DNA polymerase	BRL	USA

4. Medical equipment

4.1 Sterile plastic Syringe (5 ml)

4.2 Needles No. 20

4.3 6 ml EDTA containing tube BD Vacutainer®

4.4 Others: Gauze, Micropore, Alcohol, etc.

Method and Patients

Part 1: Prevalence and Mortality rate of severe cutaneous adverse reaction.

A retrospective study design was used. The protocol was approved by Siriraj Institutional Review Board (SIRB), Siriraj Hospital, Mahidol University. Five years retrospective data, during 2003-2007, were reviewed using electronic database of Adverse Drug Reaction Monitoring Center, Siriraj Hospital. Both inpatients and outpatients who were diagnosed by dermatologists to be SJS, TEN and HSS were included. The following data were collected from electronic database; 1) demographic data; 2) causative drugs; 3) prevalence of SJS, TEN and HSS; 4) onset time of symptom after causative drug had been administered; 5) duration of hospitalization; and, 6) clinical outcomes. Prevalence of SCAR was calculated using the number of patients who experienced SCAR compared to the total number of patients who received the drugs during 5 years (the later data were collected from Siriraj Computer Center, Siriraj Hospital). Data collected were complied on Microsoft excel sheet and subjected to descriptive statistical analysis.

Part 2: HLA-B and HLA-C locus genetic polymorphism as a marker of severe cutaneous adverse reactions in Thai patient on allopurinol.

Method

A case-control study design was used. The protocol was approved by Siriraj Institutional Review Board (SIRB), Siriraj Hospital, Mahidol University. Blood samples (5 ml) were obtained. The samples were collected in tubes containing EDTA as the anticoagulant. All whole blood samples were stored at 2-8 ^{0}C not more than 2-3 days and testing for HLA genotype by polymerase chain reaction sequence specific primer method (PCR-SSP) and confirmed *HLA-B*5801* allele by One Lambda test kid 57/58 subtype.(One Lambda, Inc., USA)

Sample Size Calculation

The sample size calculation is divided into case and the control group.

Shuen-Lu Hung et al.[21] reported that *HLA-B*5801* allele was found 15% of allopurinol tolerant control patients whereas in Thai population the frequency of *HLA-B*5801*allele was found approximately 8.4 percent.[25]

Risk of SJS and TEN in patients with *HLA-B*5801* allele was 580 times higher (OR=580). In case-control study, approximately 4 times of odds ratio should be used for sample size calculation. [56] In this study, 5 times of odds ratio had been used to calculate the sample size.

$$n = \frac{[\, Z_{\alpha} \sqrt{2\,p\,(1-p)} + Z\,\beta \sqrt{p_1\,(1-p_1) + p_2\,(1-p_2)}\,]^2}{(p_1 - p_2)^2}$$

n refers to the study population in each group.

P_1 = expected rate that interested factor found in control population, $P_1 = 0.08$

P_2 = expected rate that interested factor found in case population

$P_2 = P1 \times OR / [1 + P_1 (OR-1)] = 0.08 \times 5 / [1 + 0.08 (5-1)] = 0.303$

$P = (P_2 + P_1) /2 = (0.303 + 0.08) /2 = 0.19$

$\alpha = 0.05$ (one-sided); $Z_{\alpha} = 1.645$ $\beta = 0.2$ (two-sided); $Z_{\beta} = 0.84$

$$n = \frac{[1.645\sqrt{2(0.19(1-0.19)} + 0.84\sqrt{0.08(1-0.08) + 0.30(1-0.30)}\,]^2}{(0.08\text{-}0.30)^2}$$

$n = 27.91$

Population in the study group (n_1) and the control group (n_2), $n_1 / n_2 = k$ in this study requires $k = 1/2 = 0.5$ using the following formula.[57]

$n_2 = ½\ n\ (1+1/K)$ and $n_1 = ½\ n\ (1+K)$

$n_2 = ½\ (28)\ (1+1/0.5) = 42$

$n_1 = ½\ (28)\ (1+0.5) = 21$

This study required case at least 21 patients while 42 patients were required in the control group therefore, the least total participants required in the study was 63 patients.

Patient Selection

Case

Patient with adverse drug reactions from allopurinol whose data had been recorded in the database of Adverse Drug Reaction Monitoring Center, Siriraj Hospital, using ICD-10 computerized system (L51.1 Stevens-Johnson syndrome and L51.2 toxic epidermal necrolysis) and fulfilled the following inclusion criterias were included into the study: both inpatients and outpatients who were diagnosed by dermatologists to be SJS, TEN, HSS, and other rash from allopurinol; willing to be included in the study and signed the informed consent form. Thirty-four patients with adverse reaction from allopurinol were recruited into the study.

Control

Patients using allopurinol without adverse drug reactions who were outpatients at Siriraj hospital, Bangkok during December 2009 to March 2010 were screened into the study. The inclusion criteria were as followed: use allopurinol for more than 6 months with no evidence of adverse drug reactions from allopurinol willing to be include into the study and signed the informed consent form.

Step of testing HLA genotype by sequence specific primer method

The HLA genotype was performed in a protocol design by Department of Transfusion Medicine Siriraj Hospital as follow.

Step1. Prepared primer to detect specific alleles

Step2. DNA isolation

Step3. Optical Density Measurement

Step4. Polymerase Chain Reaction method

Step5. Gel electrophoresis

Step6. Interpreted HLA allele by key HLA-B and HLA-C

Step7. Confirm *HLA-B*5801* allele by using a DNA detection kit 57/58 (One lambda, Inc., USA)

1. Primer to detect specific allele

Primer to detect specific alleles in this study was modified from Bunce et al. for PCR-SSP technique.[58]

2. DNA Isolation

Genomic DNA was isolated from lymphocytes obtained from 5 ml of EDTA blood. The DNA was prepared by an improved salting-out method as in the following steps.

2.1 Red cell lysis: add 30 ml of Solution A in whole blood and mix for 10 minutes.

2.2 Centrifuge the tube of whole blood at 2000 g for 10 minutes.

2.3 Blood from centrifuge separate into two sections. Discard the supernatant (red cell lysis) and transfer lymphocyte that is precipitated at the bottom of tube to microtube.

2.4 Add 1.5 ml of solution A to microtube and centrifuge.

2.5 Centrifuge at 6400 rpm for 2 minute. Discard the supernatant (Repeat this step until all RBCs are lysed however, does not repeat this step more than 3 times).

2.6 Vortex the pellet to prevent clumping. Approximate the pellet size and add the appropriate volumes of reagents as listed in the follow chart

Pellet size	**100-50 µl**	**50-25 µl**	**25-10 µl**
Proteinase K	40 µl	20 µl	12 µl
ddH_2O	800 µl	400 µl	300 µl
10% SDS	300 µl	150 µl	105 µl
7.5 M Guan.HCl	300 µl	150 µl	105 µl
Ethanol precipitation			
Absolute Ethanol	4.0 ml	2.0 ml	1.0 ml

2.7 Add Proteinase K. Vortex the sample.

2.8 Add ddH_2O.Vortex the sample again.

2.9 Add 10%SDS. Mix the sample gentle by rocking the tube back-and-forth.

2.10 Add 7.5 M Guanidine HCl. Again, mix the sample gently.

2.11 After 10 minutes, mix the sample vigorously using pipettes until the mixture becomes homogeneous. Try to avoid creating bubbles while mixing with pipettes.

2.12 Incubate the sample at 68-70^{0}C for 10 minutes.

2.13 After 10 minutes, spin the sample at 14,000 rpm for 4 minutes at 4^{0}C

A. If the pellet is compact and the supernatant is clear and free of debris, continue to the next step

B. If the pellet is diffuse and the supernatant is cloudy, repeat step 12-14

2.14 Transfer the supernatant to the appropriate labeled tube by decanting or pipetting. Slowly and appropriate volume of ethanol to maintain the interface between the two phases. Gently rock the tube back-and forth until cotton-like strands of DNA appear.

2.15 Vortex the sample to tighten the pellet. Transfer the DNA to another labeled 1.5 ml microtube by drawing 800 µl of DNA-ethanol using a blue pipette-tip

2.16 Spin at 10,000 g for 2 minutes. Discard the alcohol supernatant.

2.17 Add 500 µl of 80% ethanol to the sample, vortex to loosen the pellet and let the sample stand for 1 minute.

2.18 Spin the sample at 10,000 g for 2 minutes. Discard as much of the supernatant as possible.

2.19 Add 200 µl of TE buffer to the sample. Vortex and incubate at 68-70^{0}C for 5 minutes with the cap open to evaporate the ethanol.

2.20 Cap the tube and vortex the sample. If the sample is viscous, add 200 µl of TE buffer and incubate for 2 minutes. Continue this procedure until a smooth, syrup-like consistency is achieved.

3. Optical Density Measurement

After DNA isolation from process I should bring a sample to measure the amount and quality of DNA by OD measurement. These steps should be done with spectrophotometer as following.

3.1 Dilute a sample of DNA isolation from step 1 in 1:100 concentrations, by using DNA 10 µl add ddH_2O 990 µl.

3.2 Prepare ddH_2O 1 ml for control.

3.3 Set spectrophotometer measure OD at 260 and 280 nm.

3.4 Calculate OD 260/280 ratio to observe purity and estimate concentration of DNA following this formula.

DNA concentration in µg/ml or ng/µl = OD260 x 50 x dilution factor

4. Polymerase Chain Reaction Techniques; PCR

The polymerase chain reaction (PCR) is a technique to amplify a specific region of a DNA strand. This technique can increase the number of DNA more than ever, millions of times. A PCR setup requires several component and reagents the components includes: DNA template, primer, Taq DNA polymerase, Photo mix; PCR buffer, deoxynucleotide triphosphate (dNTPs), $MgCl_2$, Glycerol and cresol red. DNA amount will amplify by continuous cycling. Each cycling consists of 3 mains step as following denaturation, primer annealing, primer extension step. Thermal cycle condition as following.

Step	Number of cycling	Temperature(^{0}C)	Time
1	-	96	2 min
2	5	96 70 72	25 sec 45 sec 45 sec
3	21	96 65 72	25 sec 50 sec 45 sec
4	4	96 55 72	25 sec 1 min 2 min
5	-	4	-

Mixture of PCR reaction

1. Prepare photomix 250 µl/test.
2. Add Tag DNA polymerase 2.8 µl/test.
3. Add DNA concentration about 100-125 µg/ml calculated from DNA concentration measure by OD measurement.
4. Add ddH_2O up to 150 µl.

5. Gel Electrophoresis

The analysis of productivity from PCR technique in this study is gel electrophoresis. Gel electrophoresis is a technique use for the separate for DNA molecule using an electric field applied to a gel matrix. DNA fragment are separated by size as they move through a gel matrix.

Step of gel electrophoresis as following

5.1 Weigh 1.95 g of agarose into a conical flask. Add 130 ml of 1XTBE, swirl to mix.

5.2 Microwave for about 2 minute to dissolve the agarose.

5.3 Leave it to cool on the bench for 10 minutes down to about 60^0C.

5.4 Add 13 µL of ethidium bromide and swirl to mix.

5.5 Insert the comb and pour the gel slowly into the gel tray.

5.6 Leave to set at room temperature for at least 30 minutes.

5.7 Pour 1XTBE buffer into gel chamber to submerge the gel to 2–5 mm depth. After the gel has solidified enter gel in the chamber with 1XTBE solution and load the first lane with marker and other lane with DNA.

5.8 The gels were run for 32 minutes when adequate migration has occurred, DNA fragments are visualized by staining with ethidium bromide using UV illumination and compared with the size marker ΦX174 phage DNA digested by Hae III.

5.9 Photographs of the gels were recorded.

6. Interpretation of HLA allele

*HLA-B*5801* allele positive when specific band had positive in lane 15 (mix 59, ampicon size 374) and lane 44 (mix 93, ampicon size 421) and *HLA-Cw*0302* allele positive specific band in lane 47 (mix C4, ampicon size 206).

Table 3.1 Show details of HLA allele detection in this study

Lane	Mix	Locus	Alleles amplified	Amplicon size
1	35	B	B*07021-023/ 04/ 07/ 09/ 11-12, B*5603	405
2	36	B	B*0705-06, B*4201-02, B*5504/08, B*5605, B*8101	405
3	37	B	B*0801/ 04-08N, B*4101-03, B*4201-02	564
4	40	B	B*4901, B*5115, B*5901	385
5	43F	B	B*1501/ 03-07/ 12/ 14/ 19-20/ 24-27/ 30/ 32-35	379
6	44.2	B	B*4406	545
7	46	B	B*2701, B*4402/ 031-032/ 04/ 07-08/ 11	383
8	47	B	B*1301-04, B*1536, B*2701, B*44031-032/ 07/ 10	504
9	50	B	B*1546, B*3519, B*4002-06/ 08-09/ 011/ 13-16/ 18-20, B*4101-03, B*4402/ 031-032/ 04-05/ 07/ 09-11, B*4501-02, B*4701-03, B*4901, B*5001-02	566
10	52	B	B*40011-012/ 07	607
11	52A	B	B*1533, B*40011-12/ 02-06/ 09-12/ 14-16/ 18-20, B*4101-03, B*4801/ 03-05	465
12	53	B	B*1401-04	389
13	54	B	B*1402-03/ 05, B*3526, B*3904	182
14	58	B	B*3801/ 021-022/ 03, B*39011/ 013/ 021-022/ 03, B*3904-05/ 061-062/ 07-15, B*67011-012	612
15	**59**	**B**	**B*5705, B*5801-02**	**374**
16	60	B	B*5701-04	351
17	62	B	B*2714, B*39061-062, B*5501-03/ 05, B*5601/ 05, B*5901, B*7301	422
18	63	B,Cw	B*4501, B*5001-02, B*5401, B*5501-03/ 05/ 07, B*5601-02/ 04, B*8201 Cw*1507	383
19	64	B	B*5508, B*5601-05	551
20	65	B	B*5401, B*5507	421
21	66.1	B	B*2702-04/ 052-053/ 06-11/ 13-14	149
22	67	B	B*1517, B*2701-02/ 04/ 052-053/ 08/ 10/ 12-14, B*3702, B*4701-03	437
23	68	B	B*3701, B*4406, B*5108	606
24	69	B	B*3701, B*3803, B*39021-022/ 08/ 13, B*4502	422
25	72	B	B*4012, B*4801/ 03-04/ 06, B*8101	567
26	72A	B	B*07021-023/ 03-06/08-11/ 13, B*0801-08N, B*1405, B*3903/14, B*4201-02, B*4801/04-06	495
27	73	B	B*1516-17	516
28	74	B	B*1304, B*1501101/ 01102N/ 012/ 02/ 04-08/ 11-16/ 19-21/ 24-28/ 31-36/ 38-40/ 43-45/ 50, B*4601, B*5701	477

Lane	Mix	Locus	Alleles amplified	Amplicon size
29	75	B	B*1304, B*1501101/ 01102N/ 012/ 03-07/ 12/ 14/ 19-20/ 24-27/ 32-36/ 38-40/ 43/ 46-47/ 49-50, B*3528, B*4003/ 20, B*4802	421
30	76	B	B*1301, B*1502/ 13/ 20-21/ 25/ 36/ 44, B*4408, B*5705	420
31	78	B	B*0709/ 11, B*1503/ 18/ 23/ 29/ 47/ 49, B*3525-26, B*3907, B*4802, B*5603	486
32	79	B,Cw	B*1503/ 09-10/ 18/ 23/ 29/ 37, B*3525, B*4802, Cw*0703	691
33	80	B	B*0710, B*1510/ 18/ 21/ 23/ 37/ 44, B*3526, B*3907/ 15	415
34	81.1	B	B*1514, B*4408	637
35	81.2	B	B*1512, B*1519	636
36	82	A,B	A*2501-02, A*2601-06/09/11N/12, A*3401 A*6601-03, A*68011-012/ 02/ 031-032/ 04-07, B*1508/ 11/ 15/ 22, B*3514, B*5603	553
37	83	B	B*1522, B*1801-05/ 07, B*3501-04/ 07-14/ 18/ 20-24/ 28, B*7801-04	128
38	84.1	B	B*3501/ 03-092/ 11/ 14/ / 15/ 17-19/ 21/ 23-25/ 27, B*5301-03	389
39	85	B	B*0708, B*0807, B*1508/ 22/ 29, B*1807, B*3501/ 03/ 05/ 07-08/ 11/ 14-15/ 17/ 19/ 21/ 23-25/ 27, B*5301-03	416
40	88	B	B*51011/ 012/ 021/ 022/ 03-09/ 11N/ 12/ 14/ 16, B*52011-012	401
41	89	B	B*51011/ 021/ 03/ 07-14/ 16, B*52012, B*5605, B*7801/ 022/ 03	487
42	91	B	B*1807, B*3521, B*51011/ 012/ 03-04/ 06/ 08-09/ 11N-14/ 16, B*5302, B*7801/ 021/ 022	588
43	92	B	B*15012, B*52011/ 012	440
44	**93**	**B**	+/-Bw4: B*0802-03, B*1301-04, B*1513/16/17/23/24/36/43, B*2701-07/09-14, B*3701-02, B*3801-03, B*4013/19, B*4402-08/10/11, B*4701/03, B*4901, B*5101-16, B*52011-012, B*5301-03, B*5701-05, **B*5801-02**, B*5901	**421**
45	94	B	+/-Bw6: B*07021-023/03-10/ 12-13, B*0801-08N, B*1401-05, B*1501-12/14-15/18-22/25-35/37-40/44-50, B*1801-05/07, B*2708/12, B*3501-26/28, B*3901-15, B*4001-16/20, B*4101-3, B*4201-2, B*4409, B*4501-02, B*4601, B*4702-03, B*4801-06, B*5001-02, B*5401, B*5501-05/07-08, B*5601-05, B*67011-012, B*7301, B*7801-04, B*8101, B*8201	404
46	95	B	B*4601	460
47	**C4**	**C**	**Cw*0302**	**206**
48	NC		Negative Control	

Statistical Analysis

Corrected data were analyzed by using SPSS statistical package 17.0 for windows. The statistical analysis for prevalence and mortality rate of severe cutaneous adverse reactions was performed by descriptive analysis. The statistical analysis between cases and controls for the clinical characteristics was performed by descriptive, fisher exact test and non parametric test. Dichotomous variables (presented as frequency, with percentage).Continuous variables (presented as mean with standard deviations). The strength of association was estimated by calculating the odds ratio and 95% confidence interval. Odds ratios were calculated with Haldane's modification, which add 0.5 to all cells to accommodate possible zero count.[59] All P values were two tailed and P values of less than 0.05 were considered to indicate possible statistical significance. A multivariate logistic regression using creates model predicted probability of allopurinol hypersensitivity.

Diagnostic Tests [60]

HLA-B*5801	**Case**	**Control**	**Total**
Positive	**a** true positive (case and positive *HLA-B*5801*)	**b** false positive (control but positive *HLA-B*5801*)	a+b
Negative	**c** false negative (case and negative *HLA-B*5801*)	**d** true negative (control and negative *HLA-B*5801*)	c+d
Total	a+c total number of case who had adverse drug reaction from allopurinol	b+d total number of allopurinol tolerant control	a+b+c+d

Calculate the proportion of patients with SCAR who also have *HLA-B*5801* positive. That calculation goes: a/(a+c). By convention, we refer to that property of "positivity in the SCAR" as ***Sensitivity***.

Calculate the proportion of patients who are allopurinol tolerant control and *HLA-B*5801* negative. That calculation goes: d/(b+d). By convention, we refer to that property of "negativity in the allopurinol tolerant control" as ***Specificity***.

Calculate the proportion of patients with *HLA-B*5801* positive who also have SCAR. That calculation goes: a/(a+b). By convention, we refer to that property of "SCAR among positives" as ***Positive Predictive Value*** **(PPV)**.

Calculate the proportion of patients with *HLA-B*5801* negative who also are allopurinol tolerant control. That calculation goes: d/(c+d). By convention, we refer to that property of "allopurinol tolerant control among negatives" as ***Negative Predictive Value*** **(NPV)**.

CHAPTER IV
RESULT

Results are presented in two parts: 1) Prevalence and mortality rate related to SCAR, which were from drug exposure during 2003-2007 of patients at Siriraj Hospital 2) Association between *HLA-B*5801* and *HLA-Cw*0302* alleles to severe cutaneous adverse reaction including other types of cutaneous reactions caused by allopurinol in Thai patients.

Part 1: Prevalence and mortality rate related to SCAR

Demographic data

SCAR was found in 136 patients. Most patients were hospitalized, (81.62%). The proportion of female and male was not different (1.2:1). The mean age was 46.68 ± 20.50 years old (range 4 months – 88 years). It was the fact that adults experienced more frequently adverse drug reaction rather than children. SCAR in adults was not different among age group. The data are summarized in Table 4.1, ;84 cases with SJS (61.76%); 3 cases with SJS overlap TEN (2.21%); 10 cases with TEN (7.35%) and 39 cases with HSS (28.68%).

Table 4.1 Demographic data of patients with SCAR (n=136)

Demographic data	SJS*N(%)	TEN N(%)	HSS N(%)	SCAR N(%)
Number of patients	87(63.97)	10(7.35)	39(28.68)	136(100)
Type of patients				
Outpatient	23(26.44)	0(0)	2(5.13)	25(18.38)
Inpatient	64(73.56)	10(100)	37(94.87)	111(81.62)
Gender Female	51(58.62)	5(50.0)	18(46.15)	74(54.41)
Male	36(41.38)	5(50.0)	21(53.85)	62(45.59)
Age (years, mean ±SD)	(48.21±18.43)	(51.1±18.79)	(42.1± 24.69)	(48.8±20.50)
median	46	41	44	45
0-12	1(1.15)	0(0)	5(12.82)	6(4.41)
12- 20	4(4.60)	0(0)	4(10.26)	8(5.88)
21-40	27(31.03)	5(50.0)	8(20.51)	40(29.41)
41-60	28(32.18)	1(10.0)	12(30.76)	41(30.14)
over 60	27(31.03)	4(40.0)	10(25.64)	41(30.14)
Number of dead	6(6.90%)	5(50.0%)	5(12.82%)	16(11.76%)

**SJS and SJS overlap TEN*

Drugs with high prevalence as the cause of SCAR

SCAR was found most frequently in anticonvulsant drug group (34.56%); the second and the third were antimicrobial (25.74%) and anti-gout (14.70%). Drug groups frequently found to be the cause of SCAR was shown in table 4.2. The top five drugs most frequently reported to be the cause of SCAR were phenytoin, allopurinol, cotrimoxazole, carbamazepine and nevirapine & phenobarbital. The highest prevalence of SJS, TEN and HSS were found with carbamazepine, allopurinol and phenytoin which the rates were 3.26, 0.21 and 2.64 per 1000 patients respectively, as shown in table 4.3.

Table 4.2 Drugs of causing SCAR

Causative drug	SJS*	TEN	HSS	Total
Anticonvulsant	21	1	25	47 (34.56)
Carbamazepine	9(1*)	0	1	11 (8.09)
Phenytoin	9	1	19	29 (21.32)
Phenobarbital	1	0	5	6 (4.41)
Sodium valproate	1	0	0	1 (0.74)
Antimicrobial	29	4	2	35 (25.74)
Sulfonamide	14(1*)	0	2	17 (12.50)
Penicillin	4	0	0	4 (2.94)
Cephalosporin	2	1	0	3 (2.20)
Carbapenem	1	1	0	2 (1.47)
Quinolone	5	1	0	6(4.41)
Glycopeptides	1	0	0	1 (0.74)
Lincosamide	0	1	0	1 (0.74)
Misc.(dapsone)	1	0	0	1 (0.74)
Allopurinol	13	2	5	20 (14.70)
Antiviral	5	0	1	6 (4.41)
Nevirapine	5	0	1	6 (4.41)
NSAIDs	3	0	1	4 (2.94)
Dipyrone	1	0	0	1 (0.74)
Ibuprofen	1	0	1	2 (1.47)
Mefenamic acid	1*	0	0	1 (0.74)
Total of five groups	71	7	34	112 (82.35)
Others	16	3	5	24 (17.65)
Total	87	10	39	136 (100)

include SJS overlap TEN

Table 4.3 Top five high risk drugs with prevalence of SCAR

Causative drug	SJS*	TEN	HSS	SCAR
Phenytoin	1.25	0.14	2.64	4.03
Carbamazepine	3.26	0	0.33	3.59
Nevirapine	2.79	0	0.56	3.35
Cotrimoxazole	2.77	0	0.37	3.14
Allopurinol	1.39	0.21	0.53	2.13
Phenobarbital	0.29	0	1.44	1.73

* *Prevalence 1:1,000 patients using the causative drug in 5 years*

Onset of symptoms, duration of hospitalization and mortality rate

Mean onset time of SCAR after the administration of causative drug was 20.12 ± 15.98 (median, 16) days (range 1 to 98 days). Mean onset times of SJS, SJS overlap TEN, TEN and HSS, were 18.29 ± 13.38 (median, 15) days, 12 ± 8.54 (median, 13) days, 13.50 ± 10.84 (median, 12) days and 26.24 ± 20.39 (median, 23) days respectively. Mean duration of hospitalization was 20.69 ± 22.71 (median, 13) days. Mean duration of hospitalization when categorized by event; SJS, SJS overlap TEN, TEN and HSS, were 18.13 ± 14.57 days (median, 12), 21.00 ± 19.15 days (median, 12), 22.50 ± 11.77 days (median, 23) and 24.60 ± 34.31 (median, 13) days respectively. HSS showed longer period of hospitalization (range 4 to 185 days). Mortality rate of SJS, TEN and HSS were 6.90%, 50.0% and 12.82% respectively. Twenty-five percent of all death cases were related to allopurinol, the highest mortality generator, the details are in table 4.4.

Table 4.4 Mortality and the causative drug

Age/Sex	Causative drug	Reaction	Complications	Cause of death
79/F	Allopurinol	SJS	Metabolic acidosis	Septic shock
83/F	Allopurinol	SJS	Septicemia	Pneumonia with septic shock
78/M	Allopurinol	SJS	Respiratory failure ARF	VAP with septic shock
42/M	Allopurinol	TEN	ARSD, ARF, VAP, DIC, Septicemia	Septic shock
31/M	Isoniazid	TEN	Acute hepatitis	fulminant hepatic failure
24/F	Isoniazid	HSS	Hepatic encephalopathy, DIC, Hypernatremia, GI bleeding	fulminant hepatic failure
77/F	Carbamazepine	SJS	ARF, Metabolic acidosis, Hyperphosphatemia, UTI	Septic shock
69/F	Cefotaxime	SJS	Pulmonary collapse, Plural effusion, Septicemia	Septicemia
75/F	Clindamycin	TEN	ARF, pneumonia, Hepatic failure, DIC, GI bleeding	Septic shock
51/M	Dipyrone[$]	HSS	Septicemia	Multiple organ failure
65/F	Ibuprofen	HSS	Respiratory failure, DVT, severe pneumonia	Respiratory failure, Septic shock
76/F	Imipenam+cilastatin	TEN	Pneumonia	Septic shock
1/M	Phenobarbital	HSS	Pulmonary edema, DIC Electrolyte imbalance	DIC, septic shock
79/F	Phenytoin	HSS	HAP, Acute pyelonephritis	HAP
40/F	Propylthiouracil	TEN	DIC, pneumonia, Acute diarrhea	Septic shock
29/M	Vancomycin	SJS	Meningitis	Brain hemiation hydrocephalus

[$] *Secondary exposure; VAP,Ventilator-associated pneumonia; ARF, Acute renal failure; ARSD, Acute respiratory distress syndrome; DIC, Disseminated intravascular coagulation; HAP, Hospital acquired pneumonia; DVT, Deep vein thrombosis; UTI, Urinary tract infection*

Part 2: Association between *HLA-B*5801* and *HLA-Cw*0302* alleles to severe cutaneous adverse reaction including other types of cutaneous reactions caused by allopurinol in Thai patients.

Demographic data

There were 82 patients participated in the study which performed during December 2009 – March 2010, 34 out of the 82 patients were recruited from patients who had adverse drug reaction from allopurinol. Within these 34 patients, severe cutaneous adverse reaction was found in 25 patients and other cutaneous reaction was found in 9 patients. Other 48 patients were recruited from patients with allopurinol tolerant control. Gender condition was also noticed, female had 11.78 times higher risk compared with male in experiencing SCAR from allopurinol (95%CI = 2.87 to 48.29, P-value < 0.001). In the same direction, 94% of allopurinol tolerant patients were male. Average duration from the start of using allopurinol until the onset of adverse drug reaction was approximately 3 weeks. Patient with hyperuricemia and patients who no dosage adjustment base on creatinine clearance had 12.93 times (95% CI = 2.52 to 66.32, P-value < 0.001) and 8.66 times (95% CI = 2.84 to 26.45, P-value < 0.001) and higher risk of SCAR when compare with allopurinol tolerant control. Patients with history of drug allergy had 2.35 times (95% CI = 0.85 to 6.57 P-value = 0.097) higher risk to SCAR. Moreover, we found that patients with underlying disease of diabetes mellitus and chronic renal insufficiency had 7.059 times (95% CI = 1.673 to 29.77, P-value = 0.006) and 4.2 times (95% CI = 1.41 to 12.46, P-value = 0.008) higher risk of SCAR respectively, as detailed in table 4.5 and type of skin reaction was shown in table 4.6.

Onset of symptoms and duration of hospitalization

Mean onset time of rash after the administration of allopurinol was 24.06 ± 17.59 days (range 1 to 85 days). Mean onset times of SJS, TEN HSS and other rash were 20.43 ± 8.52 days, 8 days, 30.88 ± 16.82 days and 24.85 ± 29.45 days respectively. Most patients 70.59% were hospitalized. Mean duration of hospitalization was 24.06 ± 17.59 days. Mean duration of hospitalization when categorized by event, i.e., SJS, TEN, HSS and other rash were 21.61± 20.13 days, 31 days, 10.25 ± 1.75 days and 16.0 ± 8.0 days respectively.

Table 4.5 Demographic data in study population

	Allopurinol tolerant control (C) (n=48)	Allopurinol induced skin reactions			P-value		
		SCAR (A) (n=25)	Other skin (B) (n=9)	Total (A+B) (n=34)	(C) VS (A)	(C) VS (B)	(C) VS (A+B)
Characteristic							
Age (years, mean ±SD	60.25 ± 12.52	63.96 ± 14.89	75.77±3.52*	67.08±13.86*	0.141	< 0.001	0.007
(min-max, median)	(32-82, 58.50)	(22-85,67)	(70-80, 77)	(22-85, 70)			
Age < 60	25 (52.08)	8 (32.0)	0 (0)*	8 (23.53)*	0.102	0.003	0.009
≥ 60	23 (47.92)	17 (68.0)	9 (100.0)	26 (76.47)			
Native Thai n (%)	19 (39.58)	15(60.0)	7 (77.78)	22 (64.70)	0.217	0.106	0.068
Thai-Chinese n (%)	28 (58.33)	10 (40.0)	2 (22.22)	12 (35.29)			
Other n (%)	1 (2.08)	0 (0)	0 (0)	0 (0)			
Male n (%)	45 (93.75)	14 (56.0)	6 (66.67)	20 (58.82)	< 0.001	0.044	< 0.001
Female n (%)	3 (6.25)	11 (44.0)*	3(33.33)*	14 (41.18)*			
Duration of drug exposure (days)							
(mean ±SD, min-max)	4.09 ± 2.50 years	23.83±13.30*	23.86±29.45*	23.84±17.58*	< 0.001	< 0.001	< 0.001
	(0.8-9.52 years)	(1-72)	(1-85)	(1-85)			
Serum creatinine (mg/dl)							
(mean ±SD, min-max)	1.22 ± 0.366	1.51 ± 0.81	1.58 ± 0.31*	1.52 ± 0.70*	0.087	0.001	0.008
	(0.8-3.0)	(0.8 – 4.5)	(1.2-2.1)	(0.8-4.5)			
Indication for allopurinol							
Gouty arthritis n (%)	46 (95.83)	16 (64.0)	8 (88.89)	24 (70.59)	0.003	0.409	0.001
Hyperuricemia n (%)	2 (4.17)	9 (36.0)*	1 (11.11)	10 (29.41)*			

Table 4.5 Demographic data in study population (cont.)

	Allopurinol tolerant control (C) (n=48)	Allopurinol induced skin reactions			P-value		
		SCAR (A) (n=25)	Other skin (B) (n=9)	Total (A+B) (n=34)	(C) VS (A)	(C) VS (B)	(C) VS (A+B)
Thiazide used							
Thiazide use	12 (25.0)	6 (24.0)	2 (22.22)	8 (23.53)	0.925	1.000	0.879
No thiazide use	36 (75.0)	19 (76.0)	7 (77.77)	26 (76.47)			
Adjust dose base on CrCl							
Recommended dose	39 (81.25)	8 (32.0)	4 (44.44)	12 (35.29)	< 0.001	0.074	< 0.001
Overdose	9 (18.75)	16 (64.0)*	4 (44.44)	20 (58.82)*			
Other drug allergy							
No drug allergy n (%)	36 (75.0)	14 (56.0)	4 (44.40)	18 (52.94)	0.097	0.109	0.038
Drug allergy n (%)	12 (25.0)	11 (44.0)*	5 (55.56)	16 (47.06)*			
Underlying disease n (%)							
Benign prostatic hypertrophy	5 (10.42)	0 (0)	1 (11.11)	1 (2.94)	0.158	1.000	0.393
Cardiovascular disease	9 (18.75)	4 (16.0)	5 (55.56)*	9 (26.47)	1.000	0.032	0.405
Chronic renal insufficiency[#]	20 (41.67)	18 (72.0)*	9 (100.0)*	27 (79.41)*	0.008	0.002	< 0.001
Diabetes mellitus	3 (6.25)	8 (32.0)*	6 (66.67)*	14 (41.18)*	0.006	< 0.001	< 0.001
Dyslipidemia	19 (39.58)	12 (48.0)	7 (77.78)	19 (55.88)	0.490	0.065	0.145
Fatty liver	6 (12.50)	1 (4.0)	0 (0)	1 (2.94)	0.410	0.575	0.230
Hypertension	34 (70.83)	19 (76.0)	9 (100)	28 (82.35)	0.639	0.095	0.231
Hyperthyroid	1 (2.08)	3 (12.0)	1 (11.11)	4 (11.76)	0.113	0.293	0.155
Osteoarthritis	4 (8.33)	3 (12.0)	0 (0)	3 (8.82)	0.685	1.000	1.000

*# Chronic renal insufficiency was defined as creatinine clearance ≤ 60 mg/dl * P value less than 0.05*

Table 4.6 Type of skin reactions

Skin reactions	Number of patients with skin reactions (%)
SJS	15 (44.1)
TEN	1 (2.9)
HSS	9 (26.5)
Exfoliative dermatitis	5 (14.7)
Fix drug eruption	1 (2.9)
Eczema	1 (2.9)
Maculopapular rash	2 (5.8)

***HLA-B*5801 and HLA-Cw*0302* alleles**

Among the 82 patients participated in the study, there were 48 patients who used allopurinol without adverse drug reaction (allopurinol tolerant patients) and vice versa in the rest 34 patients. There were 25 patients who had severe cutaneous adverse reaction and 9 patients who had other types of cutaneous adverse reactions. *HLA-B*5801* and *HLA-Cw*0302* alleles were found in all 25 patients (100%) with severe cutaneous adverse reaction. In 9 patients who had other types of cutaneous adverse reaction, *HLA-B*5801* and *HLA-Cw*0302* alleles were found in 6 and 5 patients respectively. We found that *HLA-B*5801* and *HLA-Cw*0302* alleles were positive in all 5 patients who had exfoliative dermatitis while the 1 patient who had maculopapular rash only *HLA-B*5801* allele was found. Details are shown in figure 4.1- 4.5 showed positive band in lane 15 and lane 44 indicated that *HLA-B*5801* allele is positive while positive band in lane 47 indicated that *HLA-Cw*0302* allele is positive. Summary of HLA-B*5801 and HLA-Cw*0302 alleles for case and control are shown in table 4.7.

These 48 patients who had no cutaneous adverse reactions from allopurinol, *HLA-B*5801* and *HLA-Cw*0302* alleles was found in 7 patients (14.58%) only and *HLA-Cw*0302* allele was also found in all of these 7 patients. Patient's medical records shown that 2 of the 7 patients (28.57%) who had *HLA-B*5801* allele used to experienced severe adverse drug reaction from NSAIDs, 1 patient had jaundice from using sulindac and the other patient had HSS from diclofenac. Details of these 7 patients are shown in table 4.8.

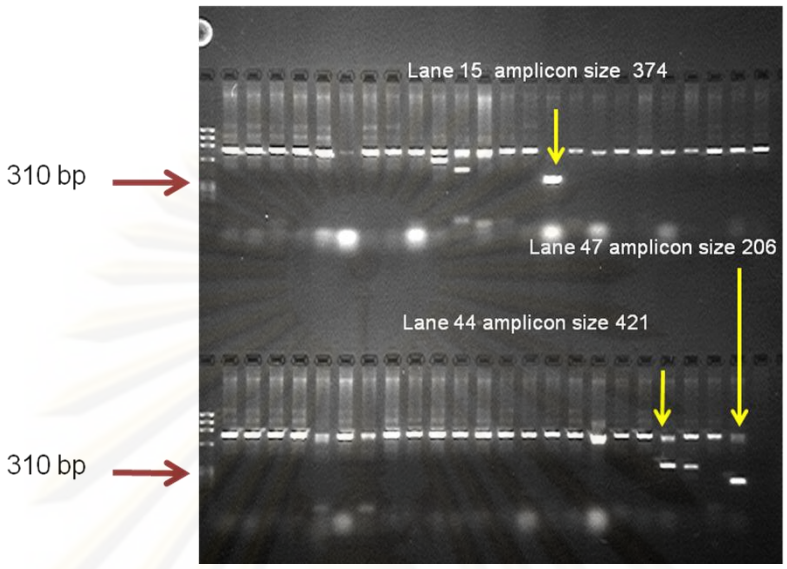

Figure 4.1 Positive *HLA-B*5801* and *HLA-Cw*0302*alleles in patient with SJS

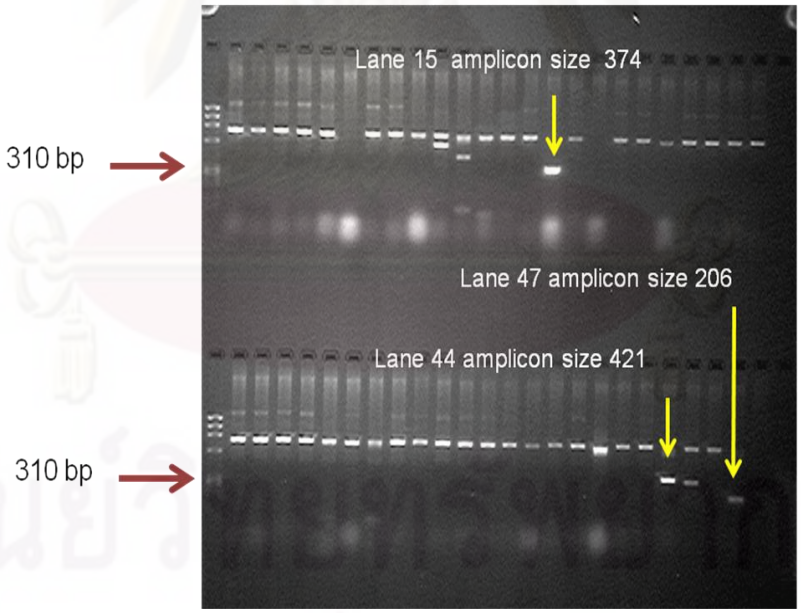

Figure 4.2 Positive *HLA-B*5801* and *HLA-Cw*0302* alleles in patient with TEN

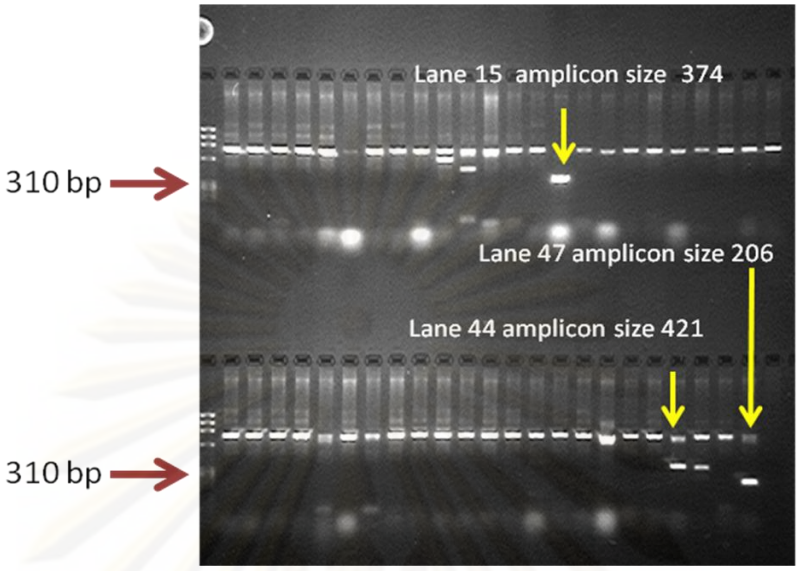

Figure 4.3 Positive *HLA-B*5801* and *HLA-Cw*0302* alleles in patient with HSS.

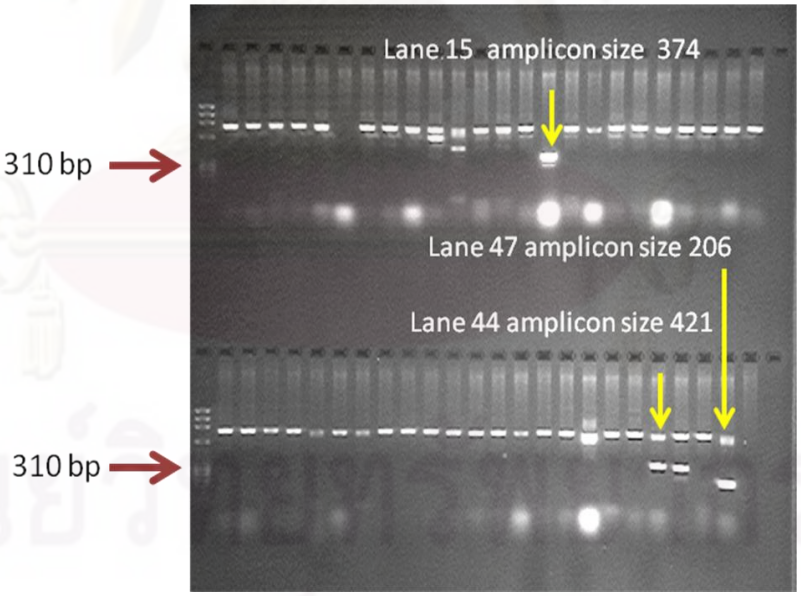

Figure 4.4 Positive *HLA-B*5801* and *HLA-Cw*0302* alleles in patient with exfoliative dermatitis.

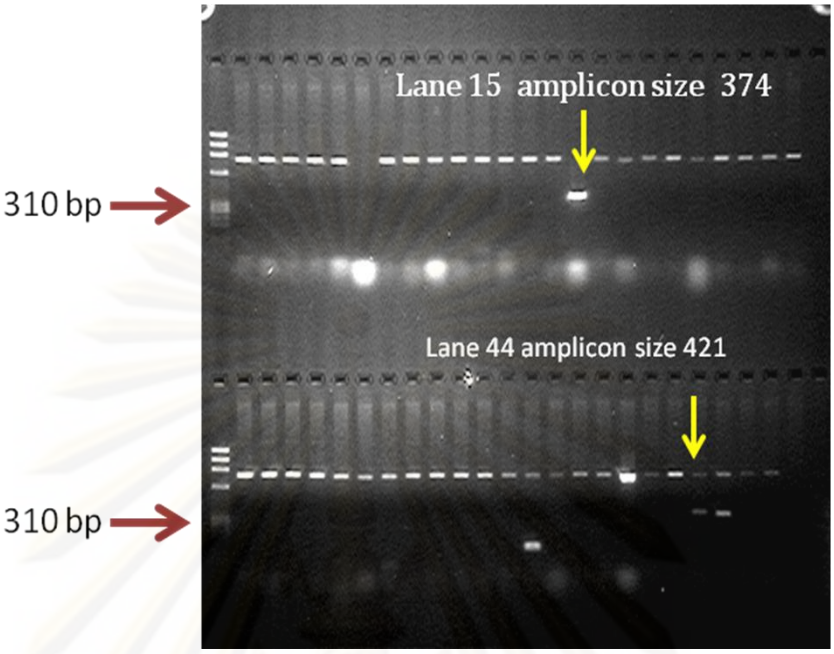

Figure 4.5 Positive *HLA-B*5801* and negative *HLA-Cw*0302* alleles in patient with maculopapular rash

Table 4.7 Summary of *HLA-B*5801* and *HLA-Cw*0302* alleles for case and control

HLA-B*5801	**Case (SCAR)**	**Control**	**total**
Positive	25 (a) true positive (case and positive *HLA-B*5801*)	7 (b) false positive (control but positive *HLA-B*5801*)	32
Negative	0 (c) false negative (case but negative *HLA-B*5801*)	41(d) true negative (control and negative *HLA-B*5801*)	41
total	25 (a+b) total number of case who had adverse drug reaction from allopurinol	48 (b+d) total number of allopurinol tolerant control	73

The sensitivity and specificity of the *HLA-B*5801* allele for prediction of allopurinol induced SCAR were 100% (25/25) and 85.41 % (41/48) respectively. The positive predictive value and the negative predictive value of the *HLA-B*5801* allele was 78.12% (25/32) and 100% (41/41), respectively.

Table 4.8 Previous drug allergy reported for allopurinol tolerant patients with *positive HLA-B*5801* and *HLA-Cw*0302* alleles

No.	Age/sex	Underlying disease	Dose (mg)	Scr (mg/ml)	Drug allergy
1	51/M	HT,DLP, gout	300	1.1	No
2	75/M	HT, gout	300	1.5	diclofenac
3	52/M	HT,DLP, gout, fatty liver, OA	200	0.8	Penicillin
4	66/M	HT, gout	200	1.6	No
5	77/M	DM, HT,DLP, gout, CKD	100	3.0	No
6	61/M	HT, gout, ankylosing spondylosis	100	0.9	sulindac
7	50/M	HT,DLP, gout	300	1.0	No

HT, Hypertension DLP, Dyslipidemia, DM, Diabetes mellitus OA, Osteoarthritis CKD, Chronic kidney disease.

Association between *HLA-B*5801* and *HLA-Cw*0302* alleles to SCAR

When calculating odds ratio by Haldane's modification, which add 0.5 to all cells to accommodate possible zero count, we found that patients with *HLA-B*5801* and *HLA-Cw*0302* alleles had 282 times higher risk to have SCAR caused by allopurinol than patients who do not have these HLA alleles as shown detail in table 4.9 A, B and C.

Table 4.9 A Association between *HLA* allele to allopurinol induced SCAR

HLA-allele	Allopurinol Tolerant Control N % (n=48)	SCAR N% (n=25)	P-value	Odds ratio
*HLA-B*5801*	7 (14.58)	25 (100.0)	< 0.001	282.2
*HLA-Cw*0302*	7 (14.58)	25 (100.0)	< 0.001	282.2
*HLA-B*15*	15 (31.25)	4 (16.0)	0.260	0.42
*HLA-B*27*	7 (14.58)	1 (4.0)	0.250	0.24
*HLA-B*38*	1 (2.08)	1 (4.0)	1.000	1.96
*HLA-B*39*	3 (6.25)	3 (12.0)	0.406	2.04
*HLA-B*40*	16 (33.33)	8 (32.0)	0.908	0.94
*HLA-B*46*	11 (22.92)	4 (16.0)	0.557	0.64

Table 4.9 B Association between *HLA* allele to allopurinol induced other type of skin

HLA-allele	Allopurinol Tolerant Control N % (n=48)	Other skin N%(n=9)	P-value	Odds ratio
*HLA-B*5801*	7 (14.58)	6 (66.67)	0.003	11.71
*HLA-Cw*0302*	7 (14.58)	5 (55.56)	0.015	7.32
*HLA-B*15*	15 (31.25)	0 (0)	0.094	0.11
*HLA-B*27*	7 (14.58)	0 (0)	0.582	0.29
*HLA-B*38*	1 (2.08)	0 (0)	1.000	1.67
*HLA-B*39*	3 (6.25)	0 (0)	1.000	0.68
*HLA-B*40*	16 (33.33)	3 (33.33)	1.000	1.0
*HLA-B*46*	11 (22.92)	2 (22.22)	1.000	0.96

Table 4.9 C Association between *HLA* allele to allopurinol induced total of skin reaction

HLA-allele	Allopurinol Tolerant Control N % (n=48)	Allopurinol induced skin reactions (n=34)	P-value	Odds ratio
*HLA-B*5801*	7 (14.58)	31 (91.18)	< 0.001	60.52
*HLA-Cw*0302*	7 (14.58)	30 (88.24)	< 0.001	43.92
*HLA-B*15*	15 (31.25)	4 (11.76)	0.062	0.29
*HLA-B*27*	7 (14.58)	1 (2.94)	0.131	0.18
*HLA-B*38*	1 (2.08)	1 (2.94)	1.000	1.42
*HLA-B*39*	3 (6.25)	3 (8.82)	0.688	1.45
*HLA-B*40*	16 (33.33)	11 (32.35)	0.926	0.96
*HLA-B*46*	11 (22.92)	6 (17.65)	0.562	0.72

Model for prediction of cutaneous adverse reaction from allopurinol

Logistic regression was performed. Among the 82 patients participated in this study. Only 79 patients data were complete and therefore were selected for the creation of the model. Univariate logistic regressions were performed, the results were shown in table 4.10. *HLA-B*5801* and *HLA-Cw*0302* alleles showed high significant and high odds ratio. The significant factors from univariate regression were further included into the multivariate logistic regression model.

Multivariate logistic regression was used to create the model for prediction of cutaneous adverse reaction from allopurinol. There were factors that related to incidence of cutaneous adverse reaction as shown in table 4.11. These factors were analyzed by forward stepwise method and found that only 3 factors related to adverse drug reaction from allopurinol including *HLA-B*5801* allele, gender and diabetes. Genetic variation, *HLA-B*5801* positive and negative, were defined as 1 and 0 respectively. Gender factor, female and male, were defined as 1 and 0 respectively. Underlying disease factor, diabetic and non-diabetic, were define as 1 and 0 respectively. Therefore, the model created as below:

Table 4.10 Risk factors for allopurinol induced rash using univariate logistic regression

Factors (N)	**P value**
*HLA-B*5801* (82)	< 0.001
*HLA-Cw*0302* (82)	< 0.001
Diabetes (82)	0.001
Gender (82)	0.001
Chronic renal insufficiency (81)	0.001
Other drug allergy (82)	0.040
Age (82)	0.011
Indication of allopurinol (82)	0.006
Thaizide use (82)	0.879
Dose of allopurinol more than 200 mg (80)	0.913
Over recommend dose (80)	< 0.001

Table 4.11 Risk factors for allopurinol induced rash in multivariate logistic regression

Factor	B	Sig.	Odds ratio	95.% C.I. for odds ratio
*HLA-B*5801*	5.242	< 0.001	189.06	13.40 – 2667.36
Diabetes	3.238	0.019	25.48	1.719 – 377.70
Gender	3.197	0.022	24.46	1.584 – 377.60
Constant	-4.793	< 0.001	0.008	

Logit (Y) = - 4.793 + 5.242 (*HLA-B*5801*) + 3.238 (diabetes)

+ 3.197 (gender)……..(1)

P (Y) = $e^{\text{logit (Y)}} / 1+ e^{\text{logit (Y)}}$……...(2)

Y = Cutaneous adverse reaction from allopurinol

Nagelkerke's R^2 (Pseudo R^2) = 0.788

Table 4.12 Association between probabilities from model prediction of allopurinol hypersensitivity and observed patients

Observed	Predicted		
	Case-control		Percentage collection
	Control	Case	
Control (n=48)	41	7	85.4
Case (n=31)	1	30	96.8
Overall percentage			89.9

From the model summary, Nagelkerke's R^2 (Pseudo R^2) is 0.788 which demonstrated that 78.8% of the variance could be explained by logistic model. The model has high accuracy, its result match of the study finding 89.9 % (71/79). Sensitivity of the equation is 96.8% (30/31), which demonstrate the power of prediction of cutaneous adverse reaction from allopurinol. Specificity is 85.4% (41/48), which demonstrates the ability of prediction of cutaneous adverse reaction from allopurinol. Example 1 demonstrated how the model could be used to predict probability of cutaneous reaction from allopurinol.

Example 1 Diabetic male patient who had positive *HLA-B*5801* allele and had been starting treatment with allopurinol.

$$\text{Logit (Y)} = -4.793 + 5.242\,(\textit{HLA-B*5801}) + 3.238\,(\text{diabetes}) + 3.197\,(\text{gender})$$

$$= -4.793 + 3.238\,(1) + 5.242\,(1) + 3.197\,(0)$$

$$= 3.687$$

$$\text{Probability (Y)} = e^{\text{logit (Y)}} / 1 + e^{\text{logit (Y)}}$$

$$= e^{3.687} / 1 + e^{3.687}$$

$$= 0.976$$

This patient had high probability of having cutaneous adverse reaction from allopurinol.

Table 4.13 Probability of cutaneous reaction from allopurinol base on model prediction

HLA-B*5801	**Gender**	**Diabetic**	**Probability**
Positive	Female	Yes	0.999
Positive	Female	No	0.975
Positive	Male	Yes	0.976
Positive	Male	No	0.610
Negative	Female	Yes	0.838
Negative	Female	No	0.169
Negative	Male	Yes	0.174
Negative	Male	No	0.008

CHAPTER V
DISCUSSION AND CONCLUSION

There were two main purposes in this present study; First, to investigate the causative drugs, the prevalence and mortality rates related to severe cutaneous adverse reaction (SCAR) during 2003-2007 using retrospective data collected from electronic database of Adverse Drug Reaction Monitoring Center and Siriraj Computer Center, Siriraj Hospital, Bangkok; second, to determine the association between *HLA-B*5801* and *HLA-Cw*0302* alleles to severe cutaneous adverse reaction induced by allopurinol in Thai patients using case control study.

Part I: Prevalence and Mortality rate of severe cutaneous adverse reactions.

SCAR was found in 136 patients during 2003-2007 including 84 cases with SJS (61.76%), 3 cases with SJS overlap TEN (2.21%), 10 cases with TEN (7.35%) and 39 cases with HSS (28.68%). When categorized by group of causative drugs, anticonvulsants shared one third of all reported SCAR. This result is consistent with a previous study in India, Malaysia and Srinagarind Hospital.[61-63] In this study, phenytoin, carbamazepine and phenobarbital were the main causative drugs. These three drugs have similarity in their chemical structure; they all are aromatic anticonvulsants which are metabolized in the liver by cytochrome P450 enzyme. The arene oxide metabolites which are the product of this metabolic pathway can cause cellular toxicity by activating self-destruction of the immune system.[64] Phenytoin had the highest prevalence of HSS; the rate was approximately 2-3 per 1,000 patients. Approximately 3-4 per 1,000 patients using carbamazepine experienced SJS were from carbamazepine usage. In this study, over 80% of adverse drug reactions from phenobarbital were found in children due to the more frequently usage of this drug in children than in adults. Special precaution of cross-reaction has to be concerned if patients experience severe adverse drug reaction with these drugs, 45 - 75% of cross–reaction had been reported.[65-66] Recently, there were few studies that showed strong association between *HLA-B*1502* allele and carbamazepine induced SJS/TEN in Han Chinese, Thai and Indian patients.[20, 53-54] The United States Food and Drug Administration (USFDA) recommend genetic screening of this allele for all

carbamazepine users in Asians [19] since high frequency of this allele has been reported in Asian population.[16]

The other drugs group frequently found to be the cause of SCAR was antimicrobial (25.74%). Sulfonamides was found to be the highest cause of SJS and HSS while previous similar study in Siriraj Hospital in 1993 revealed that penicillin was the main cause of SJS/TEN during that period.[67] This should be due to the increasing usage of cotrimoxazole for opportunistic infection prophylaxis in Human immunodeficiency virus (HIV) patients. HIV patients have higher probability of confronting with adverse drug reaction from cotrimoxazole, approximately 18-57%, as compared to the adverse drug reaction rate of 3% in overall patients. Glutathione deficiency, co-infection of Cytomegalovirus or Epstein-Barr virus in HIV patients might be the reason of this circumstance.[68] Moreover, allopurinol is the one drug that all symptoms, SJS/TEN and HSS have been reported. If HSS only was considered, allopurinol became the most often reported HSS causative drug. One out of five patients who experienced SCAR from allopurinol was dead.

Comparisons of the mean onset times of SCAR after causative drug administration revealed that HSS had longer incubation time as compare to SJS and TEN. However, mortality rate in this study was quite similar to previous studies which reported mortality rate of SJS TEN and HSS to be 5%[13], 30-50%[13] and 8-20% [40, 69-70] respectively. The overall mortality rate in this study was 11.76%. This high mortality rate indicated that severity of the event has not been decreased from the past despite evolutionary of medical care. Probably Siriraj Hospital is the tertiary care setting and 25% of the death cases with very severe clinical symptom were referred from other health care settings, hence, overall mortality rate was higher than previously reported from other setting. From retrospective study as above allopurinol revealed the highest mortality rate.

Part 2: Association between *HLA-B*5801* and *HLA-Cw*0302* alleles to severe cutaneous adverse reaction including other types of cutaneous reactions caused by allopurinol in Thai patients.

This study is a case-control study. There were 82 patients participated in the study that includes 25 patients who experienced SCAR, 9 patients who experienced other cutaneous adverse reactions and 48 patients who had no adverse drug reaction from allopurinol.

There were 25 patients in this study who experienced SCAR which could be categorized into 3 groups including SJS, TEN and HSS. HLA genotyping revealed that *HLA-B*5801*and *HLA-Cw*0302* alleles were found in all patients (100%). This finding is consistent with the study of Hung et al.[21] who studied in Han Chinese patients with SCAR and Wichittra et al.[24] who studied in Thai patients with SJS and TEN while Lonjou et al.[8] who studied in European patients with SJS and TEN revealed that *HLA-B*5801* allele was found in only 61% of these patients and Kaniwa et al.[23] who studied in Japanese patients and found *HLA-B*5801* allele in only 40% of the patients. This study demonstrated that there was a strong association between severe cutaneous adverse reaction from allopurinol and *HLA-B*5801* and *HLA-Cw*0302* alleles in Asian patients especially in Thai and Han Chinese patients. This might due to the reason that allele frequency of *HLA-B*5801* and *HLA-Cw*0302* alleles more were found frequently in Asian than in Caucasian and Japanese.[25] Summary of studies reporting *HLA-B*5801* and *HLA-Cw*0302* alleles in different ethnics as shown in table 5.1.

This study demonstrated that *HLA-B*5801* allele was also associated to exfoliative dermatitis which has been classified to be moderate to severe dermatitis; the patient had no internal inflammation and/or diagnostic criteria of HSS or DRESS have not been completely fulfilled. The results from Hung et al., Wichittra et al., and this study indicated that *HLA-B58* found in patients was all *HLA-B*5801* allele. Therefore, if the laboratory or testing kit is not available to test this specific allele *HLA-B*5801* genotyping; low to intermediate resolution method which can identify *HLA-B58* might be sufficient for screening patients with high risk to allopurinol induced SCAR and exfoliative dermatitis. *HLA-B*5801* and *HLA-Cw*0302* alleles were both found in all patients with SCAR. This demonstrated that *HLA-B*5801*

allele is usually transmitted together with *HLA-Cw*0302* allele as known as linkage disequilibrium.[71-72] In clinical practice, either *HLA-B*5801* or *HLA-Cw*0302* alleles testing can be used to identify patients with high risk for allopurinol induced SCAR. However, *HLA-B*5801* allele had been reported to be more specific.[21]

Table 5.1 Summary of studies reporting the *HLA-B*5801*and *HLA-Cw*0302* alleles in different ethnics

Study/ Ethnic/Type of skin	***HLA-B*5801***	***HLA-Cw*0302***	**Control**	**Odd ratio**
This study Thai/SCAR	100% (25/25)	100% (25/25)	14.58% (7/48) 14.58% (7/48)	282.2 282.2
Wichittra et al.[24] Thai/SJS/TEN	100% (27/27)	-	12.96% (7/54)	348.3
Hung et al. [21] Han Chinese/ SCAR	100% (51/51)	94%(48/51)	14.81% (20/135) 14.07% (19/135)	580.3 97.7
Lonjou et al. [8] European/SJS/TEN	61% (19/31)	-	1.5% (28/1882)	61
Kaniwa et al. [23] Japanese/SJS/TEN	40% (4/10)	-	0.6% (3/493)	41

Among patients with SCAR from allopurinol, there were 2 patients who also had adverse drug reaction from phenytoin. The first patient had maculopapular rash; *HLA-B*5801* and *HLA-B*1513* alleles were found from HLA genotyping screening. The second patient had TEN from phenytoin and pancreatitis from sodium valproate; HLA genotype revealed *HLA-B*5801* and *HLA-B*1505* alleles. This demonstrated that *HLA-B*1502* allele might not show up in all patients with TEN from phenytoin which supported the results previously reported by Hung et.al who mention that *HLA-B*1502* allele was found in only 30.8% (8 from 26 patients).[73]

*HLA-B*5801* allele was found up to 14.58% (7 from 48 patients) in allopurinol tolerant control. This finding is consistent with the study of Hung et al. (14.81%) and Wichittra et al. (12.96%) [21, 24] while in European and Japanese study found only 1.5% and 0.6% respectively because the frequency of *HLA-B*5801* allele was lower than Asian population.[25] Two of these patients had history of severe adverse drug reaction from NSAIDs, 1 patient had jaundice from use of sulindac and

the second patient had HSS from diclofenac. From the study of Kazeem et al. which explored patients with SJS and TEN from lamotrigine, *HLA-B*5801* allele was found to has significant associated with the adverse reaction. (P-value = 0.037) [74] This indicated that patients with *HLA-B*5801* allele may have high risk from adverse drug reaction from other drugs as well besides allopurinol.

Pathogenesis of allopurinol hypersensitivity syndrome is unclear; its etiology is related to many factors including immunology, genetics, and accumulation of oxypurinol and reactivate of latent virus.[75] Apart from *HLA-B*5801* genotyping, appropriate dosage regimen, reasonably drug use, patient's renal function, diabetes and gender were also factors significantly related to adverse drug reaction from allopurinol. This information leaded researcher to create the model to predict the adverse drug reaction from allopurinol by using logistic regression. Main factors included in the model equation for predicting adverse drug reaction cause by allopurinol were *HLA-B*5801* allele, female gender and underlying disease of diabetes mellitus. Wichittra et al.[24] also reported that these three factors were significantly associated to allopurinol induced SJS and TEN. We found that female had higher risk to allopurinol induced cutaneous adverse reaction than male. About 75% (6 from 8 patients) of diabetes mellitus patients had poor renal function and no dosage adjustment base on creatinine clearance that could decrease the excretion of oxypurinol (allopurinol metabolite) so it might put the patient to higher risk of adverse drug reaction. It is the fact that renal insufficiency is the factor underneath diabetes mellitus. While patients with chronic renal disease that drug dosage was usually adjusted according to renal function then oxypurinol was not accumulate as seen in diabetes patients who did not dosage adjustment base on renal function. However, this model should be validated before use.

Among allopurinol tolerant patients, it was noticed that *HLA-B*5801* allele was only found in male and diabetes mellitus was found in only 1 patient, dosage of allopurinol was adjusted according to renal function in 6 of 7 patients (85.71%). All patients with *HLA-B*5801* allele were using allopurinol for treating gouty arthritis. Model for calculation the probability of adverse drug reactions show that these patients had probability about 60% of cutaneous adverse reaction from allopurinol. Therefore, if allopurinol is needed, dosage adjustment according to renal function and

related conditions is important for use of allopurinol in these patients. With this finding, the following factors affect safely use of allopurinol, 1) Reasonably drug use 2) Appropriate dosage regimen based on patient's renal function 3) Screening test of *HLA-B*5801* allele before administration of allopurinol the fact that high cost of HLA genotyping, cost effectiveness should be considered. Therefore, with the study result, screening test is recommended in high risk patient especially in patients with diabetes and renal insufficiency. This is for highest patient safety. In the future, genetic screening test may be beneficial in order to prevent severe adverse drug reactions from clinically use allopurinol.

Limitation

1. Only few patients with other types of rash (besides SCAR) from allopurinol were included higher number of patients are needed to increase the power of statistical analysis before any strong conclusion could be made on patients with these type of cutaneous adverse reaction from allopurinol.

2. Several interesting factors which might affect cutaneous adverse reaction from allopurinol show statistically significant but had not been pick up by the SPSS program to be included in the predictive model, such as, recommended dose, indication of allopurinol and chronic renal insufficiency. This might due to the small number of subjects included in this study.

Further Study

In the future, convenient genotyping testing kit should be developed to make it easier to use without specialist for testing and interpreting genetic data. Since the current testing method is still complicated and the testing cost is still quite high, this may cause financial barrier when applying to clinical practice. Loop-mediated isothermal amplification method (LAMP method) is an interested technique that should be developed to reduce cost of testing to the patient. Currently, there was a study which used this technique for *HLA-B*1502* allele testing and the result was concordance with sequence base typing (SBT) and PCR-SSP method which are standard technique commonly used at present.[76] Researcher noticed that there may be some other factors related to renal function that might have some impact on adverse drug reaction from allopurinol and this can be more clearly seen in larger population.

REFERENCES

(1) Wortmann, R. L. Kelley's Textbook of Rheumatology [online]. Philadelphia : Elsevier, 2008. Available from : http://www.mdconsult.com/das/book/body/201106963-6/998044834/1807/652.html#4-u1.0-B978-1-4160-3285-4..10087-7_3217 [2010, March].

(2) Nakorn, R. N. Management of hyperuricemia and Gout. In Asavatanabodee, P. (eds.), Rheumatology for the Non-rheumatologist, 277-302. Bangkok: Cityprint publication, 2007.

(3) Dhana, N. and Jongjarearnprasert, K. Adverse Drug Reaction Report :Experience in Siriraj Hospital. 2545-2547. Siriraj Med J 57 (2005): 235-240.

(4) Limsuwan, T. Laboratory testing for adverse drug reaction diagnosis. In Nathisuwan, S., Chulawattanathon S., Chindavijak, B., Suksomboon, N., Suansanei T. (eds.), Advances in Pharmacotherapeutics and Pharmacy Practice, 88. Bangkok: Pachachon Publisher, 2008.

(5) Halevy, S., Ghislain, P. D., Mockenhaupt, M., Fagot, J. P., Bouwes Bavinck, J. N., Sidoroff, A., et al. Allopurinol is the most common cause of Stevens-Johnson syndrome and toxic epidermal necrolysis in Europe and Israel. J Am Acad Dermatol 58 (2008): 25-32.

(6) Russmann, S. and Lauterburg, B. [Life-threatening adverse effects of pharmacologic antihyperuricemic therapy]. Ther Umsch 61 (2004): 575-577.

(7) Mockenhaupt, M., Viboud, C., Dunant, A., Naldi, L., Halevy, S., Bouwes Bavinck, J. N., et al. Stevens-Johnson syndrome and toxic epidermal necrolysis: assessment of medication risks with emphasis on recently marketed drugs. The EuroSCAR-study. J Invest Dermatol 128 (2008): 35-44.

(8) Lonjou, C., Borot, N., Sekula, P., Ledger, N., Thomas, L., Halevy, S., et al. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. Pharmacogenet Genomics 18 (2008): 99-107.

(9) Markel, A. Allopurinol-induced DRESS syndrome. Isr Med Assoc J 7 (2005): 656-660.

(10) Gutierrez-Macias, A., Lizarralde-Palacios, E., Martinez-Odriozola, P. and Miguel-De la Villa, F. Fatal allopurinol hypersensitivity syndrome after treatment of asymptomatic hyperuricaemia. BMJ 331 (2005): 623-624.

(11) Roujeau, J. C., Kelly, J. P., Naldi, L., Rzany, B., Stern, R. S., Anderson, T., et al. Medication use and the risk of Stevens-Johnson syndrome or toxic epidermal necrolysis. N Engl J Med 333 (1995): 1600-1607.

(12) Duongngeon, T. Steven-Johnson syndrome and Toxic Epidermal Necrolysis. In Tida Ningsanon, J. Y. (eds.), Trongpraden reung Adverse Drug Reaction (in Thai), 150-157. Bangkok: Paramut Publisher, 2006.

(13) Chia, F. L. and Leong, K. P. Severe cutaneous adverse reactions to drugs. Curr Opin Allergy Clin Immunol 7 (2007): 304-309.

(14) Adverse drug reactions report. [online]. 2006. Available from : www.fdaolap.fda.moph.go.th/apr2006/searchapr2.asp?offset=10 [2009, March].

(15) Puttilerpong, C. Advances in Pharmacogenomics and HIV Pharmacotherapy. In Nathisuwan, S., Chulawattanathon S., Chindavijak, B., Suksomboon, N., Suansanei T(eds.), Advances in Pharmacotherapeutics and Pharmacy Practice, 168. Bangkok: Pachachon Publisher, 2008.

(16) Chung, W. H., Hung, S. I. and Chen, Y. T. Human leukocyte antigens and drug hypersensitivity. Curr Opin Allergy Clin Immunol 7 (2007): 317-323.

(17) Lugsiri, A. Emerging Role of Pharmacogenomic Testing for Serios ADRs Prediction. In Nathisuwan, S., Chulawattanathon S., Chindavijak, B., Suksomboon, N., Suansanei T(eds.), Advances in Pharmacotherapeutics and Pharmacy Practice, 106-107. Bangkok: Pachachon Publisher, 2008.

(18) Abacavir (marketed as Ziagen) and Abacavir-containing Medications [online]. 2008. Available from : www.fda.gov/medwatch/safety/2008/safety08.htm#Abacavir [2009, May].

(19) Ferrell, P. B., Jr. and McLeod, H. L. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics 9 (2008): 1543-1546.

(20) Locharernkul, C., Loplumlert, J., Limotai, C., Korkij, W., Desudchit, T., Tongkobpetch, S., et al. Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with HLA-B*1502 allele in Thai population. Epilepsia 49 (2008): 2087-2091.

(21) Hung, S. I., Chung, W. H., Liou, L. B., Chu, C. C., Lin, M., Huang, H. P., et al. HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol. Proc Natl Acad Sci U S A 102 (2005): 4134-4139.

(22) Dainichi, T., Uchi, H., Moroi, Y. and Furue, M. Stevens-Johnson syndrome, drug-induced hypersensitivity syndrome and toxic epidermal necrolysis caused by allopurinol in patients with a common HLA allele: what causes the diversity? Dermatology 215 (2007): 86-88.

(23) Kaniwa, N., Saito, Y., Aihara, M., Matsunaga, K., Tohkin, M., Kurose, K., et al. HLA-B locus in Japanese patients with anti-epileptics and allopurinol-related Stevens-Johnson syndrome and toxic epidermal necrolysis. Pharmacogenomics 9 (2008): 1617-1622.

(24) Tassaneeyakul, W., Jantararoungtong, T., Chen, P., Lin, P. Y., Tiamkao, S., Khunarkornsiri, U., et al. Strong association between HLA-B*5801 and

allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in a Thai population. Pharmacogenet Genomics 19 (2009): 704-709.

(25) Middleton, D., Menchaca, L., Rood, H. and Komerofsky, R. New allele frequency database: http://www.allelefrequencies.net. Tissue Antigens 61 (2003): 403-407.

(26) Adverse drug reaction's definition.World Health Organization [online]. Available from: http://www.who.int/medicines/areas/quality_safety/safety_efficacy/Annex1GlossaryofTerms.pdf [2010, March].

(27) French, L. E. Toxic epidermal necrolysis and Stevens Johnson syndrome: our current understanding. Allergol Int 55 (2006): 9-16.

(28) Diedre Faust, P. H. Gout and Other Crystal-induced Synovitis. In Dario M. Torre, G. C. L., Jerome J. Van Ruiswyk, Ralph M. Schapira (eds.),Clinical medicine for students, 310-313. Philadelphia: Lippincott Williams & Wilkins, 2009.

(29) Emmerson, B. T. The management of gout. N Engl J Med 334 (1996): 445-451.

(30) Eggebeen, A. T. Gout: an update. Am Fam Physician 76 (2007): 801-808.

(31) Keith, M. P. and Gilliland, W. R. Updates in the management of gout. Am J Med 120 (2007): 221-224.

(32) Klasco, RK. (Ed): DRUGDEX® System. Thomson Reuters, Greenwood Village, Colorado (Vol.144expire [6/2010]).

(33) Charles F Lacy, L. L. A., Morton P. Goldman, Leonard L. Lance. Drug Information Handbook with International Trade Names Index. 18 ed. Ohio: LEXI-COMP, 2009-2010.

(34) Bardin, T. Current management of gout in patients unresponsive or allergic to allopurinol. Joint Bone Spine 71 (2004): 481-485.

(35) Wongpuwaruk, P. Definition Classification and Mechanism of ADR. In Ningsanon, T., Yothapitak, J (eds.), Trongpraden reung Adverse Drug Reaction (in Thai), 7-8. Bangkok: Paramut Publisher, 2006.

(36) Jeffrey L. Kishiyama, A. T. T., Pedro C. Avila. Drug Allergy. In Tristram G. Parslow, D. P. S., Abba I. Terr, John B. Imboden (eds.), Medical immunology, 394-396. New York: McGraw-Hill, 2001.

(37) Descotes, J. and Choquet-Kastylevsky, G. Gell and Coombs's classification: is it still valid? Toxicology 158 (2001): 43-49.

(38) Mockenhaupt, M. Severe drug-induced skin reactions: clinical pattern, diagnostics and therapy. J Dtsch Dermatol Ges 7 (2009): 142-160; quiz 161-142.

(39) Ben m'rad, M., Leclerc-Mercier, S., Blanche, P., Franck, N., Rozenberg, F., Fulla, Y., et al. Drug-induced hypersensitivity syndrome: clinical and biologic disease patterns in 24 patients. Medicine (Baltimore) 88 (2009): 131-140.

(40) Shiohara, T., Inaoka, M. and Kano, Y. Drug-induced hypersensitivity syndrome (DIHS): a reaction induced by a complex interplay among herpesviruses and antiviral and antidrug immune responses. Allergol Int 55 (2006): 1-8.

(41) Gawkrodger, D. J. Erythroderma. Philadelphia: Elsevier, 2008.

(42) Guliz Karakayli, G. B., Ida Orengo, Ted Rosen. Exfoliative dermatitis [online].1999. Available from : http://www.aafp.org/afp/990201ap/625.html [2010, March]

(43) Habif, T. P. Clinical Dermatology [online]. Marryland : Elsevier, 2009, Available from : http://www.mdconsult.com/das/search/results/201106963-

9?searchId=998055942&kw=Exanthems%20and%20Drug%20eruption&bbSearchType=single&area=BookFast&set=1[2010, March].

(44) Roderick Nairn, M. H. Major histocompatibility complex. In Roderick Nairn, M. H.(eds.), Immunology for medical students, 57. Philadelphia: Elsevier, 2002.

(45) Richard Coico, G. S., Eli Benjamini. Immunology A Short Course. fifth ed. New Jersey: John Wiley & Sons 2003.

(46) Davis H. Margulies, K. N., Jamie Rossjohn, Jame McCluskey. Major Histocompatibility Complex (MHC) Molecules: Structure, Function, and Genetics. In Paul, W. E., Fundamental immunology, 570. Philadelphia: Lippincott Williams & Wilkins, 2008.

(47) Carpenter, C. B. Histocompatibility Systems. In Leo C. Ginns, A. B. C., Peter J. Morris(eds.), Transplantation, 60. Massachusetts: Blackwell Science, 1999.

(48) Susana G. Marino, A. J., Marcelo A. Fernandez-Vina. The Human Major Histocompatibility Complex In Maurice R.G.O'Gorman, A. D. D. (eds.), Handbook of HUMAN IMMUNOLOGY, 551-556. New York: Taylor & Francis Group, LLC, 2008.

(49) Phillip A Dyer, A. L., Helen Liggett, Karen Wood, Judith Worthington. Testing for Histocompatibility. In Forsythe, J. L. R.(eds.), Transplantation, Netherlands: Elsevier Saunders, 2005.

(50) Edgar, J. D. M. Translantation. In Edgar, J. D. M.(eds.), Immunology Master medicine, 150. Philadelphia: Elsevier, 2006.

(51) Glossary (Appendix). In David Male, J. B., David B Roth, Ivan Roitt (eds.), Immunology Philadelphia: Elsevier, 2006.

(52) Kitporka, P. Histocompatibility System in Kidney Transplantation. In Vareesangthip K., J. S., Sumethakul W., Choosil S. (eds.), Textbook of Kidney Transplantation, 17. Bangkok: Krugthep wetchasan, 2004.

(53) Man, C. B., Kwan, P., Baum, L., Yu, E., Lau, K. M., Cheng, A. S., et al. Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. Epilepsia 48 (2007): 1015-1018.

(54) Mehta, T. Y., Prajapati, L. M., Mittal, B., Joshi, C. G., Sheth, J. J., Patel, D. B., et al. Association of HLA-B*1502 allele and carbamazepine-induced Stevens-Johnson syndrome among Indians. Indian J Dermatol Venereol Leprol 75 (2009): 579-582.

(55) Chantarangsu, S., Mushiroda, T., Mahasirimongkol, S., Kiertiburanakul, S., Sungkanuparph, S., Manosuthi, W., et al. HLA-B*3505 allele is a strong predictor for nevirapine-induced skin adverse drug reactions in HIV-infected Thai patients. Pharmacogenet Genomics 19 (2009): 139-146.

(56) Thamlikitkul, V. Clinical Research: Designing and Applying the Results. Siriraj Med J 58 (2006): 1112-1120.

(57) Thabane, L. Sample Size Determination in Clinical trials [online]. 2005. Available from : www.lehanathabane.com[2009, March].

(58) Bunce, M., O'Neill, C. M., Barnardo, M. C., Krausa, P., Browning, M. J., Morris, P. J., et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). Tissue Antigens 46 (1995): 355-367.

(59) Haldane, J. B. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 20 (1956): 309-311.

(60) Gordon Guyatt, D. S., Brian Haynes. Evaluating Diagnostic Tests. In R. Brian Haynes, D. L. S., Gordon H. Guyatt, Peter Tugwell (eds.), Clinical Epidermiology, 278-280. Philadelphia: Lippincott William & Wilkins, 2006.

(61) Sharma, V. K., Sethuraman, G. and Minz, A. Stevens Johnson syndrome, toxic epidermal necrolysis and SJS-TEN overlap: a retrospective study of causative drugs and clinical outcome. Indian J Dermatol Venereol Leprol 74 (2008): 238-240.

(62) Yap, F., Wahiduzzaman, M. and Pubalan, M. Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) in Sarawak: A Four Years' Review. Egyptian Dermatology Online Journal 4 (2008): 1-13.

(63) Jantararoungtong, T., Tiamkao, S., Vanaprasath, S., Choonhakarn, C., Auvichayapat, N. and Tassaneeyakul, W. Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis in Srinagarind Hospital: A Retrospective Study of Causative Drugs and Clinical Outcome. Thai J Pharmacol 31 (2009): 41-44.

(64) Shear, N. H. and Spielberg, S. P. Anticonvulsant hypersensitivity syndrome. In vitro assessment of risk. J Clin Invest 82 (1988): 1826-1832.

(65) Mansur, A. T., Pekcan Yasar, S. and Goktay, F. Anticonvulsant hypersensitivity syndrome: clinical and laboratory features. Int J Dermatol 47 (2008): 1184-1189.

(66) Sierra, N. M., Garcia, B., Marco, J., Plaza, S., Hidalgo, F. and Bermejo, T. Cross Hypersensitivity Syndrome between Phenytoin and Carbamazepine. Pharm World Sci 27 (2005): 170-174.

(67) Leenutaphong, V., Sivayathorn, A., Suthipinittharm, P. and Sunthonpalin, P. Stevens-Johnson syndrome and toxic epidermal necrolysis in Thailand. Int J Dermatol 32 (1993): 428-431.

(68) Sonthisombat, P. Currrent issues of penicillin and sulfa drugs allergy and management. In Busba Chindavijak, N. S., Wimon Anantasakulwat, Surakit Nathisuwan, Precha Montakantikul (eds.), Achieving Patients' Safety Through Pharmaceutical Care, 198. Bangkok: Prachachon, 2004.

(69) Seth, D., Kamat, D. and Montejo, J. DRESS syndrome: a practical approach for primary care practitioners. Clin Pediatr (Phila) 47 (2008): 947-952.

(70) Eshki, M., Allanore, L., Musette, P., Milpied, B., Grange, A., Guillaume, J. C., et al. Twelve-year analysis of severe cases of drug reaction with eosinophilia and systemic symptoms: a cause of unpredictable multiorgan failure. Arch Dermatol 145 (2009): 67-72.

(71) N., P. The HLA complex. In Ratanawararak., M. (eds.), Basic and Clinical Imunology, 96. Bangkok: Chulalongkorn, 2543.

(72) Romphruk, A. V., Romphruk, A., Kongmaroeng, C., Klumkrathok, K., Paupairoj, C. and Leelayuwat, C. HLA class I and II alleles and haplotypes in ethnic Northeast Thais. Tissue Antigens (2010):

(73) Hung, S. I., Chung, W. H., Liu, Z. S., Chen, C. H., Hsih, M. S., Hui, R. C., et al. Common risk allele in aromatic antiepileptic-drug induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Han Chinese. Pharmacogenomics 11 (2010): 349-356.

(74) Kazeem, G. R., Cox, C., Aponte, J., Messenheimer, J., Brazell, C., Nelsen, A. C., et al. High-resolution HLA genotyping and severe cutaneous adverse reactions in lamotrigine-treated patients. Pharmacogenet Genomics 19 (2009): 661-665.

(75) Lee, H. Y., Pang, S. M. and Thamotharampillai, T. Allopurinol-induced Stevens-Johnson syndrome and toxic epidermal necrolysis. J Am Acad Dermatol 59 (2008): 352-353.

(76) Cheng, S. H., Kwan, P., Ng, H. K. and Ng, M. H. New testing approach in HLA genotyping helps overcome barriers in effective clinical practice. Clin Chem 55 (2009): 1568-1572.

APPENDICES

APPENDIX A

2 PRANNOK Rd. BANGKOKNOI
BANGKOK 10700

Tel. (662) 4196405-6
FAX (662) 4196405

MAHIDOL UNIVERSITY
Siricaj xaar

Siriraj Institutional Review Board

Certificate of Approval

COA no.Si 513/2009

Protocol Title : PREVALENCE AND MORTALITY RATE OF SEVERE CUTANEOUS ADVERSE REACTION IN SIRIRAJ HOSPITAL.

Protocol number : 500/2552(EC3)

Principal Investigator/Affiliation : Miss Sunicha Limkobpaiboon / Department of Pharmacy
Faculty of Medicine Siriraj Hospital, Mahidol University

Research site : Faculty of Medicine Siriraj Hospital

Approval includes :

1. แบบเสนอโครงการวิจัยเพื่อขอรับการพิจารณาจากคณะกรรมการจริยธรรมการวิจัยในคน SIRB Submission Form
2. แบบบันทึกข้อมูล Case Record Form

Approval date : October 13, 2009

Expired date : October 12, 2010

This is to certify that Siriraj Institutional Review Board is in full Compliance with International Guidelines For Human Research Protection such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

.....
(Prof. Jariya Lertakyamanee, M.D.)
Chairperson

October 22, 2009
date

.....
(Clin. Prof. Teerawat Kulthanan, M.D.)
Dean of Faculty of Medicine Siriraj Hospital

October 26,2009
.....
date

หน่วยพิมพ์โรงพยาบาลศิริราช 2341 / 3,000 เล่ม / ก.ย 51 / Mat. 10023252

2 PRANNOK Rd. BANGKOKNOI
BANGKOK 10700

Tel. (662) 4196405-6
FAX (662) 4196405

MAHIDOL UNIVERSITY
Since ๒๔๙๙

Siriraj Institutional Review Board

Certificate of Approval

COA no.Si 600/2009

Protocol Title : HLA-B AND C LOCUS GENETIC POLYMORPHISM AS A MARKER OF SEVERE CUTANEOUS ADVERSE REACTIONS IN THAI PATIENT ON ALLOPURINOL

Protocol number : 534/2552 (EC1)

Principal Investigator/Affiliation : Miss Sunicha Limkobpaiboon / Pharmacy Practice Department
Faculty of Pharmaceutical Sciences, Chulalongkorn University

Research site : Faculty of Medicine Siriraj Hospital

Approval includes :

1. SIRB Submission Form
2. Protocol
3. Participant Information Sheet
4. Informed Consent Form
5. Telephone Script
6. Case Record Form

Approval date : December 3, 2009

Expired date : December 2, 2010

This is to certify that Siriraj Institutional Review Board is in full Compliance with International Guidelines For Human Research Protection such as the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

.....
(Prof. Jariya Lertakyamanee, M.D.)
Chairperson

December 9, 2009
date

.....
(Clin. Prof. Teerawat Kulthanan, M.D.)
Dean of Faculty of Medicine Siriraj Hospital

December 9,2009
.....
date

หน่วยพิมพ์โรงพยาบาลศิริราช 2341 / 3,000 แผ่น / ก.ย 51 / Mat. 10023252

APPENDIX B

เอกสารชี้แจงผู้เข้าร่วมการวิจัย

(Participant Information Sheet)

ในเอกสารนี้อาจมีข้อความที่ท่านอ่านแล้วยังไม่เข้าใจ โปรดสอบถามหัวหน้าโครงการวิจัย หรือผู้แทนให้ช่วยอธิบายจนกว่าจะเข้าใจดี ท่านอาจจะขอเอกสารนี้กลับไปอ่านที่บ้านเพื่อปรึกษาหารือกับญาติพี่น้อง เพื่อนสนิท แพทย์ประจำตัวของท่าน หรือแพทย์ท่านอื่น เพื่อช่วยในการตัดสินใจเข้าร่วมการวิจัย

ชื่อโครงการ : การตรวจหาสารพันธุกรรมชนิดเอชแอลเอ -บีและซี ที่ใช้เป็นตัวบ่งชี้การเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอล

ชื่อผู้วิจัย : เภสัชกรหญิงศุณิชา ลิ้มกอปรไพบูลย์
นิสิตปริญญาโท สาขาวิชาเภสัชกรรมคลินิก คณะเภสัชศาสตร์จุฬาลงกรณ์มหาวิทยาลัย และเภสัชกรโรงพยาบาลศิริราช

สถานที่วิจัย : โรงพยาบาลศิริราช

ผู้สนับสนุนทุนวิจัย : คณะเภสัชศาสตร์จุฬาลงกรณ์มหาวิทยาลัย

โครงการนี้ทำเพื่อศึกษาความสำคัญของสารพันธุกรรม ชนิดเอชแอลเอ -บีและซีต่อการเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอล

การศึกษานี้จะเป็นประโยชน์ต่อทั้งตัวท่านเองและแพทย์ผู้ทำการรักษา เนื่องจากตัวท่านจะทราบความเสี่ยงทางพันธุกรรมของท่านในการเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงต่อการได้รับยาอัลโลพูรินอล และจะเป็นประโยชน์สำหรับแพทย์ และเภสัชกร เพื่อที่จะหาแนวทางป้องกันและแก้ไขปัญหาที่เกิดจากการใช้ยาซึ่งส่งผลกระทบที่รุนแรงจากการตรวจสารพันธุกรรมที่บ่งชี้ต่อการเกิดอาการไม่พึงประสงค์ที่รุนแรงจากยาดังกล่าวได้

ท่านได้รับเชิญให้เข้าร่วมการวิจัยนี้เพราะ

- ❑ ท่านเคยมีประวัติการเกิดผลข้างเคียงทางผิวหนังชนิดรุนแรงจากการได้รับยาอัลโลพูรินอล
- ❑ ท่านกำลังได้รับยาอัลโลพูรินอล โดยไม่เกิดอาการแพ้ยา

ยาอัลโลพูรินอลเป็นยาลดกรดยูริกที่นิยมใช้กันอย่างแพร่หลายในการลดระดับของกรดยูริกในเลือด เนื่องจากมีประสิทธิภาพสูง และ สามารถใช้ได้ในผู้ป่วยที่มีการทำงานของไตบกพร่อง แต่ในขณะเดียวกันพบว่ายานี้ทำให้เกิดการแพ้ยาได้บ่อยจากรายงานพบว่ายานี้ทำให้เกิดผื่นที่ไม่รุนแรงได้ประมาณร้อยละ 2 ของผู้ป่วยที่รับประทานยานี้ โดยจากรายงานของกระทรวงสาธารณสุขของประเทศไทยในปี พ.ศ.2549-2551 พบว่ามีรายงานว่ายานี้ทำให้เกิดผื่นผิวหนังชนิดรุนแรงเป็นอันดับสองรองจากยาในกลุ่มซัลฟา ในอดีตการแพ้ยาเป็นเรื่องที่ไม่สามารถป้องกันได้แต่ในปัจจุบันนักวิจัยได้ค้นพบว่าปัจจัยเสี่ยงที่สำคัญอย่างหนึ่งที่ก่อให้เกิดการแพ้ยาคือลักษณะทางพันธุกรรมของบุคคลนั้น ซึ่งในปัจจุบันพบว่าสารพันธุกรรมชนิดเอชแอลเอมีผลต่อการตอบสนองต่อยาในแง่ของการเกิดอาการไม่พึงประสงค์จากยา “เอชแอลเอ” เป็นกลุ่มของยีนที่ตั้งอยู่หลายตำแหน่งบนแขนข้างสั้นของโครโมโซมคู่ที่ 6 ของมนุษย์ มีบทบาทสำคัญเกี่ยวกับการตอบสนองของระบบภูมิคุ้มกันของ ร่างกายและพบว่ามี

ความสัมพันธ์กับการเกิดการแพ้ย า นักวิจัยพบความสัมพันธ์ระหว่างสารพันธุกรรมชนิด เอชแอลเอ-บี ตำแหน่ง 5801 และ เอชแอลเอ -ซี ตำแหน่ง 0302 ในประชากรชาวไต้หวันที่มีเชื้อชาติจีน และ กลุ่มผู้ป่วยที่มีสารพันธุกรรมในตำแหน่งดังกล่าวจะมีอุบัติการณ์ของการแ พ้ยาสูงกว่าบุคคลทั่วไป สารพันธุกรรมตำแหน่งนี้พบบ่อยในชาวเอเชียมากกว่าในประเทศอื่น โดยมีรายงานในประชากรชาวจีนร้อยละ 8.8-10.9 ในผู้ป่วยชาวไทยร้อยละ 8.4 ในขณะที่ในประชากรผิวขาวพบเพียงร้อยละ 1-6

ในโครงการวิจัยนี้จะมีผู้ป่วยที่เคยมีอาการแพ้ยาทางผิวหนังชนิดรุน แรงจากยาอัลโลพูรินอลเข้าร่วมทั้งสิ้น 20 คน และผู้ป่วยที่ได้รับยาโดยไม่มีอาการแพ้ยาทางผิวหนังอย่างรุนแรงจำนวน 60 คน โดยมีระยะเวลาที่จะทำวิจัยทั้งสิ้นประมาณ 6 เดือน เมื่อท่านตัดสินใจเข้าร่วมการวิจัยแล้วท่านจะได้รับการปฏิบัติไม่แตกต่างจากการตรวจและรักษาตามมาตรฐานปกติ เพียงแต่ผู้วิจัยจะขอถามประวัติเกี่ยวกับโรคและการใช้ยาของท่าน และท่านจะได้รับการเจาะเลือดเพื่อเก็บส่งตรวจทางห้องปฏิบัติการเพิ่มเติมจำนวน 1 ช้อนชา (5 มิลลิลิตร) เพื่อตรวจหาสารพันธุกรรมดังกล่าวข้างต้น ความเสี่ยงที่อาจจะเกิดขึ้นเมื่อเข้าร่วมการวิจัยไม่แตกต่างจากการเจาะเลือดตามปกติ โดยความเสี่ยงที่อาจจะเกิดขึ้นมีน้อยมาก และถ้าเกิดขึ้นแล้วก็สามารถหายได้เอง เช่นการห้อเลือด สำหรับค่าใช้จ่ายในการตรวจวินิจฉัยและรักษาโรคยังคงเป็นไปตามสิทธิปกติที่ท่านมี การกระทำดังกล่าวจะทำร่วมไปกับการรักษาพยาบาลตามปกติ และไม่มีการให้ยาใดๆในการวิจัยนี้

หากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัย มีข้อข้องใจที่จะสอบถามเกี่ยวข้องกับการวิจัย หรือเมื่อบาดเจ็บ /เจ็บป่วยจากการวิจัย ท่านสามารถติดต่อได้ที่ เภสัชกรหญิง ศุณิชา ลิ้มกอปรไพบูลย์ ฝ่ายเภสัชกรรม โรงพยาบาลศิริราช โทรศัพท์ที่ติดต่อได้สะดวกคือ 081-929-0094

หากท่านไม่เข้าร่วมในโครงการวิจัยนี้ ท่านก็จะได้รับการตรวจเพื่อการวินิจฉัย และรักษาโรคของท่านตามวิธีการที่เป็นมาตรฐาน หากมีข้อมูลเพิ่มเติมทั้งด้านประโยชน์และโทษที่เกี่ยวข้องกับการวิจัยนี้ ผู้วิจัยจะแจ้งให้ทราบโดยเร็วโดยไม่ปิดบัง

ข้อมูลส่วนตัวของผู้เข้าร่วมการวิจัยจะถูกเก็บรักษาไว้ ไม่เปิดเผยต่อสาธารณะเป็นรายบุคคลแต่จะรายงานผลการวิจัยเป็นข้อมูลรวม ข้อมูลของผู้ร่วมการวิจัยเป็นรายบุคคลอาจมีคณะบุคคลบางกลุ่มเข้ามาตรวจสอบได้ เช่น ผู้ให้ทุนวิจัย, สถาบัน หรือองค์กรของรัฐที่มีหน้าที่ตรวจสอบ, คณะกรรมการจริยธรรมฯ เป็นต้น และข้อมูลจะเก็บไว้เป็นเอกสาร /แผ่นซีดี/ไฟล์ไว้ต่อเป็นเวลา 2 ปีหลังสิ้นสุดการวิจัย โดยหัวหน้าโครงการวิจัยเป็นผู้รับผิดชอบในการรักษาความลับผู้เข้าร่วมการวิจัย ผู้เข้าร่วมการวิจัยมีสิทธิ์ถอนตัวออกจากโครงการวิจัยเมื่อใดก็ได้ โดยไม่ต้องแจ้งให้ทราบล่วงหน้า และการไม่เข้าร่วมการวิจัยหรือถอนตัวออกจากโครงการวิจัยนี้จะไม่มีผลกระทบต่อการบริการและการรักษาที่เป็นมาตรฐานของการรักษาโรคตามที่ท่านควรจะได้รับแต่ประการใด หากท่านได้รับการปฏิบัติที่ไม่ตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงนี้ ท่านสาม ารถแจ้งให้ประธานคณะกรรมการจริยธรรมฯ ทราบได้ที่ สำนักงานคณะกรรมการจริยธรรมการวิจัยในคน ตึกอดุลยเดชวิกรม ชั้น 5 ร.พ.ศิริราช เบอร์โทร. 02419-7000 ต่อ 6405

ข้าพเจ้าได้อ่านรายละเอียดในเอกสารนี้ครบถ้วนแล้ว

ลงชื่อ.....วันที่.....

(.....)

APPENDIX C

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย

(Informed Consent Form)

วันที่..... เดือน..... พ.ศ.....

ข้าพเจ้า.....อายุ.....ปี อาศัยอยู่บ้านเลขที่.....ถนน.....แขวง/ตำบล..... ..... เขต/อำเภอ.....จังหวัด.....รหัสไปรษณีย์..... ..... โทรศัพท์ .....

ขอแสดงเจตนายินยอมเข้าร่วมโครงการวิจัยเรื่อง การตรวจหาสารพันธุกรรมชนิดเอชแอลเอ-บีและซี (HLA-B, HLA-Cw) ที่ใช้เป็นตัวบ่งชี้การเกิดอาการไม่พึงประสงค์ทางผิวหนังชนิดรุนแรงในผู้ป่วยไทยที่ใช้ยาอัลโลพูรินอล (allopurinol)

โดยข้าพเจ้าได้รับทราบรายละเอียดเกี่ยวกับที่มาและจุดมุ่งหมายในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือ ได้รับการปฏิบัติ ประโยชน์ที่คาดว่าจะได้รับของการวิจัย และความเสี่ยงที่อาจจ ะเกิดขึ้นจากการเข้าร่วมการวิจัย รวมทั้งแนวทางป้องกันและแก้ไขหากเกิดอันตรายขึ้น ค่าใช้จ่ายที่ข้าพเจ้าจะต้องรับผิดชอบจ่ายเอง โดยได้อ่านข้อความที่มีรายละเอียดอยู่ในเอกสารชี้แจงผู้เข้าร่ วมการวิจัยโดยตลอด อีกทั้งยังได้รับคำอธิบายและตอบข้อสงสัยจากหัวหน้าโครงการวิจัยเป็นที่เรียบร้อยแล้ว

ข้าพเจ้าจึงสมัครใจเข้าร่วมในโครงการวิจัยนี้

หากข้าพเจ้ามีข้อข้องใจเกี่ยวกับขั้นตอนของการวิจัย หรือหากเกิดผลข้างเคียงที่ไม่พึงประสงค์จากการวิจัยขึ้นกับ ข้าพเจ้า ข้าพเจ้าจะสามารถติดต่อกับ เภสัชกรหญิงศุณิชา ลิ้มกอปรไพบูลย์ ฝ่ายเภสัชกรรม โรงพยาบาลศิริราช 081-9290094 หากข้าพเจ้าได้รับการปฏิบัติไม่ตรงตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถติดต่อกับประธานคณะกรรมการจริยธรรมการวิจัยในคนได้ที่ สำนักงานคณะกรรมการจริยธรรมการวิจัยในคน ตึกอดุลยเดชวิกรม ชั้น 6 ร.พ.ศิริราช โทร. (02) 419-6405-6 โทรสาร (02) 419-6405

ข้าพเจ้าได้ทราบถึงสิทธิ์ที่ข้าพเจ้าจะได้รับข้อมูลเพิ่มเติมทั้งทางด้านประโยชน์และโทษจากการเข้าร่วมการวิจัย และสามารถถอนตัวหรืองดเข้าร่วม การวิจัยได้ทุกเมื่อโดยไม่ต้องแจ้งล่วงหน้าหรือระบุเหตุผล โดยจะไม่มีผลกระทบต่อการบริการและการรักษาพยาบาลที่ข้าพเจ้าจะได้รับต่อไปในอนาคต และยินยอมให้ผู้วิจัยใช้ข้อมูลส่วนตัวของข้าพเจ้าที่ได้รับจากการวิจัย แต่จะไม่เผยแพร่ต่อสาธารณะเป็นรายบุคคล โดยจะนำเสนอเป็นข้อมูลโดยรวมจากการวิจัยเท่านั้น

ข้าพเจ้าได้เข้าใจข้อความในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และหนังสือแสดงเจตนายินยอมนี้โดยตลอดแล้ว จึงลงลายมือชื่อไว้

ลงชื่อ.....ผู้เข้าร่วมการวิจัย/ผู้แทนโดยชอบธรรม/วันที่.....

(.....)

ลงชื่อ.....ผู้ให้ข้อมูลและขอความยินยอม/หัวหน้าโครงการวิจัย/วันที่.....

(.....)

ในกรณีผู้เข้ าร่วมการวิจัยอ่านหนังสือไม่ออก ผู้ที่อ่านข้อความทั้งหมดแทนผู้เข้าร่วมการวิจัยคือ .....จึงได้ลงลายมือชื่อไว้เป็นพยาน

ลงชื่อ.....พยาน/วันที่.....

(.....)

APPENDIX D

แบบเก็บข้อมูลผู้ป่วยที่เกิดอาการไม่พึงประสงค์จากยา allopurinol

จากแฟ้มเวชระเบียนผู้ป่วยนอก โรงพยาบาลศิริราช

ข้อมูลทั่วไปของผู้ป่วย (demographic data)

1. รหัสผู้ป่วย……อายุ……ปี

   เพศ ❑ ชาย ❑ หญิง

   อาจารย์แพทย์เจ้าของไข้…… คลินิค……..

2. เชื้อชาติ/สัญชาติของผู้ป่วย…….

   บิดา…….....

   มารดา…….

3. โรคที่เป็นร่วมด้วย

   ❑ 1 โรค ❑ 2 โรค

   ❑ 3 โรค ❑ 4 โรค

   ❑ มากกว่า 4 โรค

   ระบุชื่อ

   โรค……

4. ประวัติการแพ้ยาตัวอื่นร่วมด้วย

   ❑ มี ระบุชื่อยา…….. ผลการประเมิน……

   วันที่……..

   ❑ ไม่มี

5. การรักษาในปัจจุบัน - การใช้ยาอื่นทดแทนยา allopurinol

   ❑ benzbromarone

   ❑ ไม่ได้ใช้ยาอื่นทดแทน

ข้อมูลเกี่ยวกับอาการไม่พึงประสงค์จากยา allopurinol

1. ปีพ.ศ.ที่เกิดอาการ……อายุที่เกิดอาการไม่พึงประสงค์……ปี

2. อาการไม่พึงประสงค์จากยา

❑ SJS ❑ TEN ❑ HSS ❑ Other……

3. ขนาดยาที่ใช้ก่อนเกิดอาการไม่พึงประสงค์ (dose/day)……mg/day

4. ยาที่ใช้ร่วมด้วยระหว่างที่เกิดอาการ

1. diuretic ❑ thiazide ❑ nonthiazide

2. antibiotic ❑ amoxicillin/ampicillin ❑ other……..

3. Other …….

วันที่เริ่มใช้ยา		วันที่ admit	
วันที่เกิดอาการ		วันที่ D/C	
วันที่หยุดยา		ระยะเวลาที่อยู่ ร.พ.	
Duration of drug expose			

6. ค่าการทำงานของไตขณะได้รับยา (SCr)…….mg/dl

7. ยาที่ผู้ป่วยได้รับนั้นมีข้อบ่งใช้ตามเกณฑ์หรือไม่

1. symptomatic hyperuricemia

❑ gouty arthritis

❑ stone

2. asymtomatic hyperuricemia

❑ ได้รับ chemotherapy (tumor lysis syndrome prevention)

❑ ไม่ได้รับ chemotherapy (no indication)

8. ขนาดยาที่ผู้ป่วยได้รับนั้นตรงตามขนาดยาที่แนะนำให้ปรับตามค่าการทำงานของไตหรือไม่

❑ ตามขนาดที่แนะนำ ❑ มากกว่าขนาดที่แนะนำเมื่อคำนวณตาม CrCl

9. หลังได้รับยาสามารถควบคุมระดับกรดยูริกให้ได้ตามเกณฑ์ (5-6 mg/dl) หรือไม่

❑ สามารถควบคุมได้ ❑ ไม่สามารถควบคุมได้

10. อาการนำก่อนมาโรงพยาบาล (prodrome)

❑ มี ระบุ…… ❑ ไม่มี

11. Systemic involvement

❑ acute hepatocellular injury

❑ worsening renal function

❑ hematologic disorder

12. Mucous membrane erosions, number : site

❑ oral

❑ eyes

❑ genital

13. ผลของการเกิดอาการไม่พึงประสงค์จากยา allopurinol

❑ หายเป็นปกติ

❑ หายโดยมีร่องรอยเดิม

❑ เสียชีวิต เนื่องจากสาเหตุอื่นที่ไม่เกี่ยวกับยา

❑ เสียชีวิต เนื่องจากสาเหตุจากยา

14. ระดับการประเมินอาการไม่พึงประสงค์จากยา

❑ certain ❑ possible

❑ probable ❑ unlikely

15. วันที่ทำการเจาะเลือด……

16. ผลการตรวจ HLA typing ……

APPENDIX E

แบบเก็บข้อมูลผู้ป่วยในกลุ่มควบคุมที่รับประทานยา allopurinol

จากแฟ้มเวชระเบียนผู้ป่วยนอก โรงพยาบาลศิริราช

ข้อมูลทั่วไปของผู้ป่วย (demographic data)

1. รหัสผู้ป่วย……อายุ……ปี

   เพศ ❑ ชาย ❑ หญิง

2. เชื้อชาติ/สัญชาติของผู้ป่วย…….

   บิดา…….....

   มารดา…….

3. โรคที่เป็นร่วมด้วย

   ❑ 1 โรค ❑ 2 โรค

   ❑ 3 โรค ❑ 4 โรค

   ❑ มากกว่า 4 โรค

   ระบุชื่อโรค……

4. ประวัติการแพ้ยาตัวอื่นร่วมด้วย

   ❑ มี ระบุชื่อยา…….. ผลการประเมิน…….

   วันที่…….

   ❑ ไม่มี

ข้อมูลเกี่ยวกับการใช้ยา allopurinol

5. ระดับกรดยูริกในเลือด……..mg/dl
6. ค่าการทำงานของไตขณะได้รับยา (SCr)…….mg/dl
7. ขนาดยาที่ใช้ในปัจจุบัน (dose/day)……mg/day
8. มีข้อบ่งใช้ตามเกณฑ์หรือไม่……
9. ระยะเวลาตั้งแต่เริ่มใช้ยา……..วัน/เดือน
10. วันที่เจาะเลือด……..
11. HLA genotype…….....

APPENDIX F

Table F1 SCAR Characteristics, HLA-genotypes and some related demophaphic data of the SCAR group

No.	Age /sex	Type of SCAR	indication	Dose (mg)	Scr (mg/dl)	Duration of exposure	Mucosa involvement	Internal organ damage	*HLA B*5801*	*HLA Cw*0302*
1	77/M	SJS	Hyperuricemia	200	0.8	34	(+) oral	No	Positive	Positive
2	85/M	SJS	Gouty arthritis	100	1.2	1*	(+) eye, oral, genital	No	Positive	Positive
3	50/M	SJS	Gouty arthritis	300	1.0	20	(+) eye, oral	LFI, eosinophilia	Positive	Positive
4	60/M	HSS	Hyperuricemia	300	1.5	14	No	LFI, ARF, eosinophilia	Positive	Positive
5	56/M	SJS	Gouty arthritis	300	1.2	25	(+) eye, oral, genital	LFI	Positive	Positive
6	65/M	SJS	Gouty arthritis	200	2.1	24	(+) eye	LFI	Positive	Positive
7	69/F	HSS	Hyperuricemia	N/A	0.9	20	No	LFI, eosinophilia	Positive	Positive
8	70/F	SJS	Gouty arthritis	300	1.3	21	(+) eye, genital	No	Positive	Positive
9	42/F	SJS	Hyperuricemia	600	1.4	30	(+) eye, oral	LFI	Positive	Positive
10	67/F	HSS	Gouty arthritis	200	1.2	33	(+) eye, oral, genital	LFI, ARF, eosinophilia	Positive	Positive
11	62/M	HSS	Gouty arthritis	300	2.2	27	No	LFI, ARF	Positive	Positive
12	72/M	HSS	Gouty arthritis	300	N/A	20	No	LFI, ARF	Positive	Positive
13	80/F	SJS	Gouty arthritis	300	N/A	N/A	(+) eye, genital	N/A	Positive	Positive
14	22/M	HSS	Gouty arthritis	200	1.8	35	(+) oral	LFI, ARF	Positive	Positive
15	75/M	SJS	Gouty arthritis	300	1.6	7	(+) oral, genital	LFI	Positive	Positive
16	70/F	SJS	Hyperuricemia	100	0.8	14	(+) oral	No	Positive	Positive

SJS, Steven-Johnson syndrome, TEN, Toxic epidermal necrolysis, HSS, Drug hypersensitivity syndrome, LFI, Liver function injury ARF, Acute renal failure

Table F1 SCAR characteristics, HLA-genotypes and some related demophaphic data of the SCAR group (cont.)

No	Age /sex	Type of SCAR	indication	Dose (mg)	Scr (mg/dl)	Duration of exposure	Mucosa involvement	Internal organ damage	*HLA B*5801*	*HLA Cw*0302*
17	57/F	SJS	Gouty arthritis	300	1.3	25	(+) eye, oral, genital	No	Positive	Positive
18	38/F	SJS	Gouty arthritis	300	4	20*	(+) eye, oral	No	Positive	Positive
19	67/M	HSS	Gouty arthritis	100	2.6	72	(+) oral	LFI, ARF, eosinophilia	Positive	Positive
20	80/F	SJS	Hyperuricemia	300	N/A	20	(+) eye	N/A	Positive	Positive
21	67/F	HSS	Hyperuricemia	300	0.9	27	(+) eye	LFI	Positive	Positive
22	53/M	SJS	Gouty arthritis	300	1.2	24	(+) eye, oral, genital	LFI	Positive	Positive
23	56/M	HSS	Gouty arthritis	200	1.4	30	No	LFI	Positive	Positive
24	82/F	SJS	Hyperuricemia	300	1.1	21	(+) eye	LFI	Positive	Positive
25	75/M	TEN	Hyperuricemia	300	1.3	8	(+) eye, oral	Eosinophilia	Positive	Positive

SJS, Steven-Johnson syndrome, TEN, Toxic epidermal necrolysis, HSS, Drug hypersensitivity syndrome , LFI, Liver function injury ARF, Acute renal failure

Table F2 Other type of skin characteristics, HLA-genotypes and some related demophaphic data of the other type of skin group

No	Age/sex	Type of SCAR	indication	Dose (mg)	Scr (mg/dl)	Duration of exposure	Mucosa involvement	Internal organ damage	*HLA B*5801*	*HLA Cw*0302*
1	73/M	Exfoliative	Gouty arthritis	200	2.1	21*	No	No	Positive	Positive
2	77/M	Exfoliative	Gouty arthritis	N/A	N/A	10*	No	RFI	Positive	Positive
3	78/M	Exfoliative	Gouty arthritis	200	1.8	35	No	RFI	Positive	Positive
4	80/F	Exfoliative	Gouty arthritis	100	1.0	1*	No	Eosinophilia	Positive	Positive
5	71/M	Exfoliative	Gouty arthritis	300	1.2	N/A	No	LFI, eosinophilia	Positive	Positive
6	78/M	MP rash	Gouty arthritis	100	1.4	1*	No	N/A	Positive	Negative
7	70/M	Eczema	Hyperuricemia	300	1.5	14	No	No	Negative	Negative
8	78/F	Fix drug	Stone	100	1.4	2 year	No	No	Negative	Negative
9	77/F	MP rash	Gouty arthritis	100	1.7	85	No	No	Negative	Negative

Exfoliative, Exfoliative dermatitis, MP rash, maculopapular rash, Fix drug, Fix drug eruption

Table F3 Control characterictic, HLA-genotypes and some related demophaphic data of the control group

No.	Age/sex	indication	Dose (mg)	Scr (mg/dl)	Recommended dose	Thiazide used	Diabetes	*HLA B*5801*	*HLA Cw*0302*
1	51/M	Gouty arthritis	300	0.9	Recommended	No	No	Negative	Negative
2	67/F	Gouty arthritis	300	1.1	Over dose	No	No	Negative	Negative
3	63/M	Gouty arthritis	400	1.1	Over dose	No	No	Negative	Negative
4	55/F	hyperuricemia	100	1.2	Recommended	Thiazide	No	Negative	Negative
5	57/M	Gouty arthritis	300	1.1	Recommended	Thiazide	No	Negative	Negative
6	74/M	Gouty arthritis	100	1.5	Recommended	No	No	Negative	Negative
7	51/M	Gouty arthritis	300	1.1	Recommended	No	No	Positive	Positive
8	75/M	Gouty arthritis	300	1.5	Over dose	No	No	Positive	Positive
9	70/M	Gouty arthritis	200	1.1	Recommended	No	No	Negative	Negative
10	82/M	Gouty arthritis	350	1.1	Over dose	Thiazide	No	Negative	Negative
11	80/M	Gouty arthritis	150	1.3	Recommended	No	No	Negative	Negative
12	71/M	Gouty arthritis	100	1.0	Recommended	No	No	Negative	Negative
13	55/M	Gouty arthritis	200	1.1	Recommended	No	No	Negative	Negative
14	52/M	Gouty arthritis	400	1.2	Recommended	No	No	Negative	Negative
15	72/M	Gouty arthritis	100	1.4	Recommended	Thiazide	No	Negative	Negative
16	55/M	Gouty arthritis	300	1.1	Recommended	No	No	Negative	Negative
17	50/M	Gouty arthritis	200	1.1	Recommended	No	No	Negative	Negative

Table F3 Control characterictic, HLA-genotypes and some related demophaphic data of the control group (cont.)

No.	Age/sex	indication	Dose (mg)	Scr (mg/dl)	Recommended dose	Thiazide used	Diabetes	*HLA B*5801*	*HLA Cw*0302*
18	67/M	Gouty arthritis	200	0.9	Recommended	No	No	Negative	Negative
19	49/M	Gouty arthritis	200	1.5	Recommended	No	diabetes	Negative	Negative
20	67/M	Gouty arthritis	300	0.8	Recommended	No	No	Negative	Negative
21	59/M	Gouty arthritis	150	1.7	Recommended	No	No	Negative	Negative
22	78/M	Gouty arthritis	150	1.3	Recommended	No	No	Negative	Negative
23	64/M	Gouty arthritis	300	1.0	Recommended	No	No	Negative	Negative
24	72/M	Gouty arthritis	150	1.1	Recommended	No	No	Negative	Negative
25	43/M	Gouty arthritis	300	1.0	Recommended	No	No	Negative	Negative
26	47/M	Gouty arthritis	300	1.1	Recommended	No	No	Negative	Negative
27	79/F	Gouty arthritis	150	1.9	Recommended	Thiazide	No	Negative	Negative
28	77/M	Gouty arthritis	200	1.4	Recommended	No	No	Negative	Negative
29	50/M	Gouty arthritis	200	1.0	Recommended	Thiazide	No	Negative	Negative
30	48/M	Gouty arthritis	200	1.1	Recommended	No	No	Negative	Negative
31	55/M	Gouty arthritis	200	0.9	Recommended	No	No	Negative	Negative
32	75/M	Gouty arthritis	200	1.2	Recommended	No	No	Negative	Negative
33	49/M	Gouty arthritis	300	1.4	Over dose	Thiazide	No	Negative	Negative
34	52/M	Gouty arthritis	200	0.8	Recommended	No	No	Positive	Positive

Table F3 Control characterictic, HLA-genotypes and some related demophaphic data of the control group (cont.)

No.	Age/sex	indication	Dose (mg)	Scr (mg/dl)	Recommended dose	Thiazide used	Diabetes	*HLA B*5801*	*HLA Cw*0302*
35	66/M	Gouty arthritis	200	1.6	Over dose	No	No	Positive	Positive
36	53/M	Gouty arthritis	300	0.9	Recommended	Thiazide	No	Negative	Negative
37	77/M	Gouty arthritis	100	3.0	Recommended	Thiazide	diabetes	Positive	Positive
38	61/M	Gouty arthritis	100	0.9	Recommended	No	No	Positive	Positive
39	32/M	Gouty arthritis	300	1.2	Recommended	No	No	Negative	Negative
40	33/M	hyperuricemia	300	0.8	Recommended	No	No	Negative	Negative
41	56/M	Gouty arthritis	200	1.1	Recommended	No	No	Negative	Negative
42	42/M	Gouty arthritis	300	1.1	Recommended	No	No	Negative	Negative
43	50/M	Gouty arthritis	300	1.0	Recommended	No	No	Negative	Negative
44	47/M	Gouty arthritis	200	1.3	Recommended	No	No	Positive	Positive
45	70/M	Gouty arthritis	300	1.3	Over dose	Thiazide	diabetes	Negative	Negative
46	65/M	Gouty arthritis	200	1.2	Recommended	Thiazide	No	Negative	Negative
47	58/M	Gouty arthritis	200	1.9	Over dose	Thiazide	No	Negative	Negative
48	71/M	Gouty arthritis	300	1.3	Over dose	No	No	Negative	Negative

APPENDIX G

Table G1 HLA genotype and result from model to predict probability of allopurinol hypersensitivity.

No.	*HLA-B*5801*	Gender	Diabetic	HLA-genotype	Probability
1	Positive	Male	No	15/58	0.610
2	Positive	Male	No	40/58	0.610
3	Positive	Male	No	39/58	0.610
4	Positive	Male	No	39/58	0.610
5	Positive	Male	No	40/58	0.610
6	Positive	Male	No	15/58	0.610
7	Positive	Female	No	39/58	0.975
8	Positive	Female	No	46/58	0.975
9	Positive	Female	No	40/58	0.975
10	Positive	Female	No	40/58	0.975
11	Positive	Male	Yes	15/58	0.976
12	Positive	Male	No	57/58	0.610
13	Positive	Female	No	40/58	0.975
14	Positive	Male	No	46/58	0.610
15	Positive	Male	Yes	46/58	0.976
16	Positive	Female	Yes	40/58	0.999
17	Positive	Female	Yes	07/58	0.999
18	Positive	Female	Yes	15/58	0.999
19	Positive	Male	No	46/58	0.610
20	Positive	Female	Yes	27/58	0.999
21	Positive	Female	No	40/58	0.975
22	Positive	Male	No	56/58	0.610
23	Positive	Male	Yes	51/58	0.976
24	Positive	Female	No	38/58	0.975
25	Positive	Male	Yes	40/58	0.976
26	Negative	Male	No	07/56	0.008

Table G1 HLA genotype and result from model to predict probability of allopurinol hypersensitivity. (cont.)

No.	*HLA-B*5801*	Gender	Diabetic	HLA-genotype	Probability
27	Negative	Female	No	15/27	0.169
28	Negative	Male	No	15/40	0.008
29	Negative	Female	No	15/40	0.169
30	Negative	Male	No	15/40	0.008
31	Negative	Male	No	35/46	0.008
32	Positive	Male	No	18/58	0.610
33	Positive	Male	No	40/58	0.610
34	Negative	Male	No	38/44	0.008
35	Negative	Male	No	40/44	0.008
36	Negative	Male	No	40/46	0.008
37	Negative	Male	No	39/44	0.008
38	Negative	Male	No	39/46	0.008
39	Negative	Male	No	07/15	0.008
40	Negative	Male	No	27/27	0.008
41	Negative	Male	No	40/46	0.008
42	Negative	Male	No	15/15	0.008
43	Negative	Male	No	07/35	0.008
44	Negative	Male	Yes	46/55	0.174
45	Negative	Male	No	40/51	0.008
46	Negative	Male	No	18/27	0.008
47	Negative	Male	No	13/51	0.008
48	Negative	Male	No	15/15	0.008
49	Negative	Male	No	40/51	0.008
50	Negative	Male	No	27/40	0.008
51	Negative	Male	No	15/44	0.008
52	Negative	Female	No	46/54	0.169
53	Negative	Male	No	15/55	0.008
54	Negative	Male	No	46/52	0.008

Table G1 HLA genotype and result from model to predict probability of allopurinol hypersensitivity.(cont.)

No.	*HLA-B*5801*	Gender	Diabetic	HLA-genotype	Probability
55	Negative	Male	No	13/15	0.008
56	Negative	Male	No	15/51	0.008
57	Negative	Male	No	15/27	0.008
58	Negative	Male	No	18/46	0.008
59	Positive	Male	No	13/58	0.610
60	Positive	Male	No	27/58	0.610
61	Negative	Male	No	15/46	0.008
62	Positive	Male	Yes	46/58	0.976
63	Positive	Male	No	27/58	0.610
64	Negative	Male	No	15/40	0.008
65	Negative	Male	No	40/44	0.008
66	Negative	Male	No	15/39	0.008
67	Negative	Male	No	35/51	0.008
68	Negative	Male	No	40/44	0.008
69	Positive	Male	No	44/58	0.610
70	Negative	Male	Yes	40/46	0.174
71	Negative	Male	No	18/40	0.008
72	Negative	Male	No	40/48	0.008
73	Negative	Male	No	51/54	0.008
74	Positive	Male	Yes	40/58	0.976
75	Positive	Male	No	46/58	0.610
76	Positive	Male	No	55/58	0.610
77	Positive	Female	No	58/58	0.975
78	Positive	Male	Yes	18/58	0.976
79	Positive	Male	Yes	40/58	0.976
80	Negative	Male	Yes	44/54	0.174
81	Negative	Female	Yes	44/52	0.838
82	Negative	Female	Yes	40/46	0.838

APPENDIX H

BUFFER AND SOLUTIONS

1. Solution A

Ammonium chloride	6.35	gm
EDTA	1.33	gm
Trizma base	0.92	gm
add dd H_20 up to	1000	ml

2. 10% SDS

SDS	100	gm
add dd H_20 up to	1000	ml

3. 7.5 M Guanidine HCl

Guanidine HCl	216	gm
1M Tris-HCl pH 7.6	30	ml
add dd H_20 up to	300	ml

Filtered through 0.2 um filter membrane

4. 1 M Tris-HCl (pH7.6)

Tris-HCl	121.1	gm
dd H_20	900	ml

adjust pH to 7.6 with conc. HCl

add dd H_20 up to	1000	ml

Sterilize by autoclaving

5. 80% Ethanol

Absolute Ethanol	80	ml
dd H_20 up to	100	ml

6. Proteinase K (10mg/ml)

add dd H_20 up	1000	ml
Sterilize by autoclaving		

7. **TE BUFFER (pH 8.0)**

1 M Tris- HCl pH 8.0	10	ml
0.5 M EDTA pH 8.0	2	ml
add dd H_20 up to	1000	ml

8. **1 M $MgCl_2$**

$MgCl_2$	203.3	gm
add H_20	1000	ml
Sterilize by autoclaving		

9. **10X PCR Buffer** (for Phototyping)

670 mM	Tris base pH8.8
166 mM	Ammonium Sulphate
20 mM	Magnesium chloride
1%	Tween 20

10. **5 mg/ml Ethidium Bromide**

Ethidium Bromide	50	mg
dd H_20	10	ml

11. **10X TBE Buffer**

TRIZMA base	54.0	gm
Boric acid	27.5	gm
0.5 M EDTA pH 8.0	20.0	ml
add dd H_20 up to	500	ml

12. **1.5% Agarose Gel (For Horizon 11.14)**

Agarose	1.95	gm
1X TBE	130	ml
Boil		
add 5 mg/ml Ethidium bromide	13	µl

13. **Gel Loading Buffer**

Glycerol	30	ml
Bromphenol Blue	100	mg
1X TBE up to	100	ml

14. **Marker**

Phi X 174 Hae III fragment	20	ul
dd H_2O	150	ul

VITA

Ms.Sunicha limkobpaiboon was born on eleventh of May in 1979 at Siriraj Hospital, Bangkok. After graduation from The Faculty of Pharmaceutical Science, Silpakorn University in 2002 she started to work as hospital pharmacist in Phutthisong Hospital, Burirum Province for two years and then work in Department of Pharmacy, Siriraj Hospital, Mahidol University in April 2004. She had been enrolled in a study program for Master degree of Pharmacy Practice Department, Faculty of Pharmaceutical Sciences, Chulalongkorn University since June 2008.