GENETIC ASSOCIATION OF *SLC2A9* VARIANTS WITH GOUT SUSCEPTIBILITY IN MALAY POPULATION

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By

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LIST OF ABBREVIATIONS

µl	:microlitre
µmol	:micromole
ABC	:ATP-binding cassette
ABCG2	:ATP-binding cassette sub-family G member 2
ACR	:American College of Rheumatology
ALLO	:Allopurinol
AMP	:Adenosine monophosphate
ATP III (NCEP)	:National Cholesterol Education Program Expert Panel III
BMI	:Body mass index
BP	:Blood pressure
BW	:Wash buffer
CI	:Confidence interval
CKD	:Chronic kidney disease
CVD	:Cardiovascular disease
dbSNP	:Database SNP
dNTP	:Deoxynucletide triphosphate
DHR (PDZ)	:Disc large homologous region
DM	:Diabetes mellitus
eNOS	:Endothelial nitric oxide synthase
GC	:Guanine cytosine
GCKR	:Glucokinase regulatory protein
GLGF	:Glycine-leucine-glycine-phenylalanine

GLUT9	:Glucose transporter member 9
GLUT9N	:Glucose transporter family 9 with a shorter N terminal region
GWAS	:Genome wide association studies
HDL	:High density lipoprotein
HPFS	:Health Professional Follow up study
HPL	:Hyperlipidemia
HPT	:Hypertension
HWE	:Hardy Weinberg equilibirium
IDF	:International Diabetes Federation
IL-6	:Interleukin-6
IL-1β	:Interleukin-1β
IMP	:Inosine monophosphate
IqR	:Interquartile range
LD	:Linkage disequilibirium
LDL	:Low density lipoprotein
LRRC16A	:Leucine rich repeat containing 16A
MAF	:Minor allele frequency
MCT	:Intracellular monocarbpxylates
MetS	:Metabolic syndrome
MRP	:Multidrug resistance associated protein
MSU	:Monosodium urate
MTP	:Metatarsal phalangeal
NALP3	:PYD domains-containing protein 3
NO	:Nitric oxide

NPT1/ vOAT1	:Voltage driven organic anion transporter 1
OA	:Organic anion
OAT4	:Organic anion transporter 4
OAT10	:Organic anion transporter 10
OD	:Optical density
OA	:Osteoarthritis
OR	:Odd ratio
PDZK1	*:*PDZ Domain containing 1
RA	:Rheumatoid arthritis
RR	:Relative risk
SUA	:Serum uric acid
SLC2A9	:Solute carrier family 2, facilitated glucose transporter member 9
SLC5A8	:Solute carrier family 5 (sodium/monocarboxylate cotransporter), member 8
SLC5A12	:Solute carrier family 5 (sodium/monocarboxylate cotransporter), member 12
SLC13A3	:Solute carrier family 13 (sodium-dependent dicarboxylate Transporter) member 3
SLC16A9	:Solute carrier family 16, member 9
SLC17A2	:Solute carrier family 17, member 2
SLC17A3	:Solute carrier family 17 (organic anion transporter), member 3
SLC22A12 (URAT1)	:Solute carrier family 22 (organic anion/cation transporter), member 12
SMCT1	:Sodium-coupled (Na +-coupled) transporter

SNP	:Single nucleotide polymorphism
T2DM	:Type2 diabetes mellitus
TE Buffer	:Tris and EDTA buffer
TBE	**:**Tris/Borate/EDTA
TG	:Triglycerides
TNF-α	:Tumor necrosis factor-α
U	:Unit
UA	:Uric acid
QTL	:Quantitave trait loci
WHO	:World Health Organization
XO	:Xanthine oxidase

KAJIAN GENETIK VARIAN SLC2A9 DALAM POPULASI MELAYU TERHADAP GOUT

ABSTRAK

Gout adalah sejenis penyakit sendi yang terbentuk disebabkan oleh pengumpulan asid uric (berbentuk jarum) di bahagian sendi tertentu. Ianya berlaku apabila paras asid urik dan ketepuan fisiologi meningkat melebihi paras cecair dalam badan. Perkumuhan asid urik dalam buah pinggang dikawal oleh gen *SLC2A9* yang bertindak sebagai pengangkut asid urik dan terlibat dalam pembentukan benjolan pada penyakit gout. Oleh sebab itu, objektif utama kajian ini adalah untuk menguji perkaitan empat varian genetik dalam gen *SLC2A9* dan ciri-ciri demografi gout di kalangan populasi Melayu. Sampel-sampel darah diambil dari darah keseluruhan sebanyak 3 mililiter dan diekstrak menggunakan Qiagen Extraction Kit. Sementara itu, *genotyping* ke atas keempat-empat genetic varian yang diberi nama *rs11942223, rs16890979, rs5028843* dan *rs3733591* dijalankan dengan menggunakan *Polymerase Chain Reaction-Restriction Fragment Length Polymorphism* (PCR-RFLP). Purata kekerapan alel yang kecil (MAF) dan nisbah ganjil (OR) untuk 89 sampel pesakit adalah 0.018 dan 4.374 dimana ia menunjukkan kuasa penyelidikan ini adalah sebanyak 80%. Keseimbangan Hardy-Weinberg dikira dengan menggunakan perisian program *SHEsis* yang menyediakan nisbah ganjil (OR) dan 95% *confidence interval*. Data kami mengesahkan peranan gen *SLC2A9* terhadap kecenderungan ke atas gout dalam keturunan Melayu yang dilihat oleh kesan risiko (OR>2.0). Alel minor *rs3733591, rs1194223* dan *rs5028843* mempengaruhi aktiviti SLC2A9 di dalam artikular kondrosit seterusnya meningkatkan risiko pemendapan kristal uric asid dan pembentukan

tophi. Secara keseluruhan, gabungan perkaitan haplotaip 1/2/1/1, 1/1/2/1 dan 1/1/1/2 di dalam kajian kami telah menunjukkan perkembangan risiko ke atas gout.

ABSTRACT

Gout is an inflammatory arthritis that is caused by the formation of multiple needles like uric acid crystals in joints. It occurs when serum uric acid (SUA) levels rise and the physiological saturation is exceeded in body fluids. Renal urate excretion is controlled by the *SLC2A9* gene which acts as a renal urate transporter and involves in the developing of tophaceous gout. Therefore the main objective of this study is to test genetic association of four common variants of *SLC2A9* gene and demographic features of gout in Malay population. Blood samples were taken from 3ml whole blood according to Qiagen Extraction Kit and genotyping was conducted utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for 4 SNPs namely *rs5028843, rs3733591, rs11942223* and *rs16890979.* The average Minor Allele Frequency (MAF) of 0.018 with OR of 4.374 requires 89 samples of cases that project 80% power of study. Hardy Weinberg equilibirium was calculated by using SHEsis online software providing odd ratios and 95% confidence interval. Our data confirmed the role of the *SLC2A9* gene of susceptibility of gout in the Malay descendents in Malaysian population which was seen by the effect of susceptible risk of (OR>2.0). Our study showed that the minor allele of *rs3733591, rs1194223* and *rs5028843* have influenced on the activity of *SLC2A9* in articular chondrocytes increases the risk for deposition of MSU crystals and tophi formation. In summary, the association of 1/2/1/1, 1/1/2/1 and 1/1/1/2 haplotypes confer haplotypes which reflects the increase of gout development.

CHAPTER 1

INTRODUCTION

1.1 Research locale

1.1.1 Background of the research

Gout is an inflammatory arthritis that is linked with low quality of life (Roddy *et al.,* 2007 and Lee *et al.,* 2009). According to Niskanen *et al.,* (2004), gout is a disease caused by the formation of multiple needles like uric acid crystals in joints.

It occurs when serum uric acid (SUA) level rises and the physiological saturation is exceeded in body fluids. The formation of monosodium urate (MSU) crystals will occur in and around articular joints. The evidence in several studies had clearly shown the relation of uric acid levels with hypertension, inflammation, increased risk of cardiovascular disease and metabolic syndrome such as gout.

The prevalence of gouty arthritis may vary depending on geographical area, race and gender. In the USA, the prevalence of gout increased from 2.9/1,000 in 1990 to 5.2/1,000 in 1999 (Wallace *et al.,* 2004). Epidemiological studies undertaken in UK suggested the prevalence of gout per 1,000 was 2.6 in 1975 (Currie, 1979), 3.4 in 1987 and 9.5 in 1993 (Steven, 1992). In Asian countries, successive surveys had been taken in

China (Qingdao), which reported an increased trend from 3.6/1,000 in 2002 (Nan *et al.*, 2006) to 5.3/1,000 in 2004 (Miao *et al.,* 2008). Previous reports have revealed the incidence of hyperuricemia and gout is higher in Asians who live in the USA compared to those groups living in their homeland. For instance, Filipinos who lived in Hong Kong or in the USA have significantly higher number of gouty patients than those living in their country of origin (Emmerson, 1983).

In Malaysia, a developing country, there is a shortage of research and the limited studies being done are likely to be the same with other developing countries. Darmawan *et al.*, 1992 reported that the environmental factors might also be one of the factors contributing to the manifestation of gouty arthritis. Based on gender, males are much more susceptible to gout than females and most of the cases occurred at the age of over 40 years old. The onset of gout in female patients occurred at an older age in conjunction with menopause and was more frequently associated with renal sufficiency, diuretic therapy and monoarticular involvement (Chen *et al.*, 1989). Estrogens in females have uricosuric effects, therefore gout is very rarely observed among younger females (Anton *et al.*, 1986).

Many drugs have been introduced to gouty patients which may increase or decrease the serum uric acid (SUA) levels. Allopurinol is the widely used drug for gout in the long term management. It reduces uric acid by inhibiting the enzyme of xanthine oxidase (XO) which is responsible for the production of urate (Stamp *et al.*, 2010). However concurrent medications such as colchicines, non steroidal inflammatory agents

also need to be administered in the first three to six months to prevent gout flares due to the usage of allopurinol.

Gout is a multifactorial disease caused by both environmental and genetic factors. Studies have implicated the excessive alcohol consumption, dietary and genetic factors showed association with the development of gout. In terms of genetic factors, many candidate genes and involved in the development of arthritis which includes *SLC2A9, ABCG2, SLC22A12* and *URAT1* gene (Tu *et al.*, 2010).

Polymorphisms in those genes have been observed to be associated with the susceptibility of gouty patients among European and Asian populations with comparable risk alleles between these populations (Tu et al., 2010). Contribution of various mutations was varied between different populations and it may be due to long-term activity of environmental factors and past events, such as immigration and endemic disease.

In 2004, Portis and his colleagues (Portis *et al.*, 2004) reported the incidence of gout is among aborigines, together with the adoption of a more Western lifestyle. For instance, the Hmong which is one of the Asian ethnic groups from the mountainous region of China, who started the migration after the Vietnam conflict in 1975 (Lemoine *et al.*, 2005). Therefore, further research and studies on the combination between genetic and environmental factors are needed to enhance the better understanding towards molecular pathogenesis of gouty arthritis.

1.1.2 Rationale of the study

There has been a growing interest in the studies of gouty arthritis in population worldwide (Nan *et al.,* 2006). However the studies on the genetic association among Malay population are still lacking. Due to the lack of data, a case-control study was conceded in order to test the genetic associations within *SLC2A9* gene in gouty arthritis among Malay population.

This study was done based on the genome-wide association studies (GWAS) which have been carried out in worldwide populations such as Maori, Germany, Han Chinese, Caucasians and Sardinia Chianti cohorts (Li *et al.,* 2007). GWAS is an active scanning marker of genomes which will select genetic variations associated with the selective disease. This scanning marker is useful in contributing to the better strategies of detecting and treating the particular disease once the genetic associations are initiated.

Many candidate genes have been identified for several complex diseases (Moffatt *et al.,* 2007), for instance gouty arthritis. Recent studies of GWAS have identified SNPs in a genomic region on chromosome 4. The *ABCG2* gene and *SLC2A9* gene are the two most common genes which interpose the regulation of uric acid synthesis (Yang *et al.,* 2005) and proved significant association with gouty arthritis and other complex metabolic diseases. Thence, this study was conducted to replicate the GWAS analysis which have been done on several population studies and compared with the Malay population. It is hoped that this apprentice study in Malaysia will provide evidence from association to causation on the etiology of gout.

1.1.3 Objectives

1.1.3.1 General objective

To test the genetic association of four common variants of *SLC2A9* gene and demographic features of gout in Malay population

1.1.3.2 Specific objectives

1. To determine the association of demographic data (age, BMI and family history) with serum urate level

2. To determine single association of SLC2A9 single nucleotide polymorphisms with gout development

3. To determine the role of haplotype in gouty arthritis

4. To identify other possible variants within *SLC2A9* gene using fine mapping approach in Haploview software.

1.2 Genetics of metabolic syndrome

1.2.1 Metabolic syndrome

The Metabolic syndrome (MetS) is defined by the grouping of cardiovascular risk factors and caused an increased mortality for cardiovascular disease (Zimmet *et al.,* 2005). This grouping has been said to be as "Syndrome X, the deadly quartet, and the insulin resistance syndrome". It has been reported that insulin resistance is a reason that caused the abnormal pathophysiologic condition (Tsouli *et al.,* 2006). Multiple studies

have initiate that individuals with metabolic syndrome are at an increased chance of getting cardiovascular diseases (Grundy *et al.,* 2005 and Wilson *et al.,* 2005).

Hyperuricemia reflects insulin resistance (Carnethon *et al.,* 2003; Coasta *et al.,* 2002). Increased serum uric acid levels which subsequently cause gout often comes together with obesity, dyslipidemia, and hypertension. In Taiwanese adults, it has been reported that there is an encouraging association between metabolic syndrome and serum uric acid level (Lohsoonthorn *et al.,* 2006) but little information for the Malaysian adults is available.

Subsequently, several criteria were used in addition with World Health Organization (WHO) as to identify individuals who have the risks namely, the National Cholesterol Education Program Expert Panel III (ATP III) and the International Diabetes Federation (IDF) (Alberti *et al.,* 2006). **Table 1.1** shows the criteria proposed for common clinical diagnosis which are insulin resistance, body weight, lipid, blood pressure, glucose and others of the clustered syndrome.

Table 1.1: Criteria Proposed for Clinical Diagnosis of the Metabolic Syndrome

Clinical measure	**WHO (1998)**	**NCEP/ATP III (2001)**	**IDF (2005)**
Insulin resistance	IGT, IFG, T2DM or low insulin sensitivity, plus any two of the following	None but any three of the following features	None
Body weight	Males: waist to hip ratio >0.90; females : waist to hip ratio >0.85; and/or BMI >30kg/m^2	WC ≥102cm in men or ≥ 88cm in women	Increased WC (population specific) plus any two of the following
Lipid	TG> 150mg/d L and/or HDL-C<35mg/d L in men or <39mg/d L in women.	TG>150mg/d L	TG>150mg/d L
Blood pressure	≥140/90mm/Hg	≥130/85mm/Hg	≥130mm/Hg systolic
Glucose	IGT, IFG, or T2DM	≥110 mg/dL (includes diabetes)‡	≥100 mg/dL (includes diabetes)
Other	Microalbuminuria		

Abbreviations:

IGT=Impaired glucose tolerance

TG=Triglyceride

WC=Weight code

IFG=Impaired fasting glucose

HDL=High Density Lipoprotein

T2DM=Type 2 diabetes mellitu

Based on WHO, ATP III and IDF definitions, the prevalence of MetS among the Malaysian adults was 32.1, 34.3 and 37.1%, respectively. Regardless of the definition used, Malaysia seemed to record a higher prevalence compared to other Asian countries, such as India (Deepa *et al.,* 2007), Hong Kong (Ko *et al.,* 2006) and China (Gu *et al.,* 2005) where prevalence ranged from 6.1 to 18.3%, when based on ATP III definition, and increased to 9.6 to 25.8%, when data were analyzed using IDF criteria. The numbers observed in Malaysians was similar to other non-Asian populations, such as the United States (34.4%) and in Iran (35.6%) (Ervin *et al.,* 2009).

Despite the higher prevalence rates of MetS in Malaysia when compared to other Asian countries, studies on various populations in Malaysia itself are still scarce (Mohamud *et al.,* 2010). In Europe, the prevalence rates of metabolic syndrome have a range of 20-30% (Qiao, 2006 and Grundy, 2008) whereas the prevalence rates among migrant Asians have a range of 14-49% (Misra and Khurana, 2009).

1.2.2 Pathophysiology of metabolic syndrome

The metabolic reaction of cellular, chemical and soluble protein components in the human metabolism play a fundamental role to maintain normal body functions. However, the pathogenesis of metabolic syndrome remains unclear (Eckel *et al.,* 2005).

Tumor necrosis factor-α (TNF- α) is the best candidate to correlate with metabolic syndrome abnormalities. This multifunctional cytokine has been suggested to lead to the production of proinflammatory cytokine but is also trigger cell signaling by

interaction with a TNFα receptor. This in return leads to insulin resistance (Fukuchi *et al.*, 2004). Increase of adipose tissue leads to increase of immune cells and play a part in inflammation.

Inflammation which includes redness and an increased local supply of white blood cells is the body's response to injury. The purpose is to defend against infections and repair damaged tissue. The incident of having chronic inflammation will contributes to an increased risk of gout and several other MetS which are hypertension, artherosclerosis and diabetes (Whitney *et al.,* 2011).

1.2.3 Risk factors of metabolic syndrome

MetS has becoming more common in Asian and the United States. The etiology of the progression of metabolic syndrome is complex (**Figure 1.1**) and is determined by the interaction of both genetics and environmental factors.

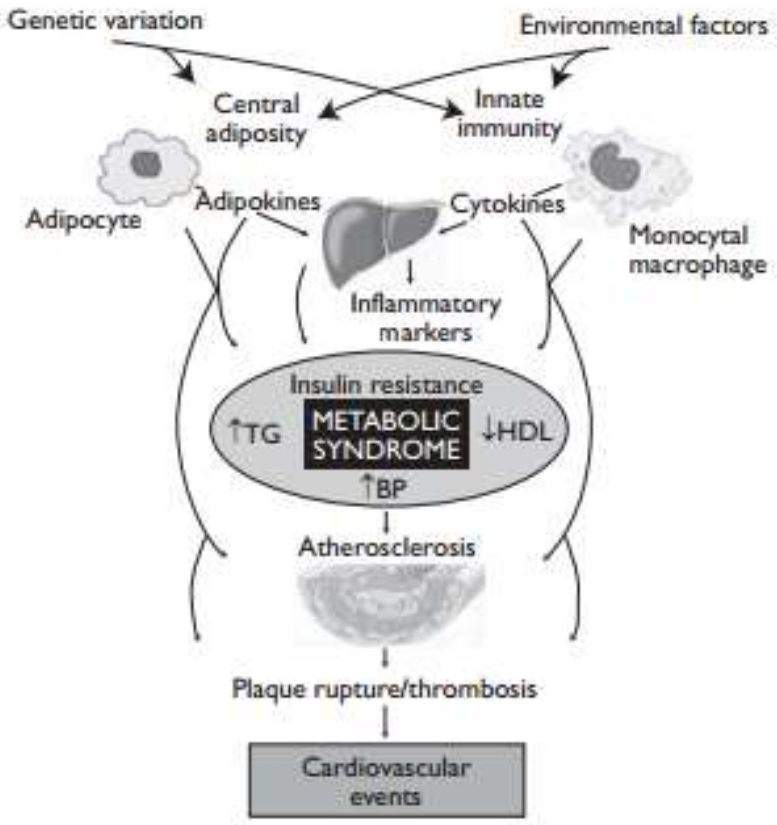

Figure 1.1: The pathophysiology of atherosclerotic cardiovascular diseases in the metabolic syndrome. Common genetic variants and environmental factors may lead to the development of atherosclerosis by affecting central adiposity, innate immunity, glucose and lipoprotein metabolism, and vascular function. BP = blood pressure; HDL= high-density lipoprotein; TG =triglycerides (American Heart Association, 2006).

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1.2.3.1 Environmental factor interaction

MetS has been classified as a lifestyle disease. Its prevalence is increasing worldwide as a consequence of the obesity epidemic. The two most significant risk factors that contribute to the disease is central obesity (extra weight around the middle and upper parts of the body) (Fauci *et al.*, 2008) and also the insulin resistance (body uses insulin effectively than normal insulin) (Misra *et al.*, 2009).

Based on the World Health Organization (http://www.who.int), approximately 1 billion adults are overweight (body mass index > 25 kg/m^2), and 300 million of these are considered clinically obese (body mass index > 30 kg/m^2). The identification of an overweight person is identified by a person with having too much fat, extra muscle and water. Other risk factors that topped the list are aging at where the MetS affect 44% of the US population more than the age of 50. Women are prone to have an increased risk of having the syndrome rather than men in most populations (Fauci *et al.*, 2008).

Thus, the factors contributing to recent changing patterns in MetS prevalence in most of geographic region may provide interesting insights into tackling the ever burden of cardiovascular risk.

1.2.3.2 Genetic factor interaction

In addition to these environmental factors, the genetic component in the pathogenesis of the MetS has also been investigated. Genetics components have been included as a risk factor to understand the genotype-phenotype interactions of this syndrome. These genetic variants may act in accordance with other variants and environmental factors.

1.2.4 Genetic analyses in metabolic syndrome

Seevral evidence suggests that metabolic syndrome is the result of the relationship between several genes and prosperous environment. It is apparent that all components in the syndrome are strongly inherited.

1.2.4.1 Application of genomewide scan and association approaches for complex human diseases

Genomewide scan is an application to locate for genetic loci that gives effect to human diseases (Wiggs *et al.,* 2000). Through this application, the genetic regions will be easily localized with regards to the disease-predisposing genes (Wray *et al.,* 2013).

Firstly, the chromosomal region will be identified whether it is linked to the disease or not, later on the linkage disequilibrium (LD) mapping will be carried out to

constrict down the region of interest. The identification of the genes and the variants that cause MetS is conducted by positional cloning without influencing the biologic role of the gene.

The susceptibility of the candidate genes can also be tested using the association studies. Genes are selected based on its potential role towards the pathogenesis of its variant or its location within a region of interest in the gene which identified by the screening of genome wide scan linkage. Basically, the association studies are being done based on genetic variation. Genetic variation has been described as a genetic difference among individuals of the same species (Barrett *et al.,* 2008). Mating patterns, genetic drift and migration are the reasons that lead to the increased and decreased genetic variation (Keinan *et al.,* 2007).

Mating patterns are mostly referred to non random mating due to most organisms choose to mate based on certain characteristics. There are two forms of non random mating which are inbreeding and outbreeding. Inbreeding happens to mate together with similar genotypes rather than with different genotypes (Keller *et al.,* 2002). On the other hand if there is an increased chance for the individuals with certain genotypes to mate with another particular genotype it is called outbreeding.

Other than this, genetic drift can also occur by changing the relative allele frequency in a population. This phenomenon can increase or decrease over time. Population bottleneck in a genetic drift happens when a group of individuals in a

population pass away which are avoided from mating. This in return will lead to drastic decrease of population sizes (Parmesan, 2006). Genetic drift plays an impart role in the species evolution because it result in the loss of rare alleles and the size of the gene pool decrease will cause a new population to be different in original later on (Hallatschek *et al.*, 2010).

Lastly is the incidence of migration. Migration is referred to the movement of organisms to different location, which often means the movement of individuals into or out of a certain population. Sudden changes of alleles can happen if the migrating individuals stay and mate with the individuals in the new location (International HapMap, 2010)

1.2.4.2 Interpretation of genetic association studies and detection using GWAS (Genomewide Association Study).

The field of genetics serves as one of the most powerful and straight approach to explore the basis of human disease. The genetic approaches have led to recognition of variations in human genome based on single-nucleotide polymorphisms (SNPs). The identified variations allow the GWAS to recognize any significant relationship between genetic and clinical aspects. This in turn triggers a cascading reaction of clinical treatment based on the markers used for diagnosis and prognosis of the said disease.

GWAS is capable to generate large sample sets of potential genes that are believed to have functional significance on the manifestation of a disease (Yamamoto and Yamada 2007) and screening thousands of SNPs in the genome at a time. These studies are now common and are beginning to identify regions which contain disease susceptibility loci (Lowe *et al.,* 2007).

The primary step of GWAS is by localizing the likely candidates of causal variants which will be validated subsequently perform the re-sequencing to identify polymorphisms in the associated region and to search the LD within the region. So genotyping is vital in order to localize the associated polymorphisms in the disease. Accurate and precise identification will lead to statistically powerful investigations between genotype and phenotype correlations (Lowe *et al.,* 2007).

However there are several reasons suggesting that most of the findings reported on associations are inaccurate (Ioannidis *et al.,* 2001). The main reasons for this include inadequate sample size, genetic heterogeneity of human populations, nature of the illness and associated presence of confounding risk factors (Kruglyak et al.2001).

Some SNPs are functional, which may raise or reduce the risk of illness than others. Functional SNPs, including missense coding SNPs and non exonic regulatory SNPs,that might be implicated in splicing mechanism are more likely to be evolutionarily deleterious or beneficial. There are many more unknown variants expected to contribute to general diseases. Therefore, the discovery of the disease associated with genetic

variants and their basic biologic pathways is not an easy task, and is a main obstacle in human genetic studies (Barnes *et al.*, 2007).

1.3 Gouty arthritis

1.3.1 Definition of gouty arthritis

Gout is an inflammatory arthritis that has risen in prevalence for recent decades (Roddy *et al.*, 2007). This prevalent metabolic disorder is characterized by chronic hyperuricaemia, a condition of elevated serum uric acid (SUA) levels (>6.8 mg/dL or ≥360 mmol/L) (Terkeltaub, 2010). The increment of SUA levels can result in the formation of monosodium urate monohydrate crystals (tophi). It is deposited in the soft tissues in and around the joints, thus invigorating an inflammatory response (Terkeltaub *et al.*, 2010 and Wortmann *et al.*, 2002). This in turn will lead to acute arthritis with severe pain, most commonly affecting the mobility (Mclean *et al.*, 2003). The articular and peri-articular inflammation in chronic gout can lead to the degradation of osseous tissue (**Figure 1.2**) and the most commonly affected region is at the base of the big toe.

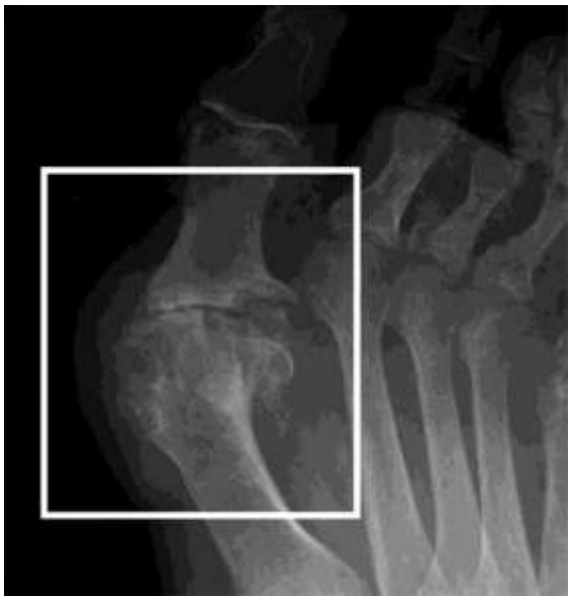

Figure 1.2: Radiograph of gout in human foot as shown in rectangular box showed damage to the osseous tissue. This image suggests a significant relationship between the presence of the gouty tophus and bone erosions in asymptomatic chronic tophaceous gouty arthritis on high resolution ultrasonography. It is the eroding tophus that is crucial for the development of bone erosions in gouty arthritis (Dalbeth *et al.*, 2014).

The gold standard of diagnosing gouty arthritis is to make obvious the incidence of monosodium urate monohydrate (MSU) crystals in synovial fluid when the patient was combating the attack (Wallace *et al.,* 2004). Janssens *et al.* (2010) reported that the synovial fluid analysis was rarely being performed by them to demonstrate the presence of urate crystals due to the lack of expertise itself to demonstrate and limitation of access to the polarizing microscope.

Synovial fluid examination has become the best practice in clinical settings; however it may not be practical especially given the incidence of gout and massive sample size that are required within the population researches. Besides that, if we were to look up in several studies, the terms such as gout flare, chronic gouty and also acute gouty be frequently being used, but to the best of our knowledge it seemed to be that these terms have been differently used by different authors. Therefore, in order to aid the study results, reasonable clarification variety of manifestations and stages of the disease may require.

1.3.2 Epidemiology of gout

Gouty arthritis, which is considered as a metabolic syndrome is the major health problem and the number is increasing worldwide. It is reported that around 20-25% of the population in the world has metabolic syndrome (Alberti *et al.,* 2006) and the prevalence differs according to geographic location, nationality, population characteristics and the

criteria of metabolic syndrome itself (Huang and Jayakar, 2010 and Romaguera *et al.*, 2010). Malaysia has been listed as the top ten countries with the highest prevalence of metabolic syndrome such as gout and diabetes mellitus (Shaw *et al.*, 2010).

If based on genders, previous studies have originated with the intention of metabolic syndrome with uric acid was higher among men than women in Americans with (35.1% versus 32.6%) (Erum, 2009), Europeans (32.2% versus 28.5%) (Qiao, 2006), Malaysian (54.7% versus 45.3%) (Moy and Bulgiba, 2010), Koreans (21.7% versus 11.4%) (Hwang *et al.*, 2009), and Japanese (11.6% versus 4.0%) (Kuzuya *et al.*, 2007). **Figure 1.3** shows the prevalence rates in UK for gout patients that are predominant in men.

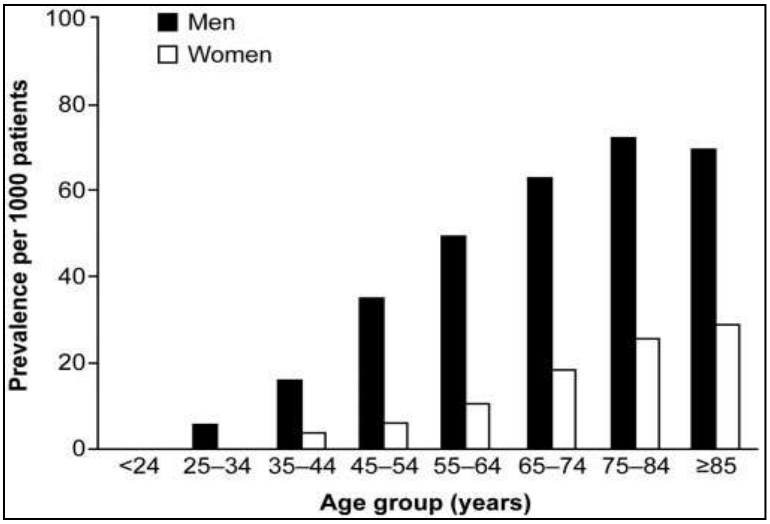

Figure 1.3: Prevalence of gout in the UK based on gender and age. The graph showed that gout is mainly a disease of older men in the UK. Gout is apparent in women after the menopause (Mikuls *et al.*, 2005).

The epidemiological connecting hyperuricemia and gout was recognized over 150 years ago with an increasing prevalence particularly in the elder age group. In the United States, the prevalence of gout in the US population after the age of 75 years of old between the year 1990 and 1999, the increased from 21 per 1000 to 41 per 1000 population (Wallace *et al.,* 2004). A year later, a second study conducted in the United Kingdom in an adult population, estimated over 7% at the age of 75 years old developed gout (Mikuls *et al.,* 2005). The same tendency has been found in the studies in China (Lin *et al.,* 2006 and Yoo *et al.,* 2005) with the proof in Qingdao, China in which the increase of gout has been reported as an increase trend from 3.6/1000 in 2002 (Nan *et al.,* 2006) to 5.3/1000 in 2004 (Miao *et al.,* 2008). It is indeed still hard to draw a clear cut of statistical epidemiology of gout among the Malays since more information and studies are needed to enhance the better understanding of the pathogenesis and epidemiology of gout.

1.3.3 Symptoms and clinical aspects of gout

Gout has been recognized as the most readily amendable of all common systemic rheumatic disease (Bieber *et al.,* 2004) leading to joint destruction and disability if untreated (Sundy *et al.,* 2007). It is basically an acute inflammatory monoarthritis generally lead to the great toe metatarsal phalangeal (MTP) joint (Cameron *et al.,* 2005) which is known as podagra. Besides toe, other joints affected are ankle, knee and wrist. The development of rigorous pain and tenderness of gout reach in 6-24 hours of onset and will spontaneously resolve within several days to two weeks. The extensive of first

attack commonly affected a single joint but oligo-articular and polyarticular gout can happen markedly in the older patient (De Leonardis *et al.,* 2007).

The metabolic disorder of gouty arthritis can elevate serum uric acid level that for years before an actual gouty attack occurs. The excessive of uric acid will lead to the development of monosodium urate (MSU) crystals which can be seen in **Figure 1.4**, and subsequently caused formation of tophi. A vascularity of connective tissues, especially the cartilage, is believed to be the major predisposing factor for the deposition of urate. Nevertheless, the MSU crystals may also be deposited in the synovial membrane, particularly when the inflammation occurs (Dalbeth *et al.,* 2008).

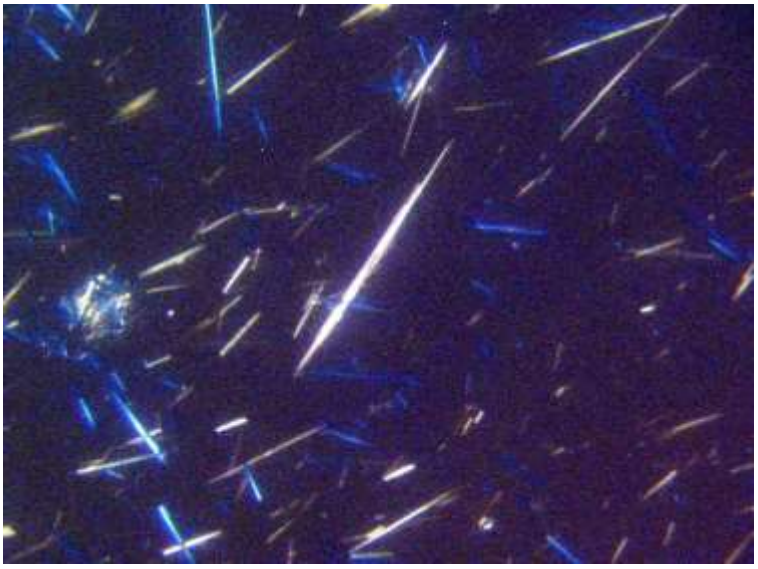

Figure 1.4: Findings of gout for MSU crystals under a polarizing microscope. A section has been stained with nonaqueous alcoholic eosin staining (NAES) to demonstrate birefringent crystals in gout (Shidham *et al.*, 2001).

Tophus (singular: tophi) which is composed of MSU crystals in matrix of lipids, protein and mucopolysaccharides are known as the trademark of chronic gout and occurs inside subcutaneous tissues and the joints. Dalbeth *et al.* (2010) reported that tophus are usually non delicate, however it may cause mechanical obstructions of joint movement and muscoskeletal disability. This is in accordance with the study done by Thiele *et al.* (2005), tophi will grow mutely for years and demonstrated by the ultrasonographic "double contour sign" (a thin coating of monosodium urate (MSU) on the surface of cartilage) in asymptomatic joints.

The basis of criteria to assess the prevalence of gout in epidemiologic studies has been described since 1960 to ease the rheumatologists in classifying the disease. In 1963, the Rome criteria were proposed during a symposium on population studies, (refer to **Table 1.2**). This criteria together with the New York's; which are the adjustment of the Rome criteria and are based on the expertise, intended for the application of epidemiologic studies (Johnson *et al.,* 2007). The criteria heavily leaned on the presence of tophi and the detection of MSU crystals in synovial fluid. This gives reason why both criteria sets are barely used in the large epidemiologic studies.