第84回総会シンポジウム

Ⅱ. 結核における宿主遺伝要因研究の現状と展望

座長 [1]慶長 直人 [2]山口 悦郎

キーワーズ：結核，遺伝要因，疾患感受性

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   Rika Yuliwulandari (Department of Human Genetics, Graduate School of Medicine, The University of Tokyo; Faculty of Medicine, Yarsi University, Jakarta Indonesia)
2. Association of *IL12RB1* polymorphisms with susceptibility to and severity of tuberculosis in Japanese: a gene-based association analysis of 21 candidate genes
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4. Genome-wide linkage analysis for tuberculosis in Thais: the way forward with human genetics
   Surakameth Mahasirimongkol (National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand)

ヒトの疾病を予防し，早期に診断，治療し，治癒に導くためには，鍵となる分子や分子機構を解明することが重要である。しかしながら，ヒトの疾患病変部を直接，基礎研究に用いることが難しい場合，動物モデルから得られた知見からヒトの病態を推定する以外には，従来，よいアプローチの方法がなかった。近年，ゲノム分野の急速な発展が，多くの疾患の発症素因解明に確かな貢献を果たし始めている。感染症分野もその例外ではない。

結核については，結核菌に対する宿主側の感受性，抵抗性を研究することにより，接触者の感染防御，感染者の効果的な発症阻止，薬剤抵抗性，難治性，重症結核に対する新しい治療法の糸口が見いだされるかもしれない。ところが，現在，この領域を専門とするわが国の研究者は少なく，むしろ，結核の高蔓延国を多く含むアジア各国の研究者が，自国の切実な問題として，結核における宿主遺伝要因の探索に真剣に取り組んでいる。

本シンポジウムでは，Rika Yuliwulandari先生に，インドネシアにおける結核発症候補遺伝子研究の進捗について，九州大学（現：産業医科大学医学部小児科学教室）の楠原浩一先生に，主にTh1免疫系候補遺伝子から結核の発症，重症化に関わる遺伝子の研究について，Nguyen Thuy Thuong Thuong/Nguyen Thi Hue先生には，ベトナムの結核性髄膜炎に着目したゲノムワイド関連解析に関するデータを，Surakameth Mahasirimongkol先生には，タイの結核罹患同胞対分析の結果をお話しいただいた。

この分野におけるわが国の現状と，東南アジアの国々の若手研究者による，結核症に対するヒト感受性遺伝子研究の急速な進展を目の当たりにして，各研究者がどのような共通理解のもとに，何を求めて研究に取り組んでいるのか，さらに，わが国のアジアへのさらなる貢献の可能性を探る機会となったものと考える。

[1]国立国際医療センター研究所呼吸器疾患研究部，[2]愛知医科大学医学部内科学講座呼吸器・アレルギー内科

連絡先：慶長直人，国立国際医療センター研究所呼吸器疾患研究部，〒162–8655 東京都新宿区戸山1–21–1
（E-mail: keicho@ri.imcj.go.jp）
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GENETIC PREDISPOSITION TO TUBERCULOSIS IN ASIA
— Present Approach and Future Directions —

Chairpersons: [1]Naoto KEICHO and [2]Etsuro YAMAGUCHI

Identification of key molecules or risk factors is essential for disease control. Although we had limited measures to this approach in the past, recent progress in human genome research has enabled us to specify essential genes or molecular mechanism for development of a variety of human diseases including infectious diseases.

Tuberculosis remains a crucial problem in Asian countries. Especially recent spread of drug-resistant tuberculosis is a potential threat all over the world. Under such circumstances, a few outstanding Asian investigators are now seriously and actively searching for host genes involved in tuberculosis, often in collaboration with foreign institutes, although we have little opportunity to know their findings directly.

In this symposium, we learned their current consensus about usefulness of host genetic research in tuberculosis and their ultimate goals of studies from four distinguished guest speakers from Indonesia, Vietnam, Thailand and Japan. They are young pioneers in the field of host genetics of tuberculosis. I believe this symposium provided a chance for mutual understanding and future collaboration in the field of host genetics around tuberculosis among Asian countries.

Key words: Tuberculosis, Genetic predisposition, Disease susceptibility

[1]Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, [2]Division of Respiratory Medicine and Allergology, Department of Medicine, Aichi Medical University School of Medicine

Correspondence to: Naoto Keicho, Department of Respiratory Diseases, Research Institute, International Medical Center of Japan, 1−21−1, Toyama, Shinjuku-ku, Tokyo 162−8655 Japan. (E-mail: keicho@ri.imcj.go.jp)

1. Polymorphisms of HLA Genes in Western Javanese (Indonesia) and Their Association to Tuberculosis

Rika YULIWULANDARI[1, 2], Tripanjiasih SUSMIARSIH[2], Kouichi KASHIWASE[3], Fumiaki NAKAJIMA[3], Helmia HASAN[4], Yulino AMRIE[5], and Katsushi TOKUNAGA[1]

[1]Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, [2]Faculty of Medicine, Yarsi University, Jakarta Indonesia, [3]Tokyo Red Cross Blood Center, Tokyo, Japan, [4]Department of Respiratory Disease, Dr. Soetomo Hospital/Faculty of Medicine, Airlangga University, Surabaya Indonesia, [5]Respiratory Hospital, Cisarua Indonesia

Abstract Human Leukocyte Antigen (HLA) genes diversity has been studied in Western Javanese (Indonesia) and their role in susceptibility to tuberculosis (TB) was examined. HLA-A, -B and -DRB1 were genotyped in 237 unrelated healthy Western Javanese (Indonesia) and 216 PTB patients by the high-resolution polymerase chain reaction-Luminex method. The most frequently detected alleles at the serological level were HLA-A24 (f=0.69), -B15 (f=0.68), and -DR12 (f=0.59). The phylogenetic tree analysis showed that Western Javanese (Indonesia) was closest to Southeast Asian populations. Based on the allele positivity, HLA-DR4 and -DR13 showed a weak association to PTB (p=0.01 pc=ns and p=0.03 pc=ns, respectively). This study suggests that HLA genes do not strongly contribute to the development of PTB in Western Javanese population.

Introduction

Human leukocyte antigen (HLA) class I and class II genes exhibit the highest degrees of polymorphisms in the human genome. The major role of HLA molecules is the presentation of a various self and non-self antigen peptides to T lymphocytes[1]. Since allele and haplotype frequencies of the HLA loci differ considerably among various human populations, identification of HLA types has become a valuable tool for anthropological studies in addition to their essential roles in disease susceptibility or resistance including tuberculosis (TB)[1].

HLA polymorphisms at 3 loci in Indonesian have not been well investigated. The population comprise 41.7% Javanese, 15.4% Sundanese, and 42.9% others. It is worthwhile to

further study the genetic link of the population to other Asian populations based on HLA genes diversity. In addition, as other Asian countries with large populations such as China and India, Indonesia also suffers from a high tuberculosis burden, an on going major public health problem in the world. Based on the World Health Organization (WHO) report in 2009, the incidence of TB in Indonesia is 228/100,000 population/year[2]. HLA associations with respect to *Mycobacterium tuberculosis* infection susceptibility and protection will be important to know the role of the gene in the defense mechanisms against the pathogen in the population.

Material and Methods

This study was approved by the ethics committees of the Faculty of Medicine, Yarsi University, Jakarta Indonesia and the Graduate School of Medicine, University of Tokyo Japan. In total 237 unrelated healthy individuals and 216 PTB patients in West Java, Indonesia participated to this study. PTB was diagnosed by the presence of acid-fast bacilli (AFB) in direct sputum smears and by standard clinical and radiological examination.

Genomic DNA was extracted from peripheral lymphocytes using a QIAamp™ DNA Blood Mini Kit (Qiagen Sciences, Maryland, USA). HLA -A, -B and -DRB1 loci were typed with a Luminex Multi-Analyte Profiling system (xMAP) and WAK-Flow HLA typing kit (Wakunaga, Hiroshima, Japan), as previously described[3].

Allele frequencies were calculated by the direct counting method. Genetic distances among the populations were calculated by the modified Cavalli-Sforza (DA) distance method[4]. A phylogenetic tree was constructed by the neighbor-joining (NJ) method using DISPAN software[5]. Principal component analysis (PCA) was performed using XLSTAT-PRO software (http://www.xlstat.com/en/support/tutorials/pca.html). For these analyses, four-digit sequence level allele frequencies for HLA-A, -B, and -DRB1 were used (data is not shown). The allele frequencies for other Asian populations were obtained from HLA data base (http://www.allelefrequencies.net).

The association between HLA and TB was examined by comparing the HLA allele positivity frequencies between controls and TB patients using the χ^2 test. The Fisher exact test was applied for expected numbers <5. A probability level of $p<0.05$ was considered statistically significant. Obtained p-values were further adjusted (p_c) by applying the Bonferroni correction for the number of comparisons for each locus.

Results

The frequency of HLA-A, -B, and -DRB1 allele positivity in PTB patients and controls at the serological level is shown in Table. All alleles with frequencies lower than 0.01 in the any of the 2 subject groups were classified as "others". The most frequent HLA-A, -B and -DR alleles were A24 ($f=0.69$), B15 (0.68) and DR12 (0.59) in addition to DR15 (0.49), respectively (Table). All frequent alleles are also commonly found in other Southeast and Northeast Asian populations.

The phylogenetic tree constructed by the NJ method based on HLA-A, -B and -DRB1 allele frequency at the four-digit sequence level in various populations (Fig.) showed that the Western Javanese (Indonesia) population was located in Southern East Asian group cluster, especially closest to Southeast Asian populations such as Malay, Filipino, Thai, and Vietnamese.

In the association analysis between the HLA alleles with PTB, only HLA-DR4 and HLA-DR13 showed a significant result ($p=0.01$ $pc=0.18$ and $p=0.03$ $pc=0.45$, respectively).

Table HLA-A, -B, -DRB1 serology-level allele positivity in controls and primary TB patients

No	Allele	Control		Case		p	pc
		n	f	n	f		
1	A1	12	0.05	5	0.02	ns	
2	A2	64	0.27	68	0.3	ns	
3	A3	11	0.05	9	0.04	ns	
4	A11	73	0.31	69	0.30	ns	
5	A24	163	0.69	157	0.69	ns	
6	A26	5	0.02	2	0.01	ns	
7	A29	5	0.02	4	0.02	ns	
8	A30	5	0.02	12	0.05	ns	
9	A32	2	0.01	4	0.02	ns	
10	A33	70	0.3	64	0.28	ns	
11	A34	33	0.14	29	0.13	ns	
12	Others	1	0	6	0.02	ns	
1	B7	12	0.05	10	0.04	ns	
2	B13	11	0.05	15	0.07	ns	
3	B15	161	0.68	143	0.63	ns	
4	B18	37	0.16	32	0.14	ns	
5	B27	14	0.06	10	0.04	ns	
6	B35	54	0.23	63	0.28	ns	
7	B38	25	0.11	22	0.10	ns	
8	B40	22	0.09	24	0.11	ns	
9	B44	42	0.18	34	0.15	ns	
10	B51	32	0.14	35	0.15	ns	
11	B52	5	0.02	7	0.03	ns	
12	B56	7	0.03	8	0.04	ns	
13	B58	26	0.11	23	0.11	ns	
14	Others	11	0.05	5	0.02	ns	
1	DR1	6	0.03	5	0.02	ns	
2	DR3	17	0.07	12	0.05	ns	
3	DR4	18	0.08	32	0.14	0.01	0.18
4	DR7	60	0.25	44	0.19	ns	
5	DR8	3	0.01	5	0.02	ns	
6	DR9	10	0.04	5	0.02	ns	
7	DR10	7	0.03	6	0.03	ns	
8	DR11	10	0.04	6	0.03	ns	
9	DR12	141	0.59	134	0.59	ns	
10	DR13	6	0.03	15	0.07	0.03	0.45
11	DR14	12	0.05	16	0.07	ns	
12	DR15	117	0.49	116	0.51	ns	
13	DR16	15	0.06	15	0.07	ns	

n: number of individual carrying the particular allele
ns: not significant

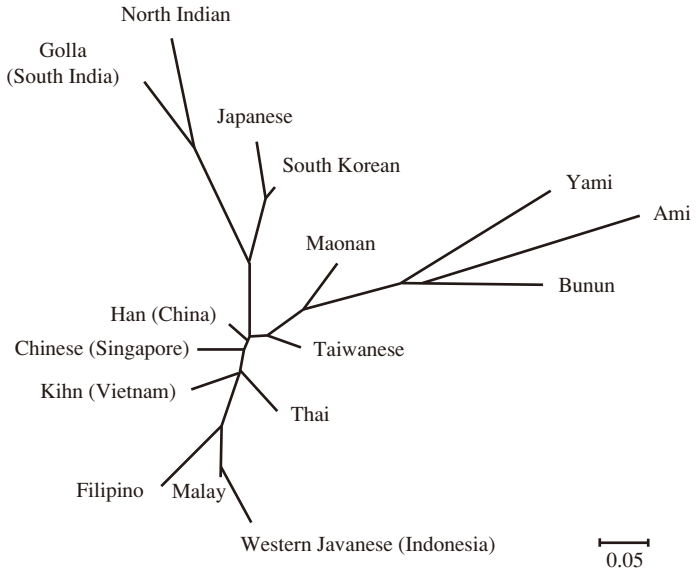

Fig. Phylogenetic tree constructed using the neighbour-joining method based on DA genetic distances obtained from the four-digit sequence-level allele frequencies for HLA-A, -B and -DRB1 (Yuliwulandari et al., 2009).

Discussion

The present study reported a comprehensive 3 loci HLA allele frequency in Western Javanese (Indonesia) using a high-resolution DNA typing method. A total of 18 HLA-A, 40 HLA-B, and 20 HLA-DRB1 alleles defined at four- to eight-digit level were identified in the population[3]. At the serological level, the most frequent HLA-A allele found in this study was HLA-A24 that accounted for 68.8% of the control samples. At the HLA-B locus, the most frequent allele was HLA-B15 (f=0.68). This serology group is also found in a high frequency in all East Asian populations[6], although considerable heterogeneity is seen in HLA-B15 allele distribution between northern and southern groups of East Asian populations in which the northern group has more frequent of HLA-B*1501 than -B*1502 whereas the southern group shows opposite situation[7]. At the HLA-DRB1 locus, the most frequent alleles was HLA-DR12 (0.59) followed by -DR15 (0.49). HLA-DR12 is commonly observed in all Southern East Asian groups including Southeast Asian populations but is infrequent in northern East Asian groups, including Japanese and Korean[7]. Both allele frequency and phylogenetic tree analyses demostrated the genetic affinities of the studied population to other East Asian populations, especially Southeast Asian populations. The present results were in concordance with archeological, anthropological, and linguistic studies in Indonesia[8].

HLA polymorphisms have been reported to be associated to the susceptibility and resistance to PTB even though the results remain conflicting. A study in Indonesian patients have reported the association between HLA-DR2 and -DQw1 and sputum smear-positive PTB (attributable risk=36% and 39%, respectively), while DQw3 was negatively associated with the disease (preventive fraction=57%)[9]. The association between DR2 (HLA-DR*15 and HLA-DR*16) and tuberculosis has also been demonstrated in Indian and Poles[10][11]. However, the present study could not confirm the association of DR2 reported either in Indonesia or in other countries.

In the analyses of single HLA alleles in the HLA class I loci, no allele was significantly associated with PTB. However two alleles of HLA class II locus, HLA-DR4 and -DR13, showed a weak association to the development of the disease (p=0.01 pc=ns and p=0.03 pc=ns, respectively. HLA-DR4 was reported to be strongly associated with the historical TB patients either alone or in the present of HLA-B14 allele in Southern Italy (p=0.000004, pc=0.0008)[12]. The allele has also been well known associated with various clinical subsets of rheumatoid arthritis and systemic lupus erythematosus[13] and to a high responsiveness to antigens specific to *Mycobacterium tuberculosis* (MTB) but not to antigens shared with other mycobacteria[14]. Because DR4 is associated with the regulation of the immune response to MTB and with rheumatoid arthritis (RA), it is possible that RA is the modern day manifestations of the genetic selective pressure exerted by tuberculosis epidemics of the recent past[14]. HLA-DR13 has been reported to be associated with susceptibility to TB in South African[15]. HLA-DR13 is also consistently associated with Hepatitis B viral clearance globally[16]. The protectiveness of HLA-DR13 is proposed to be either due to proficient antigen presentation by DR13 molecules or linked polymorphisms in neighboring immune regulatory gene[16]. The weak association observed in this study need to be replicated in other TB sample set either in the same population or in other populations to confirm the role of both alleles in the disease susceptibility.

Conclusion

The present study reported the genetic link between Western Javanese (Indonesia) and other Asian populations based on HLA allele frequency. The studied population was closest to southern East Asian group, especially Southeast Asian populations.

A weak association of HLA-DR4 and -DR13 to the susceptibility of PTB in Western Javanese (Indonesia) was observed in the study. The similar finding has also been reported in other populations. Therefore, the weak association result in this study needs to be validated using another study with larger number of samples either in the same or other populations.

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2. Association of *IL12RB1* Polymorphisms with Susceptibility to and Severity of Tuberculosis in Japanese: a Gene-based Association Analysis of 21 Candidate Genes

Koichi KUSUHARA[1, 2], Ken YAMAMOTO[3], and Toshiro HARA[2]

[1]Department of Pediatrics, University of Occupational and Environmental Health School of Medicine, Kitakyushu, Japan, [2]Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [3]Division of Molecular Population Genetics, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

Abstract To identify host genetic factors involved in the susceptibility to TB in Japanese, we performed a gene-based association analysis of 21 candidate genes on 87 TB patients and 265 controls using marker single nucleotide polymorphisms (SNPs). Among a total of 118 marker SNPs, 3 of *IL1B* and 2 of *IL12RB1* showed

association with TB. Non-synonymous coding SNPs (cSNPs) were not identified in *IL1B*. Association studies on 4 non-synonymous cSNPs of *IL12RB1* (641A/G, 1094T/C, 1132C/G, 1573G/A) in linkage disequilibrium showed that 3 of them were significantly associated with the development of TB. Haplotype analysis on the 4 cSNPs demonstrated that frequency of ATGG haplotype was significantly lower in TB patients than in controls. When TB patients were divided into 2 subgroups according to the severity of lung disease, advanced subgroup showed a prominent association with the 3 cSNPs. These data suggested that genetic variants of *IL12RB1* confer genetic susceptibility to TB, and are associated with the progression of the disease, in Japanese.

Introduction

TB is the second commonest cause of death from infectious disease after HIV/AIDS worldwide. Only about 10% of the individuals infected with *Mycobacterium tuberculosis* develop TB, whereas the remaining 90% stay free from the disease throughout their life. In addition to these clinical observations, epidemiological studies, and twin and adoption studies support the role of host genetic factors in the susceptibility to TB. For example, the concordance rates among identical twins were approximately 20 to 40% higher than those among non-identical twins. Previous case-control association studies demonstrated the association of several genes, such as HLA, NRAMP1 or SLC11A1, and vitamin D receptor genes, and IL-1 locus, with the susceptibility to TB. A linkage analysis on sib-pairs conducted in Africa has mapped TB susceptibility loci to chromosomes 15q11−13 and Xq26, although another genome-wide scan for Brazilian TB patients did not replicate it.

In the present study, we screened 21 candidate genes for TB susceptibility in Japanese by a gene-based association analysis using marker single nucleotide polymorphisms (SNPs) and subsequently analysed the association between TB and non-synonymous coding SNPs (cSNPs) adjacent to the positive marker SNPs in terms of susceptibility and disease severity.

Materials and Methods

Subjects

The study population comprised 87 unrelated Japanese patients with TB (mean age: 52.7±21.1 years; 18 women and 69 men) and 265 unrelated healthy Japanese individuals (mean age: 56.5±12.7 years; 112 women and 153 men), who resided in Kyushu Island in the southern part of Japan. All the TB patients had been given a diagnosis of pulmonary TB on the basis of clinical symptoms and chest radiographic findings with bacteriological confirmation (culture, 82 patients; smear and/or polymerase chain reaction [PCR], 5 patients). Patients with known immunodeficient states, such as HIV infection and undergoing immunosuppressive therapy were excluded. Lung disease on standard posterior-anterior chest radiograph of each patient was graded according the International Classification of Tuberculosis[1].

Screening of the candidate genes

Genomic DNAs were extracted from whole blood. Twenty-one candidate genes selected for analysis consisted of 3 genes whose association with TB has been observed in Japanese and/or other ethnic population (SLC11A1, VDR and IL-1 β genes), 14 genes associated with IL-12/IFN-γ axis (IFN-γ, IFN-γR [IFN-γ receptor] P, IFN-γR2, IL-12 p40, IL-12p35, IL-12R [IL-12 receptor] β1, IL-12R β2, signal transducer and activator of transcription [STAT]-1, IL-18, IL-18R [IL-18 receptor], IL-23p19, IL-23R [IL-23 receptor], IL-27p28 and IL-27R [IL-27 receptor, WSX-1] genes), 3 genes associated with tumor necrosis factor (TNF)-α signaling (TNF-α, TNFRSF [TNF receptor superfamily] 1 A and TNFRSF1B genes), and ubiquitin protein ligase E3A (UBE3A) gene, a putative TB susceptibility gene in chromosome 15q11−13 based on the sib-pair linkage analysis[2]. All of them are located on autosomal chromosomes. These candidate genes were screened by association analysis of marker SNPs, which were validated by the TaqMan™ Validated SNP Genotyping Assays (Applied Biosystems, Foster City, CA). A total of 118 marker SNPs with 62−23 572 base pair (bp) interval within each gene (median 5633 bp interval) were genotyped by Assays-On-Demand™ primer and probe sets (Applied Biosystems) using ABI PRISM 7900HT (Applied Biosystems) according to the manufacturer's protocol.

SNPs detection and genotyping by PCR sequencing

For genes with two or more marker SNPs exhibiting significant allele association with TB (cut-off at $P<0.05$), we subsequently searched for adjacent cSNPs by PCR and direct sequencing. Genomic DNAs extracted from whole blood of 24 TB patients randomly selected from the total TB population were used. Twenty-four samples are sufficient to detect SNPs with minor allele frequencies over 5%. To analyse exons 1−7 and 3'UTR of *IL1B* adjacent to 3 marker SNPs with positive association (rs1143629, rs1143643 and rs3917368), we constructed eight pairs of oligonucleotide primer pairs according to the human *IL1B* gene sequence (GenBank Accession No. AY137079). To analyse exons 1−17 of *IL12RB1* adjacent to 2 marker SNPs with positive association (rs2305739 and rs 383483), we constructed 17 pairs of oligonucleotide primer pairs according to the human *IL12RB1* gene sequence (Gen Bank Accession No. AY771996). Data were collected and analysed using the ABI DNA Sequencing Software Version 3.6. cSNPs were identified using the SeqMan II software version 4 (DNASTAR Inc., Madison, WI, USA). Among the cSNPs identified, non-synonymous cSNPs were selected for the second-round association study. Genotyping of 641A/G, 1094T/C, 1132C/G and 1573G/A SNPs of *IL12RB1* was performed by PCR and direct sequencing using primer pairs for exons 7, 10 and 13.

Statistics

Chi-square tests were employed to evaluate statistical differences in genotype distributions and allele frequencies of each SNP between TB and control groups. Genotype distributions of tested SNPs were compatible with the Hardy-Weinberg equilibrium. P values less than 0.05 were considered statistically significant. Linkage disequilibrium (LD) was evaluated by Lewontin's D' (|D'|) running all pairs of bi-allelic loci. All statistical analyses including haplotype estimation and association by χ^2 test were performed by using SNPAlyze version 3.2 software (DYNACOM, Mobara, Japan).

Results

In the association studies on 118 marker SNPs, among the 100 marker SNPs with minor allele frequency of 0.1 or greater, 3 maker SNPs of *IL1B* and 2 of *IL12RB1* showed association.

Three marker SNPs of *IL1B* gene were associated with TB. However, no non-synonymous cSNP was identified in exons 1−6 of *IL1B*. rs1143643 in intron 5 and rs3917368 in 3'downstream, both of which exhibited strong association with TB, were in complete LD. We then performed severity-stratified association study of rs3917368. When TB patients were divided into two subgroups according to the severity of lung disease, the advanced subgroup showed prominent associations with GG genotype and G allele of rs3917368, suggesting that the two SNPs were, probably indirectly, associated with progression of TB in Japanese (Table 1).

In the next step, we analyzed *IL12RB1* gene. Adjacent to 2 marker SNPs showing association with TB, there were 4 non-synonymous cSNPs, 641A/G, 1094T/C, 1132G/C and 1573G/A. We performed association studies of these 4 cSNPs (Table 2[3]). For 641A/G SNP, frequencies of GG genotype and G allele were significantly higher in TB patients than in controls. For 1094T/C and 1132G/C, frequencies of CC genotype and C allele were significantly higher in TB patients than in controls. The genotype and allele frequencies of 1132C/G SNP were exactly the same as those of 1094T/C SNP. The remaining SNP, 1573G/A, showed no association. These data suggested that the 3 non-synonymous cSNPs of *IL12RB1* were associated

Table 1 Genotype and allele frequencies of *IL1B* rs3917368 SNP (3'downstream) in TB patient subgroups classified by disease severity

IL1B SNPs	Controls	TB Moderate	OR [95% CI]	p	TB Advanced	OR [95% CI]	pc
rs3917368							
Genotype freq.							
AA	58 (22%)	3 (9%)			6 (11%)		
AG	138 (52)	19 (56)	2.66 [0.76−9.34]	0.11	23 (43)	1.61 [0.62−4.16]	0.64
GG	67 (25)	12 (35)	3.46 [0.93−12.9]	0.051	24 (45)	3.97 [1.32−9.05]	0.017
Total	263	34			53		
Allele freq.							
A	254 (48)	25 (37)			35 (33)		
G	272 (52)	43 (63)	1.60 [0.95−2.71]	0.073	71 (67)	1.89 [1.22−2.94]	0.008

Table 2 Genotype and allele frequencies of *IL12RB1* 641A/G, 1094T/C, and 1132C/G SNPs in TB patients and controls[3]

IL12RB1 cSNPs	Control	TB	OR [95% CI]	p-value
641A/G				
Genotype freq.				
AA	98 (38%)	23 (27%)		
AG	120 (47)	41 (48)	1.46 [0.82−2.59]	0.20
GG	37 (15)	22 (26)	2.53 [1.43−5.08]	0.0078
Total	255	86		(2×3 : p=0.030)
Allele freq.				
A	316 (62)	87 (51)		
G	194 (38)	85 (49)	1.59 [1.12−2.25]	0.0087
1094T/C (1132C/G)				
Genotype freq.				
TT (GG)	96 (37%)	20 (23%)		
TC (GC)	125 (48)	44 (51)	1.69 [0.93−3.05]	0.080
CC	39 (15)	23 (26)	2.83 [1.43−5.73]	0.0032
Total	260	87		(2×3 : p=0.013)
Allele freq.				
T (G)	317 (61)	84 (48)		
C	203 (39)	90 (52)	1.67 [1.18−2.36]	0.0034

Table 3 Estimated frequencies of haplotypes constituted by 4 cSNPs of *IL12RB1* in TB patients and controls[3)]

Haplotype	Frequency		qui-square	p
	Control (n＝249)	TB (n＝86)		
ATGG	0.598	0.483	7.46	0.0063
GCCA	0.026	0.058	3.85	0.022
GCCG	0.339	0.436	5.23	0.050
Others[#]	0.037	0.023		

[#]haplotypes with frequencies＜0.03

Table 4 Genotype and allele frequencies of *IL12RB1* 641A/G, 1094T/C and 1132C/G SNPs in TB patient subgroups classified by disease severity[3)]

IL12RB1 SNPs	Controls	TB Moderate	OR [95% CI]	p	TB Advanced	OR [95% CI]	pc
641A/G							
Genotype freq.							
AA	98 (38%)	13 (38%)			10 (19%)		
AG	120 (47)	14 (41)	0.88 [0.39−1.96]	0.75	27 (52)	2.21 [1.02−4.78]	0.083
GG	37 (15)	7 (21)	1.43 [0.53−3.85]	0.48	15 (29)	3.97 [1.64−9.63]	0.0028
Total	255	34			52		
Allele freq.							
A	316 (62)	40 (59)			47 (45)		
G	194 (38)	28 (41)	1.14 [0.68−1.91]	0.62	57 (55)	1.97 [1.29−3.02]	0.0030
1094T/C (1132C/G)							
Genotype freq.							
TT (GG)	96 (37%)	12 (35%)			8 (15%)		
TC (GC)	125 (48)	15 (44)	0.96 [0.43−2.15]	0.92	29 (55)	2.78 [1.22−6.36]	0.025
CC (CC)	39 (15)	7 (21)	1.44 [0.53−3.92]	0.48	16 (30)	4.92 [1.95−12.4]	0.00068
Total	260	34			53		
Allele freq.							
T (G)	317 (61)	39 (57)			45 (42)		
C	203 (39)	29 (43)	1.16 [0.70−1.94]	0.57	61 (58)	2.12 [1.39−3.23]	0.00088

with the development of TB in the Japanese population.

LD analysis of the 4 cSNPs showed almost complete LD among 641A/G, 1094T/C and 1132G/C and modest LD between any of the 3 cSNPs and 1573G/A. To see if a particular haplotype constituted by these 4 cSNPs was associated with TB, haplotype frequencies were estimated and association analysis was done. As shown in Table 3[3)], frequency of ATGG haplotype was significantly lower in TB patients than in controls. This further supported the association between *IL12RB1* and the development of TB.

When TB patients were divided into two subgroups according to the severity of lung disease, the advanced subgroup showed prominent associations with GG genotype and G allele of 641 A/G SNP, and with CC genotype and C allele of 1094 T/C or 1132C/G SNP (Table 4[3)]). These data suggested that the 3 non-synonymous cSNPs of *IL12RB1* were also associated with the progression of TB in the Japanese population.

Discussion

In the present gene-based association analysis of 21 candidate genes for TB susceptibility, 3 of the 4 non-synonymous cSNPs of *IL12RB1* in LD showed significant association with TB. Furthermore, the frequency of ATGG haplotype in TB patients was significantly lower than that in controls, and advanced subgroup of TB patients showed a prominent association with the 3 SNPs. These data suggested that genetic variants of *IL12RB1* confer genetic susceptibility to TB, and are associated with the progression of the disease in Japanese.

Homozygous recessive mutations in *IL12RB1* preclude the surface expression of IL-12 receptor β1 and IFN-γ secretion by T and NK cells. The lack of IFN-γ secretion results in susceptibility to poorly virulent mycobacteria. As some patients with IL-12 receptor β1 deficiency have been reported to develop clinical TB in the absence of any personal or familial history of clinical disease by poorly virulent mycobacteria[4)〜6)], it is possible that any functional polymorphisms of *IL12RB1* gene may affect the genetic control of *M. tuberculosis*.

There have been some reports on the association of *IL12RB1* gene and TB susceptibility. Akahoshi et al.[7)] reported the association of the same cluster of the SNPs analyzed in this study with TB in Japanese. They also revealed functional difference of CD2 positive cells from healthy individuals

homozygous for susceptible haplotype. In contrast, studies in Morocco[8] and Korea[9] did not demonstrate any association between the same cluster and TB. -2C/T SNP, one of the 2 SNPs reported to be associated with TB in the Moroccan study, was included in the screening step of the present study, but no association was observed. Our study, together with the study by Akahoshi et al.[7], strongly suggested that *IL12RB1* confers genetic susceptibility to TB in Japanese. On the other hand, contribution of *IL12RB1* on TB susceptibility might vary among different ethnic groups.

There are two major approaches to identify genetic predisposition, candidate gene approach and genome-wide approach. Because direct association analysis using functional variants of candidate genes is limited by incomplete knowledge about functional variation at present, indirect association mapping using marker SNPs has been considered to identify genes conferring susceptibility to common diseases such as myocardial infarction and rheumatoid arthritis. As an intermediate approach, we performed a gene-based SNPs mapping to screen 21 candidate genes and identified *IL12RB1* as probable host genetic factor for the susceptibility to TB in Japanese. Due to relatively small sample size in our study, confirmation by replication study is necessary to overcome the correction for multiple comparisons. Furthermore, genome-wide approach needs to be done in Japanese in the future. As our attempt to collect sib-pair samples from all over Japan was unsuccessful, genome-wide SNPs mapping seems to be a practical way.

In conclusion, gene-based association study on 21 candidate genes suggested that genetic variants of *IL12RB1* confer genetic susceptibility to TB, and are associated with the progression of the disease in Japanese. It would be warranted to examine whether the same association is observed in other ethnic groups.

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3. Human Genetic Susceptibility to Tuberculous Meningitis in Vietnamese Population

Nguyen Thuy Thuong THUONG[1], Sarah DUNSTAN[2,3]

[1]Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, [2]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [3]Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, UK

Abstract A case-control study of TLR2 candidate found that genotype 597CC was associated with susceptibility to tuberculous meningitis (TBM) (OR＝3.26)[1]. SNP C558T on TIRAP was found to be associated with increased susceptibility to TBM in this same Vietnamese cohort[2] (OR＝3.02). The co-inheritance of TLR2 SNP T597C and TIRAP SNP C558T increases susceptibility to TBM (OR＝5.4). The interaction between polymorphisms on TLR2 and TIRAP in TB patients and *M. tuberculosis* strains shows that individuals with the

C allele of TLR2 T597C are more likely to have TBM caused by the Beijing genotype (OR＝1.91) than other individuals. TBM patients with CC genotype of TLR2 T597C have higher risk of disease caused by the Beijing genotype (OR＝4.48). This provides evidence that *M. tuberculosis* genotype influences clinical disease phenotype[3]. A genome-wide case-control association study of 250,000 SNPs indicated that there are SNP marker profiles which are specifically associated with the susceptibility to, or protection from, clinical phenotypes of TB (data not yet published). Microarrays were used to determine gene expression profiles of over 38,500 genes from *ex-vivo M. tuberculosis* stimulated macrophages isolated from latent (LTB), PTB and TBM. These results suggest that distinct macrophage responses are associated with different clinical forms of tuberculosis and that the innate immune response may regulate clinical outcomes[4]. Overall, the work presented in these studies contributes to the current knowledge of the genetic basis of TB, and more specifically of TBM, and provides novel insights into the molecular pathogenesis of TB.

Introduction

Tuberculous meningitis (TBM) results from the haematogenous dissemination of *Mycobacterium tuberculosis* (*M. tuberculosis*) from the lung to the brain leading to the most severe form of tuberculosis (TB) with high morbidity and mortality. Currently there is an incomplete understanding of the immunopathogenesis of TBM and the host genetics affecting TBM development. The overall hypothesis of our studies is that in TBM patients, a failure of some immune responses involved in controlling *M. tuberculosis* may partially explain the dissemination of *M. tuberculosis* from the lung to other tissues such as the brain. The role of the host's genes in the development of TBM was therefore investigated by assessing (1) genetic variation in human immune genes and (2) gene expression level.

The association of TBM and SNPs on human immune candidate genes

Since there are up to 30,000 genes in the human genome, and it is unlikely that more than a few hundred make a meaningful contribution to variation in any single phenotype, a priori probability that any gene selected at a random will influence a given trait is very low[5]. Candidate genes, with higher prior odds for phenotypic involvement are mostly selected based on known biology of the disease. Candidate genes for human studies can also be selected from genes identified as influencing related traits in animal models. There are two tuberculosis genes, SLC11A1 (formerly NRAMP1) and SP110[6][7], that were identified by positional cloning in mice and then assessed in human disease. Positional information from genome-wide scans for linkage or association could indicate regions with a high probability of containing a susceptibility gene. In TB studies two genomic regions, 17q11-q21 and 2q35, carrying susceptibility genes were assessed for their association with TB[8][9]. These studies showed the signal of linkage for PTB in loci of particular genes: NOS2A, CCL18, CCL4, STAT5B and SLC11A1[9][10]. Candidate gene studies contribute by identifying the association between particular genes and tuberculosis in different populations.

In innate immunity, TLRs are essential in recognition of *M. tuberculosis*[11][12]. Genes of the TLR family have been examined for an association with TB in certain populations[2][13][14]. Due to the central role of TLR2 in the recognition of TB, we investigated the association of the polymorphism on genes of TLR pathway and different clinical forms of TB including TBM (n＝157) and PTB (n＝183). These two phenotypes are critical in TB disease. TBM is the most severe form and is representative of disseminated TB, while PTB is the most common form for localization of TB in the lung. The controls for both TB groups were umbilical cord blood samples from babies after birth. This group comprised 400 cord blood samples and was used as a population control providing the underlying genetic variation of the Vietnamese majority population. We found genetic association of TLR2 genotype 597CC of SNP T597C with susceptibility to TBM (TBM vs. control, OR＝3.26, 95％CI 1.72−6.18), with an association which is enhanced in those with more severe disease as well as those with miliary TB (controls vs. pulmonary TB OR ±95％ CI; 1.31±0.06−2.83, P＝0.497; controls vs. TBM without miliary OR±95％ CI: 2.31±1.06−5.06, P＝0.032; and controls vs. TBM with miliary OR±95％ CI: 5.28± 2.20−12.65, P＜0.0001). The molecular mechanism by which this synonymous polymorphism might affect susceptibility to severe TBM is not known. It may be that the C allele of SNP T597C has a high level of linkage disequilibrium with the allele of the GT microsatellite that affects gene regulation. The disease represents the most extensive manifestation of haematogenously disseminated TB, but the underlying mechanism of bacterial dissemination is the same for all forms of extrapulmonary TB. Given the central role of TLR2 in mediating immune responses, it is possible that TLR2 genetic variants differentially regulate inflammatory pathways and influence the intra-cerebral inflammatory responses that determine severity and outcome from the disease.

The Toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) is an adaptor that mediates signals from Toll-like receptors activated by mycobacteria. We have reported SNP C558T on TIRAP to be associated with increased susceptibility to TBM in this same Vietnamese cohort (controls vs. TBM; OR±95％ CI; 3.02±1.79−5.09, P＜0.001), but this SNP is not associated with pulmonary TB (controls vs. PTB; OR±95％ CI: 1.55±0.85−2.82, P＝0.22)[2]. In comparison to the 558CC genotype, the 558TT genotype was associated with altered levels of *ex-vivo* whole blood and *in-vivo* cerebrospinal

fluid cytokines, which suggested that TIRAP influences disease susceptibility by modulating meningeal inflammation.

Co-inheritance studies of TLR2 SNP T597C and TIRAP SNP C558T show that these two SNPs affect the risk of the development of TBM. When the polymorphism occurs in TIRAP only (TLR2 597T_TIRAP 558T), the risk for TBM is 3.2 (OR±95% CI; 3.2±1.77−5.75, P<0.0001). If individuals have both SNPs (TLR2 597C, TIRAP 558T), they have a higher risk of TBM (OR±95% CI; 5.4±1.34−21.77, P=0.008). These findings imply that these significant associated SNPs may have a combined effect and increase the risk of the development of TBM.

M. tuberculosis is another factor in TB development and manifestation. The interaction between polymorphisms on these immune genes (TLR2 and TIRAP) in different TB phenotypes (TBM and PTB) and *M. tuberculosis* strains was studied by comparing the frequency of alleles and genotypes of SNPs on the host genes and *M. tuberculosis* lineages. There was no significant association on TIRAP C558T and any *M. tuberculosis* lineage. However there was both allelic and genotypic associated in the comparison between TLR2 T597C polymorphism and the East-Asian/Beijing strain. Individuals with the C allele of TLR2 T597C were more likely to have TBM caused by the East-Asian/Beijing genotype (control vs. TBM East Asian/Beijing; OR±95% CI; 1.91±1.28−2.86, P=0.001) than other individuals. TBM patients with CC genotype of TLR2 T597C have higher risk of disease caused by the East-Asian/Beijing genotype (control vs. TBM East Asian/Beijing; OR±95% CI; 4.48±2.00−10.04, P<0.001). The study provides evidence that *M. tuberculosis* genotype influences clinical disease phenotype, particularly TBM. Together, these findings suggest that *M. tuberculosis* strain interacts with the host variation of the immune system at a genetic level, to influence the TB clinical phenotype.

Human genetics and TB in large-scale studies

We used two approaches, genome-wide scans and microarrays, to investigate the association between the host and TB at molecular genetic and transcriptional levels.

The objective of genome-wide association studies is to screen the entire human genome at high density in certain population samples. Currently marker sets of 500,000 SNPs are now commercially available as whole genome panels. SNPs can be selected in a variety of ways (e.g. with a focus on gene location only, via haplotype tagging) or at random throughout the genome[15]. Recently a genome-wide association study has been performed in 14,000 cases of seven common diseases and 3,000 shared controls in the British population. This study has found strong signals associated with each disease, which offers new avenues for exploring the pathophysiology of these important disorders. A genome-wide association study of TB is part of this larger project and the data analysis is ongoing[16]. A genome-wide case-control association study of 250,000 single nucleotide polymorphisms (SNPs) in our Vietnamese cohort indicated that there are SNP marker profiles which are specifically associated with the susceptibility to, or protection from, clinical phenotypes of TB, including TB meningitis and pulmonary TB. This study helped us highlight genes and gene regions associated with different clinical phenotype of TB (data not yet published).

The identification of genes that might be implicated in disease only partly explains the biological pathways that lead to disease. The fuller picture requires knowledge of gene expression and gene product function in the integrated environment of a living organism. For tuberculosis, although a number of studies have identified possible genes involved in TB pathogenesis, the molecular effects of these polymorphisms on macrophage function, leading to variation in manifestations of TB, are mostly unknown. Identification of gene expression profiles using microarrays is a powerful approach to obtain a full understanding of all the major genetic contributions to disease susceptibility at a transcriptional level.

Expression microarrays were used to examine gene expression profiles in the immune response to tuberculosis. The majority of previous microarray studies in tuberculosis have examined healthy donors, cell lines, or murine cells[17)~21)]. There has only been one previous study that compares gene expression profiles of individuals with different clinical forms of tuberculosis such as recurrent, acute and active TB[22]. However, no study has attempted to distinguish different clinical forms of active tuberculosis such as pulmonary and meningeal diseases.

We used microarrays to determine gene expression profiles of >38,500 genes from *ex-vivo M. tuberculosis* stimulated macrophages isolated from 12 subjects with 3 clinical TB phenotypes; latent, pulmonary and TBM. 1608 genes were differentially expressed by >2-fold and 199 genes differentially expressed by >5-fold in TB-stimulated macrophages. Furthermore, we were able validate our findings by real-time RT-PCR in an independent data set of an additional 30 individuals. Our study is the first to compare transcriptional profiles of individuals with tuberculous meningitis with individuals with other forms of TB. We identified genes that were distinctly expressed in MDMs from individuals with a history of TBM. After bacilli invade the host lung within the pulmonary alveolar macrophage, they replicate and disseminate to the regional lymph nodes. During this early stage of infection, before the development of adaptive immunity, the bacteria can spread haematogenously to other organs in the body and cause extrapulmonary disease, such as TBM[23)24)]. This step may be determined by the nature and extent of the innate immune response activated by infected macrophages. We found that several MDM immune response genes (IL1B, IL12B, TNF, TNIP3, CXCL10, CXCL11, CCL12, and CCL1) were up-regulated in TBM subjects in comparison to those with PTB and LTB. In addition, some genes, such as *MMP1* and *HAS1*, were found with enhanced expression in PTB in comparison to TBM. These genes are involved in degrading the extracellular matrix

and could mediate a role in granuloma formation and bacillus containment, which could influence dissemination and development of TBM[25]. Although the relationship between the inflammatory response and TBM pathogenesis is only partially understood, excessive immune activation may be intimately associated with disease severity and outcome.

Our results suggest that transcriptional profiling has the potential to distinguish different clinical forms of TB. With tools currently available, clinicians are unable to identify the subset of latently infected patients who will develop active disease. Furthermore, there are no techniques available to prospectively identify individuals at risk of the devastating consequences of TBM versus more treatable forms of TB such as localized pulmonary disease. Further studies in this area could lead to prognostic tests that could alter treatment algorithms with more accurate prognostic information. In addition, such studies may lead to novel molecular insight into TB pathogenesis.

Conclusion

We used genetic approaches to address the question of why some individuals have disseminated TB resulting in TB meningitis, while others have localized pulmonary TB. The association between genetic variants with susceptibility to PTB versus TBM and the gene expression profile of macrophages in their early response to *M. tuberculosis* were investigated. We demonstrated a strong association of TLR2 SNP T597C with the development of TBM and miliary tuberculosis, suggesting that primary recognition of a pathogen and appropriate activation of innate immunity can influence the extra-pulmonary dissemination of *M. tuberculosis*. Our genome wide study is the first study, on a genome-wide scale, to examine the genetic susceptibility to different clinical forms of TB, particularly to TB meningitis.

The findings from this project contribute to the current understanding of genetic variation and gene expression in relation to the susceptibility of individuals to different clinical forms of TB. In particular the combination of genome-wide association and microarrays studies provide genetic information which can be used to identify the critical mechanisms of protective immunity against TB disease. Our results can also help to explain why out of the *M. tuberculosis* infected individuals who develop active disease, some have pulmonary TB and some develop disseminated extra-pulmonary disease, such as TBM. A complete understanding of disease mechanisms and protective immunity is essential to drive future vaccine development and advances in TB treatment.

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4. Genome-wide Linkage Analysis for Tuberculosis in Thais: the Way forward with Human Genetics

Surakameth MAHASIRIMONGKOL

Medical Genetics Section, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand.

Abstract Linkage analysis of Thais tuberculosis were discussed and compared with existing linkage analyses of tuberculosis. Linkage analysis in Thais suggested chromosome 5q as a novel tuberculosis susceptibility locus, possibly specific to Asian. Order subset analysis by age at onset suggested two regions on chromosome 17p and 20p as susceptibility loci for younger age at onset tuberculosis. These analyses supported the disease patterns differed between young and old tuberculosis patients.

Introduction

Despite advances in understanding of host-pathogen interaction in various animal models of tuberculosis; the understanding of these interactions in human infected with *M. tuberculosis* remains elusive. Because of the specificity of *M. tuberculosis* to primates, genetic epidemiology approach that search for association at specific variations in human genome with tuberculosis is a straightforward method to identify these at risk variations in natural population.

In order to analyze the gene-environment interaction that is very important for genetic analysis of infectious disease, complex statistical analyses are required for the analyses that taken into account various risk factors determining outcomes of *M. tuberculosis* infection. These risk factors include; environmental factors such as socioeconomics status, demographic variables, distribution of *M. tuberculosis* strains and HIV epidemiology; and endogenous factors such as chronic illnesses, immunosuppressive treatment and immunosenescence.

Candidate gene association study; the study of difference in distribution of variation within gene(s) between tuberculosis patients and normal population had been carried out for *HLA-DQ*, *NRAMP1*, *IFNG* and various other candidate genes[1)~3)]. Only few genes demonstrate consistent evidences of association. Host genetic control of tuberculosis is likely followed the common disease common variants hypothesis; at least a few other genes with intermediate to large effects are remained to be discovered. The unbiased searching strategy for novel genetic risks in human genome is feasible with current microarray based genotyping technology. Two genome-wide gene mapping studies are utilized for the search of genetic risks for complex traits; genome-wide linkage analysis (genome-wide scan) and genome-wide association study (GWAS)[4)]. Linkage analysis is the study of segregation of alleles within affected family member, the result of analysis is region of chromosomes that highly likely to contain at risk gene of particular disease or phenotype.

Genome wide linkage analyses of tuberculosis

Linkage analysis in leprosy *per se*, identify *PARK2/PACRG* as candidate gene for leprosy[5)6)]. *PARK2* previously associated with familial form of Parkinson disease. Discovery of *PARK2/PACRG* as genetic risks of leprosy is very fascinated for research in host genetic control of mycobacterium infection; this is the proof of concept that genetic epidemiology approach is useful for identification of novel genetic risks for mycobacterium disease. Without linkage analysis and subsequent fine mapping study, it is nearly impossible for a researcher to suspect *PARK2/PACRG* as a risk factor for leprosy. Moreover, fine mapping of second linkage evidences of Leprosy identified lymphotoxin-alpha (*LTA*) as the genetic risk for early onset Leprosy in major histocompatibility complex (MHC) class

III[7].

Studies in leprosy encourage the search for tuberculosis susceptibility gene but linkage analyses for tuberculosis are not quite successful[8]. At the time of this report, five linkage analyses have not yet demonstrated any consistently replicated susceptibly genes of tuberculosis[9)~14)]. However, significant linkage in Moroccan population is promising as candidate region for a major determinant gene for tuberculosis, at least in Moroccan population.

Material and Methods

The analysis and result of genome-wide nonparametric linkage analysis in Thais is briefly discussed here. We performed high-throughput microarray-based SNP genotyping platform, a GeneChip array comprised of 59,860 bi-allelic markers, in 93 Thai families with multiple siblings. In total, 195 individuals affected with tuberculosis were genotyped by these arrays. Non-parametric linkage analysis was carried out with MERLIN; a statistical program for linkage analysis. Linkage analysis accounting for linkage disequilibrium (LD) was also carried out to reduce the false positive results from LD between near-by markers. Ordered subset analysis using minimum age at onset of tuberculosis in families was carried out with FLOSS software.

Results

Nonparametric linkage analysis revealed a region on chromosome 5q showing suggestive evidence of linkage with tuberculosis (Z (lr) statistics＝3.01, LOD score＝2.29, empirical P-Value＝0.0005). Additionally, two candidate regions on chromosome 17p and 20p were suggested by ordered subset analysis using minimum age at onset of tuberculosis as the covariate (maximum LOD score＝2.57 and 3.33, permutation p-value＝0.0187 and 0.0183, respectively). We demonstrate a region in 5q might contain susceptibility gene to tuberculosis, but this evidence is not satisfied the genome-wide significant level. Linkage evidences in Thais tuberculosis families and from other studies (Table), are the subjects of further fine mapping study. These regions should be given special interest in genomewide association study of tuberculosis.

Table Candidate loci for tuberculosis susceptibility genes

Chromosome region	Population
15q12-13 Xq26	Gambians (Bellamy et al.)[9]
10q26 11q12 20p12	Brazilians (Miller et al.)[10]
8q12-13	Moroccan (Baghdadi et al.)[11]
6p21-q23 20q13.31-33	South Africans Malawians (Cooke et al.)[12]
7p22-7p21	Ugandan (Stein et al.)[13]
5q31-33	Thais (Mahasirimongkol et al.)[14]

Discussion

From power calculation, hundreds to thousands families of at least two family members affected with diseases are required for linkage analysis of complex traits[15]. Family ascertainment is the most demanding part of linkage analysis. It is difficult and nearly impossible for a research team to collect more than a few hundreds of affected families. Inadequate number of families is the main factor cited for failure of current linkage analyses to provide linkage statistics that surpassed the appropriate genome-wide significant level. We might overcome this sample sizes problem with meta-analysis of existing linkage analyses[16].

Genetic heterogeneity for tuberculosis susceptibility genes

Genetic heterogeneity is characterized by a phenotype or disease that occurs with mutation in at least two different genes. Such an examples, is the mutations in *BRCA1* and *BRCA2*; both of these gene mutations ultimately leads to breast cancers in affected family members. Genetic heterogeneity is an important factor results in decrease of power for linkage analysis. Whenever two groups of similar diseases but different causative genes were considered together as one group in gene mapping study; this group of samples will hamper any statistics methods employed for gene mapping study assuming a single locus homogeneity model.

Heterogeneity due to populations

One interesting hypothesis, for non-overlapping of linkage evidences from linkage analyses is heterogeneity due to populations under each study. Apart from low number of families; the Africans, Brazillians and Thais population are distant populations that might acquire difference genetic risks to tuberculosis during recent migration. Linkage analysis in Thai tuberculosis suggested that 5q31-33 is a candidate region for tuberculosis susceptibility gene. This region had never been identified in other linkage analyses, and raised the possibility that this locus is specific to Thais tuberculosis.

Evidence of heterogeneity by age at onset

Tuberculosis in children and adults are distinct in a few aspects. In younger age group, patients frequently develops disseminated form of infection; TB meningitis, military tuberculosis, extrapulmonary TB. This is suggested that the disease is developed soon after infection as a result of specific immunodeficiency to *M. tuberculosis*. When the disease develops in adult, the disease tends to confine to the respiratory system, *M. tuberculosis* infection is generally well controlled for several years, symptoms eventually develops many years later after infection. It is hypothesized that immunopathogenesis underlying these two forms of tuberculosis are immunologically and genetically distinct[17]. If the genes responsible for TB in young-

er and older patients are difference, this evidence helps to confirm this hypothesis.

Ordered subset analysis (OSA) is a statistical approach to evaluate effect of a covariate on the overall linkage evidences. In Thais tuberculosis sibpairs families, the OSA that focused on family with minimum age at onset less then 24, provide two new linkage evidences on chromosome 17p and 20p. The significant of the OSA for tuberculosis in affected families suggest that the immunogenetics of younger onset patients and the older patients is distinct. The reasons for heterogeneity by the age at onset might be the difference in the exposure to the epidemic *M. tuberculosis* strains, the effect of vaccination with BCG or other differential environmental exposures by the age group.

Various immunological and bacteriological factors indicated that specific strain or subspecies of *M. tuberculosis* are unique in its interacting with host[18)~20)]. The facts that these strains of *M. tuberculosis* are distinct had not been taken into account in previous genome-wide analysis.

The way forward

Fine mapping of the 5q region is the priority for our research; additional families with at least one affected child and their parents (TRIOs) had been enrolled for the fine mapping of this linkage peak. The result of fine mapping may yield a candidate region that specific to South East Asian populations.

Genome-wide association of tuberculosis study is carrying out in Japanese, Korean, Thais, Vietnamese, Gambians and several African populations. The findings from GWAS study should provide further insight into the immunopathogenesis of this complex infectious disease. The collaborative efforts among Asian scientists on identification of tuberculosis susceptibility genes would provide insights into how the hosts response to this historic infectious agent.

Genome-wide association study is being carried out within our research collaborative network among Asian human genetics for TB. We are looking forward to the discovery of novel genetic risks and waves of replication study for the results from these genome-wide association analyses. Heterogeneity in tuberculosis susceptibility might be reduced by controlling for age, sex and exposure to specific *M. tuberculosis* strains. Specific host-pathogen interaction is not a far-fetched target, possibly achievable within next few years. It can be foreseen that identification of specific defects in host and virulence factors in pathogen might influence the current prevention and treatment strategy.

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