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Research Paper

Genetic susceptibility to Tuberculosis: Interaction between *HLA-DQA1* and age of onset

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A B S T R A C T

Several genome-wide association studies (GWAS) identified new single nucleotide polymorphisms (SNPs) with susceptibility to Tuberculosis (TB). However, many of them were not replicated across ethnic groups. The cause of this phenomenon of genetic heterogeneity is uncertain. Here, we attempted to replicate and evaluate the mechanism that causes genetic heterogeneity in several putative TB predisposition loci found by previous GWAS, including chromosome 18q, *ASAP1*, *DUSP14*, and *HLA-DQA1*. A Chinese cohort of 1200 TB patients and 1280 population controls were genotyped. The results showed that genetic predisposition to TB might operate in an age-specific manner. While no significant association was found in the whole samples, a SNP of *HLA-DQA1*, rs9272785, showed suggestive association within the young-onset TB subgroup (onset at 20–40 years of age, $N = 396$). The results provide support for the hypothesis that there are different pathogenesis mechanisms causing clinical TB disease in different age groups, and that genetics probably play a substantial role only in young-onset TB.

1. Introduction

Tuberculosis (TB) is a significant global health concern, and Hong Kong is not an exception. The World Health Organization (WHO) estimated that one-third of the world population was infected with tubercle bacilli (World Health Organization, 2017). However, in many cases, bacilli remain latent and not everyone develops clinical infection. TB is also important among the HIV-infected population as it is the leading cause of death accounting for as high as one in four HIV-related deaths. Although chemotherapy controls the disease, TB, among infectious diseases, is still the topmost killer of adults because resistant strains are constantly emerging (Centre for Health Protection, Department of Health, 2017). It is expected that TB will continue to be a major world health issue in the coming decades (World Health Organization, 2017).

The importance of host susceptibility in the progression to active TB disease has been the focus of early research studies (Casanova and Abel, 2002; Cottle, 2011; Dorman and Holland, 1998; Newport et al., 1996; Vidal et al., 1993). For example, animal studies identified that *Nramp1* determined resistance to infection on exposure to mycobacteria (Shaw et al., 1997; Vidal et al., 1993). These model animal studies provided strong evidence for genetic susceptibility to TB in human. Therefore, several groups started to adopt the familial linkage or candidate gene association study approaches. Private and rare mutations were found for severe familial TB infections that arose from immune defects caused by immune response genes. For example, disseminated bacillus Calmette-Guérin (BCG) infection could be caused by private mutations in interferon-gamma receptor-1 (*IFNGR1*), interferon-gamma receptor-2 (*IFNGR2*), interleukin-12B (*IL12B*), interleukin-12 receptor beta (*IL12RB1*) and signal transducer and activator of transcription-1 (*STAT1*), reviewed in (Stein et al., 2017; van Tong et al., 2017). However, these familial forms of predisposition to infection are rare and these mutations did not account for the susceptibility to TB in the general population.

Then, based on the hypotheses of the involvement of these genes in TB, candidate gene association studies were carried out using a case-control setting. Various DNA markers including single nucleotide polymorphisms (SNPs) and microsatellites have been studied. However, it was soon recognised that the candidate gene approach might be limited by the investigators' hypotheses and that the results cannot be generalised to other studies (Stein et al., 2017; van Tong et al., 2017).

Abbreviations: TB, Tuberculosis; GWAS, Genome wide association study

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But as extensive genotyping of SNPs was soon made possible by microarray technology, genome-wide association studies (GWAS) have been done to study susceptibility to TB.

The Wellcome Trust Case Control Consortium (WTCCC), the first largest GWAS consortium in the UK which reported 7 diseases in its first publication, reported the first GWAS in 2007. Subsequently, in 2010, the WTCCC TB group reported the first TB GWAS result (Thye et al., 2010). The main finding was that a locus (rs4331426) in chromosome 18q was associated with TB (p-value = 6.8×10^{-9}). However, whether the association also holds true for other populations is uncertain, as the samples studied in the WTCCC were only African. Replication of the results in other populations is essential to confirm this susceptible locus in chromosome 18q.

Our team replicated this SNP (rs4331426) in chromosome 18q in Chinese TB patients and found surprising results. Firstly, compared with African patients, the allelic frequencies of the high risk allele (G) were lower in Chinese (Wang et al., 2013). Furthermore, the associated odds ratio (OR) was reversed, that is the risk allele in African became protective in Chinese. Therefore, we suspected that there was heterogeneity (also known as genetic heterogeneity) across ethnic groups. However, there is little understanding of the cause of genetic heterogeneity. Some researchers believe that it is partly due to the differences in genetic background across ethnical groups. These differences may be useful for further dissection of the underlying causative variant. For instance, rs43331426 might only represent a proxy marker and the true causative variant may still need to be identified (Chimusa et al., 2014; Dai et al., 2011; Ji et al., 2013; Lee et al., 2016; Miao et al., 2016; Xue et al., 2016).

Therefore, we performed an in-depth analysis of this region on chromosome 18q11.2 in an attempt to gain a deeper understanding of its association with TB found in Chinese patients. Furthermore, subsequent GWAS loci were also examined, including those in the *ASAP1*, *DUSP14*, and *HLA-DQA1* gene regions.

2. Methods

2.1. Hong Kong Chinese TB patients (N = 1200)

The patients from the territory-wide Chest Clinic of Hong Kong Tuberculosis and Chest Service were invited to participate (Wang et al., 2013). All patients, of whom were Hong Kong Chinese, had to fulfil the criteria for established TB for inclusion into the study, i.e. (1) smear positive for *M. tuberculosis* and/or (2) culture positive for *M. tuberculosis* and/or (3) clinical TB with the diagnostic criteria of the International Union against Tuberculosis and Lung Diseases with clinical-radiological and histological grounds and a clinical response to treatment. Patients with HIV or other cases of immunodeficiency were excluded. Written informed consent was obtained from all patients. Clinical parameters about the extent and severity of disease were collected from clinical worksheets. Collection of whole blood samples from 1200 TB patients was approved by both the research ethics committees of the NTE cluster (The Chinese University of Hong Kong) and the Department of Health. Germline DNA were extracted from the peripheral blood samples.

2.2. General population control samples (N = 1280)

A general population cohort within which both sexes were of equal number was used to compare the genotype frequencies against the patients group. They were part of an existing population reference sample cohort (Healthy Community Elderly Cohort) (Jiang et al., 2010; Tang et al., 2010). Individuals with any past history of TB and chronic lung disease of any causes were excluded.

The absence of TB infection was based on self-reported past history as we could not take a chest X-ray for the control subjects for study purposes. This kind of compromised recruitment criteria were also commonly used in other studies (Chimusa et al., 2014; Khor et al., 2010).

2.3. Selection of tagging SNPs and genotyping method

2.3.1. TagSNP for the chromosome 18q11 locus

The chr.18q11 locus is located within a linkage disequilibrium (LD) block of ~200 kbp. As there is a high degree of correlation between SNPs within this LD block, the tagSNP approach was used to genotype the DNA variants in this locus. The data of all Han Chinese (CHB) SNPs within this region were obtained from the International HapMap project. Using a spectral decomposition algorithm, tagging SNPs in the loci near rs4331426 were identified to represent other SNPs in this locus (Meng et al., 2003). Each of the identified tagging SNPs represents a significant proportion of variation in this segment of LD block in the chr.18q11 locus under evaluation. We identified 18 tagSNPs in this region for genotyping, 17 of which were successfully genotyped.

2.3.2. Genotyping method

A melting temperature(Tm)-shift allele-specific genotyping method previously described by Germer et al. (Germer and Higuchi, 1999) was used. Our laboratory has developed and used this technique in our recent publications (Jiang et al., 2010; Tang et al., 2010). In brief, a set of three primers, composed of two allele-specific primers and a common reverse primer, was used in each genotyping reaction. The two allele-specific primers contained additional sequences of different lengths at the 5′ end, and will produce different melting temperatures due to differences in the GC compositions of the additional 5′ sequences. Genotyping results were confirmed by using the HapMap samples of known genotypes as quality controls (QC) in each reaction batch.

2.3.3. Additional newly reported GWAS loci for genotyping

In addition, new GWAS papers have been published recently (Barreiro et al., 2011; Curtis et al., 2015; Sveinbjornsson et al., 2016). Representative SNPs from these newly reported genes, namely *HLA-DQA1*, *ASAP1,* and *DUSP14*, were also genotyped.

2.4. Statistical analysis

Single SNP association analysis was performed by Chi-square using dominant and recessive modes. SNPs with significant genotype association were further analysed to obtain the corresponding OR and their confidence intervals (C.I.). Bonferroni correction was used to correct for the number of SNPs analysed in each gene. Sample size determination was based on the conditions that allelic frequency was 0.3 with an OR of 1.5, the sample size was ~1000 controls, and 1000 patients would have 80% power. Power calculation was performed by the software CaTS using an additive mode (Skol et al., 2006).

3. Results

3.1. Details of case samples

In total, DNA samples from 1200 Han Chinese TB patients were studied prior to genotyping. 40 samples failed QC after DNA extraction, so only 1160 samples of good quality DNA were available for genotyping. All patients fulfilled the standard inclusion criteria for clinical TB. The mean age was 48 years for TB cases and male to female ratio was 65%:35%. There was a large age range from 20 to 83 years old and the interquartile range were 31 and 63 years old for the 25th and 75th percentiles, respectively. There were 396 patients, accounting for 34% of studied samples, that were defined as young-onset TB, with the definition of young-onset being disease onset at 20 to 40 years old.

3.2. Details of population control samples

1280 control samples, which were healthy Chinese consisting of an

Table 1
The genotypes and associations with TB of the (a) chromosome 18q tagging SNPs, (b) *ASAP1* tagging SNPs, (c) *DUSP14*, and (d) *HLA-DQA1*.

	SNPs	Cases			Population Control			Genetic Association *p*-value
		AA	AB	BB	AA	AB	BB	
(a) Chromosome 18q tagging SNPs	rs542228 (A = G, B = T)	913	215	15	986	268	16	0.37
	rs9960552 (A = C, B = T)	369	561	219	445	609	214	0.19
	rs4800131 (A = C, B = A)	281	590	269	291	627	340	0.15
	rs7235269 (A = G, B = A)	132	533	482	142	571	553	0.71
	rs4330012 (A = G, B = A)	572	241	172	672	276	197	0.96
	rs12456774 (A = G, B = A)	583	450	98	650	516	98	0.68
	rs11662668 (A = G, B = A)	300	559	282	309	631	330	0.51
	rs2140037 (A = C, B = T)	548	476	119	601	539	129	0.92
	rs8091189 (A = G, B = C)	274	544	303	331	624	311	0.16
	rs1013483 (A = C, B = T)	322	555	262	380	626	265	0.40
	rs4592764 (A = T, B = A)	42	342	712	35	370	865	0.15
	rs299245 (A = G, B = C)	516	505	122	553	576	139	0.75
	rs12959243 (A = C, B = T)	305	553	280	309	619	325	0.46
	rs1546724 (A = C, B = A)	288	528	267	309	626	326	0.49
	rs6507258 (A = G, B = T)	92	467	585	104	514	642	0.98
	rs7228876 (A = C, B = T)	565	433	85	658	505	100	0.99
	rs7239677 (A = C, B = T)	58	357	653	54	421	782	0.44
(b) *ASAP1* tagging SNPs	rs4733775 (A = G, B = A)	170	575	402	171	619	401	0.71
	rs10089819 (A = C, B = T)	100	498	565	97	568	543	0.12
	rs17210536 (A = C, B = T)	62	379	702	62	421	729	0.69
(c) *DUSP14*	rs712039 (A = C, B = T)	231	593	319	281	663	331	0.41
(d) *HLA-DQA1*03*	rs9272785 (A = G, B = A)	532	444	78	631	470	111	0.14

equal number of men and women, came from a community elderly cohort with mean age of 72.5 ± 5.2 years (S.D.). Self-reported history excluded all causes of chronic lung disease and specifically history of TB.

3.3. Genetic association analysis of GWAS hits: using all samples

Table 1 shows the genotypes and associations with TB of chromosome 18q tagging SNPs, *ASAP1* tagging SNPs, *DUSP14*, and *HLA-DQA1*. A whole group analysis of 1160 TB cases and 1280 controls was performed. Genotype association was analysed with Chi-square. None of them reached statistical significance.

3.4. Genetic association analysis of GWAS hits: using young-onset TB samples

As we observed a bimodal distribution of age of onset of clinical TB, we analysed a subgroup of young-onset TB as defined by age of onset between 20 and 40 years. Table 2 shows the genotypes and associations with young-onset TB of chromosome 18q tagging SNPs, *ASAP1* tagging SNPs, *DUSP14*, and *HLA-DQA1*.

The best potential association was found at rs1013483 ($p = 0.06$), where a significant association of rs9272785 in *HLA-DQA1* with young-onset TB ($p < 0.01$) was reported. On the other hand, SNPs in the chromosome 18q locus were not significant.

3.5. Odds ratio (OR) of high risk alleles for young-onset TB

We replicated the association in the young-onset TB (20–40 years old) subgroup with the *HLA-DQA1* SNP rs9272785. rs9272785 represents a missense variant (Ala210Thr) of the *HLA-DQA1* gene. In the conventional HLA allele nomenclature, it is represented as *HLA-DQA1*03*. Tables 3 and 4 show the genotype frequencies of *HLA-DQA1*03* (rs9272785) in young-onset TB using population controls and late-onset TB (> 40 yr) as reference groups, respectively. The OR in dominant mode was 1.33 (C.I. 1.05 to 1.68) for *HLA-DQA1*03* (rs9272785) in young-onset TB.

3.6. Haplotype analysis of the chromosome 18q locus

As predicted by our study design, there was little LD between the tagging SNPs for genotyping. The program Haploview was used to analyse the LD and haplotype structure of the Chromosome 18q locus (Fig. 1). Two small LD blocks could be identified. The SNP rs1013483 was also weakly associated with one of the LD blocks. Haplotype association test by permutation did not identify any additional significant results in both the whole group and subgroup analyses (Young-onset TB).

4. Discussion

The chromosome 18q locus and the SNP rs4331426 represent the first GWAS hits of genes with susceptibility to TB (Thye et al., 2010). This was a long-waited-for finding which carried an OR of 1.2 associated with the G allele in the original GWAS report. We performed one of the first replication studies using our Hong Kong Han Chinese TB samples and found surprising results (Wang et al., 2013). On the one hand, the association with TB was insignificant. On the other hand, both the allelic distribution and the direction of effect were different between African and Chinese. The G allele became a protective allele in Chinese. This effect was accompanied by huge differences in allelic frequencies and haplotype structures between Chinese and the African population studied in the original report. Subsequent replication studies yielded similar results with ours, where either there was a lack of association or the G allele was protective (Chimusa et al., 2014; Dai et al., 2011; Ji et al., 2013; Lee et al., 2016; Miao et al., 2016; Xue et al., 2016). Therefore, we suspected that rs4331426 was not the main culprit (functional SNP) to account for TB susceptibility, and other genes or functional elements may be the real underlying cause of this GWAS signal. There are several reasons why GWAS could not be replicated in subsequent replication studies. They include heterogeneity of the alleles/haplotypes and heterogeneity of the population, which are due to differences in genetic background across populations.

Genetic heterogeneity like this case is a common observation in the post-GWAS era. While many GWAS hits could be replicated across

Table 2
The genotypes and associations with young-onset TB of the (a) chromosome 18q tagging SNPs, (b) *ASAP1* tagging SNPs, (c) *DUSP14*, and (d) *HLA-DQA1*.

	SNPs	Young-onset TB			Population control			Genetic association p-Value
		AA	AB	BB	AA	AB	BB	
(a) Chromosome 18q tagging SNPs	rs542228 (A = G, B = T)	311	72	5	986	268	16	0.55
	rs9960552 (A = C, B = T)	127	189	75	445	609	214	0.47
	rs4800131 (A = C,B = A)	92	204	92	291	627	340	0.42
	rs7235269 (A = G, B = A)	49	177	164	142	571	553	0.72
	rs4330012 (A = G, B = A)	181	82	71	672	276	197	0.17
	rs12456774 (A = G, B = A)	198	153	34	650	516	98	0.77
	rs11662668 (A = G, B = A)	112	182	93	309	631	330	0.19
	rs2140037 (A = C, B = T)	185	163	41	601	539	129	0.97
	rs8091189 (A = G, B=C)	92	180	114	331	624	311	0.14
	rs1013483 (A = C, B = T)	107	179	103	380	626	265	0.06
	rs4592764 (A = T, B = A)	14	126	233	35	370	865	0.11
	rs299245 (A = G, B=C)	168	180	41	553	576	139	0.95
	rs12959243 (A = C, B = T)	115	179	93	309	619	325	0.14
	rs1546724 (A = C, B = A)	109	171	91	309	626	326	0.17
	rs6507258 (A = G, B = T)	34	157	199	104	514	642	0.95
	rs7228876 (A = C, B = T)	193	150	33	658	505	100	0.86
	rs7239677 (A = C, B = T)	19	130	217	54	421	782	0.54
(b) *ASAP1* tagging SNPs	rs4733775 (A = G, B = A)	50	192	140	171	619	401	0.54
	rs10089819 (A = C, B = T)	25	162	197	97	568	543	0.09
	rs17210536 (A = C, B = T)	23	116	237	62	421	729	0.33
(c) *DUSP14*	rs712039 (A = C, B = T)	77	201	111	281	663	331	0.48
(d) *HLA-DQA1*03*	rs9272785 (A = G, B = A)	164	175	26	631	470	111	P < 0.01*

* Only *HLA-DQA1*03* (rs9272785) remained significant after Bonferroni correction for multiple testing.

Table 3
The genotype frequencies and OR of *HLA-DQA1*03* (rs9272785) in young-onset TB. Remarks: OR (dominant mode) = 1.33 (C.I. 1.05 to 1.68).

rs9272785				
Genotypes	GG	GA	AA	
Young-onset TB	164	175	26	P < 0.01
Population control	631	470	111	

Dominant mode			Recessive mode		
GG	GA + AA		GG + GA	AA	
164	201		339	26	
631	581		1101	111	
		$P = 0.02$			N.S.

Table 4
Genotype frequencies and OR of *HLA-DQA1*03* (rs9272785) in young-onset TB using the remaining TB patients as reference group.

rs9272785				
Genotypes	GG	GA	AA	
Young-onset TB	164	175	26	$P < 0.02$
Other TB (age > 40)	368	269	52	

Dominant mode			Recessive mode		
GG	GA + AA		GG + GA	AA	
164	201		339	26	
368	321		637	52	
		P < 0.01			(N.S.)

studies, some were confined to particular populations or ethnic groups. The underlying cause for such heterogeneity is not certain, and many reasons may contribute. For example, different genetic backgrounds across populations is the most commonly used explanation. It certainly worked in our case of the chromosome 18q SNP rs4331426, which showed a huge difference in allelic frequencies between Africans (a common SNP) and Chinese (a rare SNP). However, other instances of genetic heterogeneity were also observed even when the allelic frequencies were comparable. Therefore, other mechanisms are yet to be found. There is no other choice but to use a set of tagSNPs to comprehensively study this gene desert region to find out the true underlying functional element. We had the TB cases sample collection and with power calculation, we believed that there was sufficient power to carry out this task.

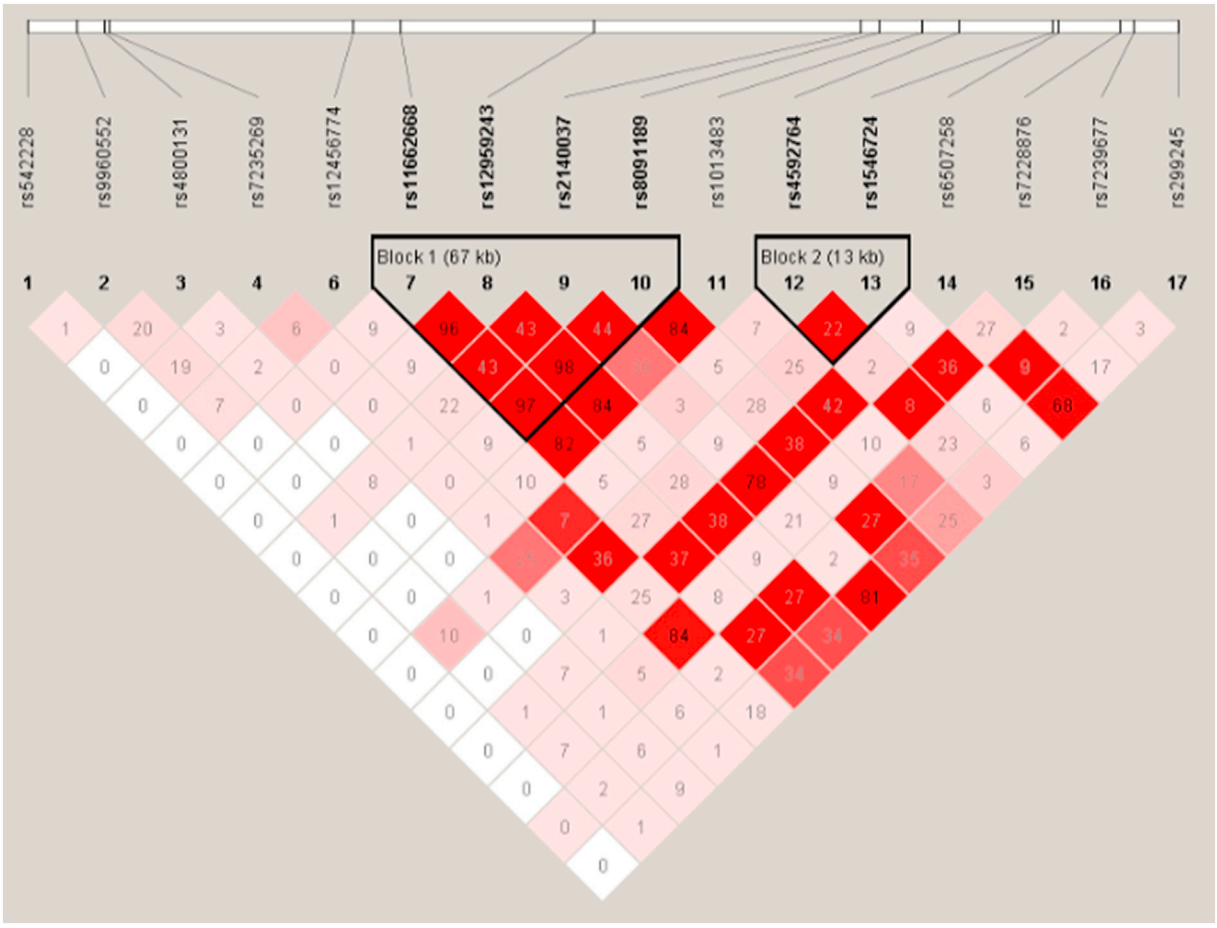

Fig. 1. The LD block structure of the chromosome 18q locus.

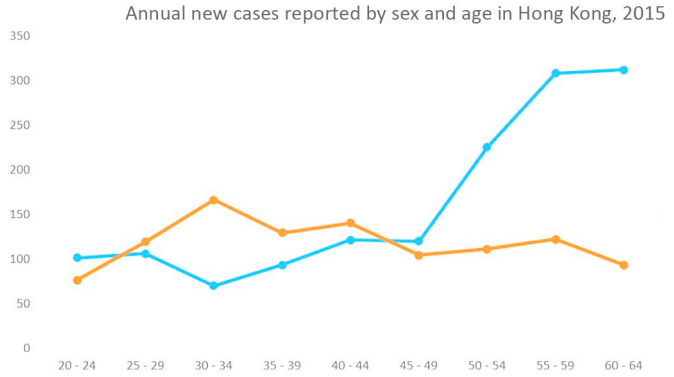

Fig. 2. Annual new cases reported by sex and age in Hong Kong, 2015. The blue line represents male and the orange line represents female patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In the whole cohort analysis, none of the SNPs in chromosome 18q reached statistical significance, though several of them were borderline. Given the power analysis of our sample size, it is suggested that the effect size in the overall TB cohort might be very small even if there was an association.

4.1. Different pathogenesis for TB onset at different age groups

An interaction of age of onset with TB had long been reported. In a genetic study, Mahasirimongkol et al. studied a small group of young TB whose ages were younger than 45. Young TB were contrasted with old TB in a GWAS setting in a case-only analysis and they reported an association at chromosome 20q12 (Mahasirimongkol et al., 2012; Nakauchi et al., 2016). Their observation indicated the possibility of different pathogenic mechanisms predisposing to TB for different age groups.

In fact, we also observed a bimodal age distribution in the case samples. It suggests that young-onset and late-onset TB might be the result of different pathogenesis and immune dysfunctions. The overall territory-wide age distribution of reported cases in Hong Kong showed a similar bimodal age distribution, in which the disease was more prominent in females. A bimodal distribution like this one has also been observed in other Asian countries, including China, Japan, and Thailand (Centre for Health Protection, Department of Health, 2017; World Health Organization, 2017). Fig. 2 presents annual new cases of TB reported by sex and age in Hong Kong, 2015.

This observation led to the attempt to analyse the young-onset subgroup for genetic association to the disease. In the chromosome 18q locus, none of the SNPs reached a significant association in the young-onset subgroup analysis after correction for multiple testing.

4.2. Replication of latest functional and GWAS hits

While we are working on this chromosome 18q locus, other GWAS and functional genetic studies reported SNPs in the *ASAP1*, *DUSP14, and HLA-DQA1* genes which might also cause predisposition to TB. Therefore, we also studied the association of these additional SNPs in our cohort. Although *ASAP1* was reported to have a robust association with TB in the original paper studying Russian patients, its association in the African sample was weak. Here, we and others suggest that the role of this gene in the Chinese population is also weak (Hu et al.,

2016). Another functional study identified *DUSP14* as a TB susceptibility gene, but it was not replicated in our study (Barreiro et al., 2011). The functional study was based on the eQTL response of dendritic cells to TB infection. Although it may be part of the normal response to TB, it may not be the key determinant for an individual to develop clinical infection.

On the other hand, the hypothesis that the *HLA-DQA1* loci predisposed to TB is not new. However, most if not all previous attempts had small sample sizes and used old allele-typing methods. A recent GWAS of European TB patients, with Icelander TB as the discovery set, identified SNPs in the *HLA-DQA1* loci as predisposition genes and all of them reached genome-wide significance (Sveinbjornsson et al., 2016). One of them, rs9272785, a missense variant (Ala210Thr) of the *HLA-DQA1* gene which is represented as *HLA-DQA1*03* in the conventional HLA allele nomenclature, is also prevalent in Chinese. We replicated the association of this SNP in the young-onset TB (20–40 years old) subgroup but not in the whole TB sample set. The OR (dominant mode) was 1.33 (C.I. 1.05 to 1.68). Thus, given that *HLA-DQA1*03* is a prevalent allele in Chinese, we conclude that it may play a considerable role in young-onset TB.

4.3. Differential pathogenesis of clinical TB across age group leads to genetic heterogeneity

We observed an example of age-genetic interaction in this TB study. It is likely that such interaction represents the underlying interaction of genetic and environmental factors on TB reactivation in different phases of the human life cycle. During young age, TB is commonly a primary infection rather than reactivation and thus genetic predisposition may have a stronger role. On the other hand, TB reactivation is more important in late-onset of the disease, where other factors like medical comorbidities and life style (environmental) factors may be more important.

5. Limitations

The associations reported here were of borderline significance ($p < .05$) after correction for multiple testing.

6. Conclusions

Genetic heterogeneity and interaction may cause different results in the replications of GWAS hits. Here, we showed an example of genetic heterogeneity and interaction which may be related to age-specific differential pathogenesis of TB. *HLA-DQA1*03* may only predispose to young-onset TB but may have little effect in TB of older age groups.

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