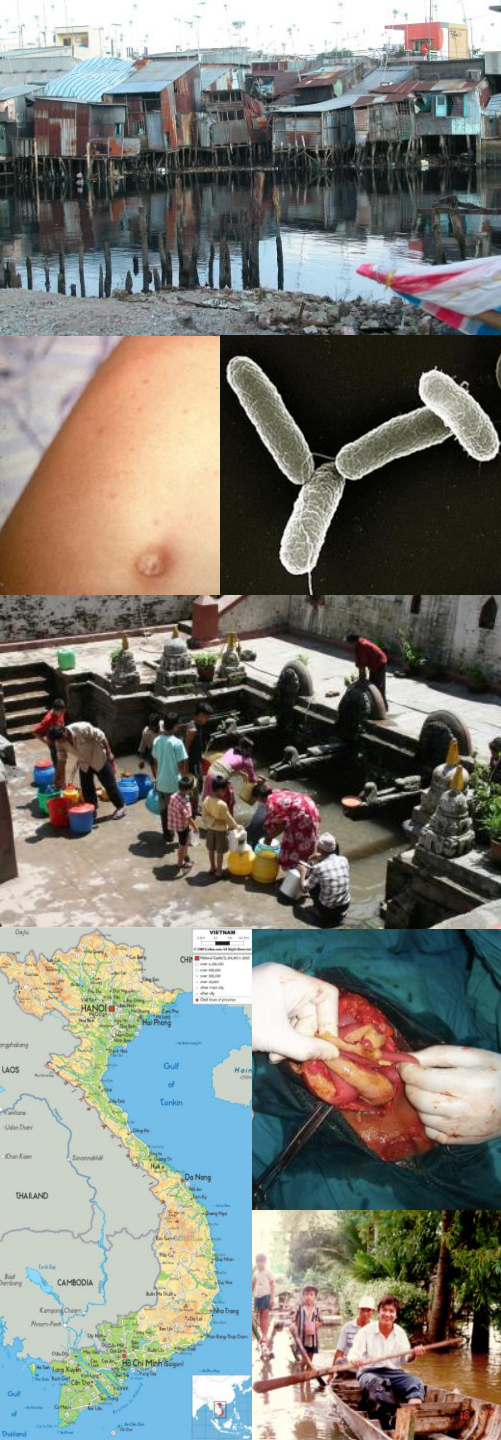


# HLA and resistance to enteric fever

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## Human genetic susceptibility to infection

- inter-individual differences in disease susceptibility
- ability to develop adequate immunity to bacterial pathogens is unequally distributed
- some success in identifying genetic component of susceptibility to various infectious diseases, eg. malaria, HIV
- enteric fever has been somewhat neglected
- vast literature on murine susceptibility



# Typhoid genomics



- advances in genomic research - unbiased, random screens across human genome to identify disease genes
- large cohorts of typhoid cases and controls needed
- ideally multiple ethnic groups

## Cohort collection

- clinical studies in Vietnam performed between 1992 and 2002
- clinical studies in Nepal performed between 2005 and 2014
- blood samples for DNA were collected at the time of study enrollment
- demographic and clinical information was recorded
- DNA collection of cases and controls from Nepal and Vietnam is largest available



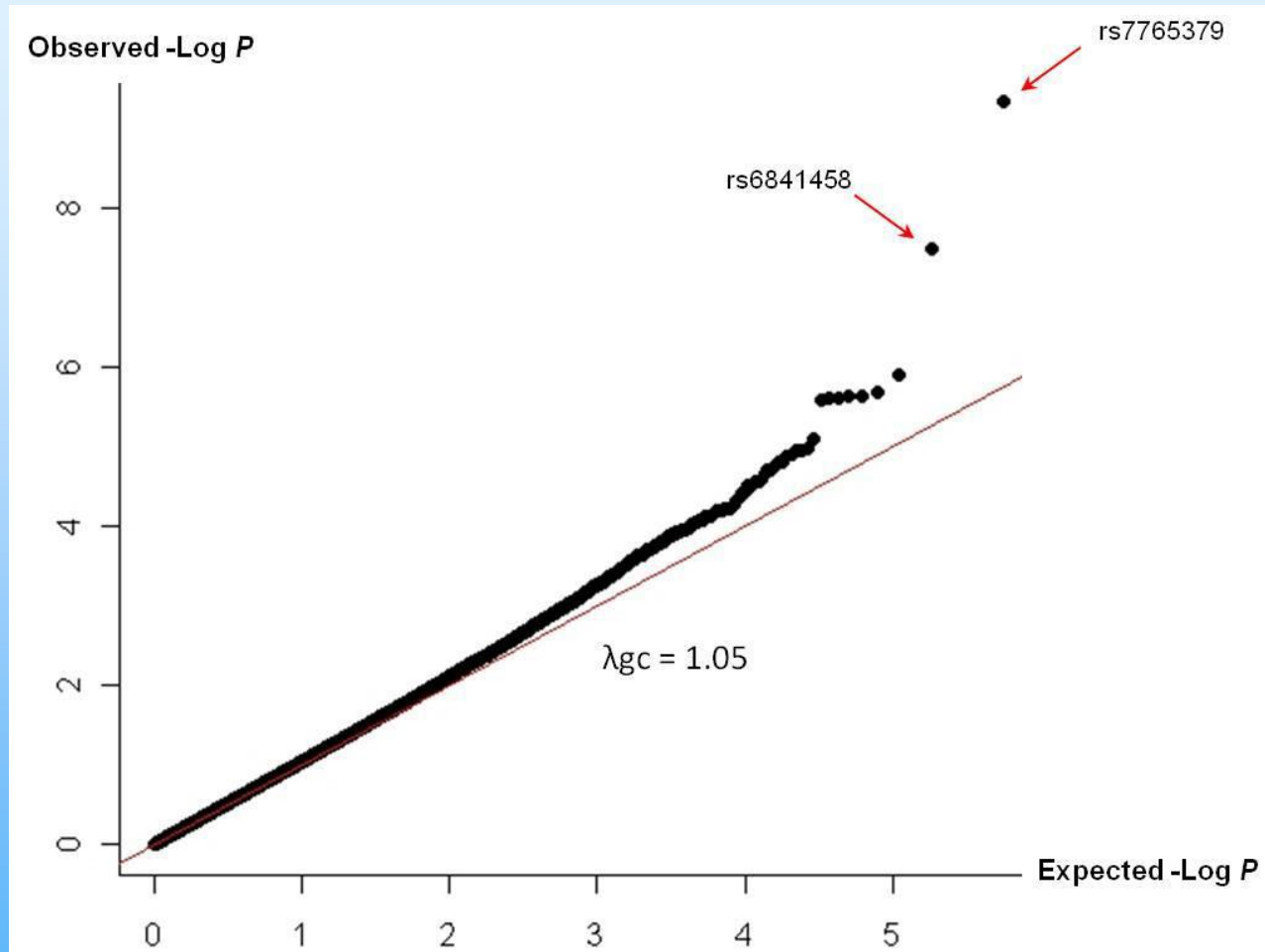
# Typhoid GWAS



- First large-scale, unbiased search for human genes affecting a person's risk of typhoid
- 432 blood culture–confirmed (*S.Typhi*) enteric fever patients and 2,011 controls from Vietnam
- genotyped on Illumina OmniExpress BeadChip, 660W BeadChip, Human Exome BeadChip
- 709,725 SNP markers passed quality control filters and were tested for association (642,445 from GWAS chip, 67,280 from exome chip)



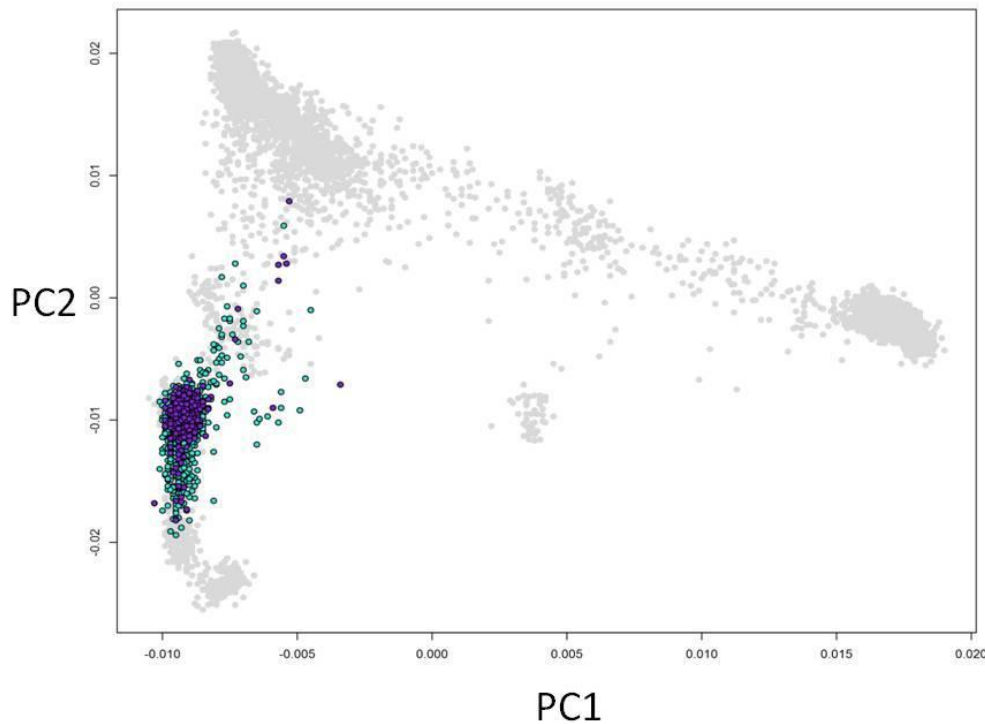
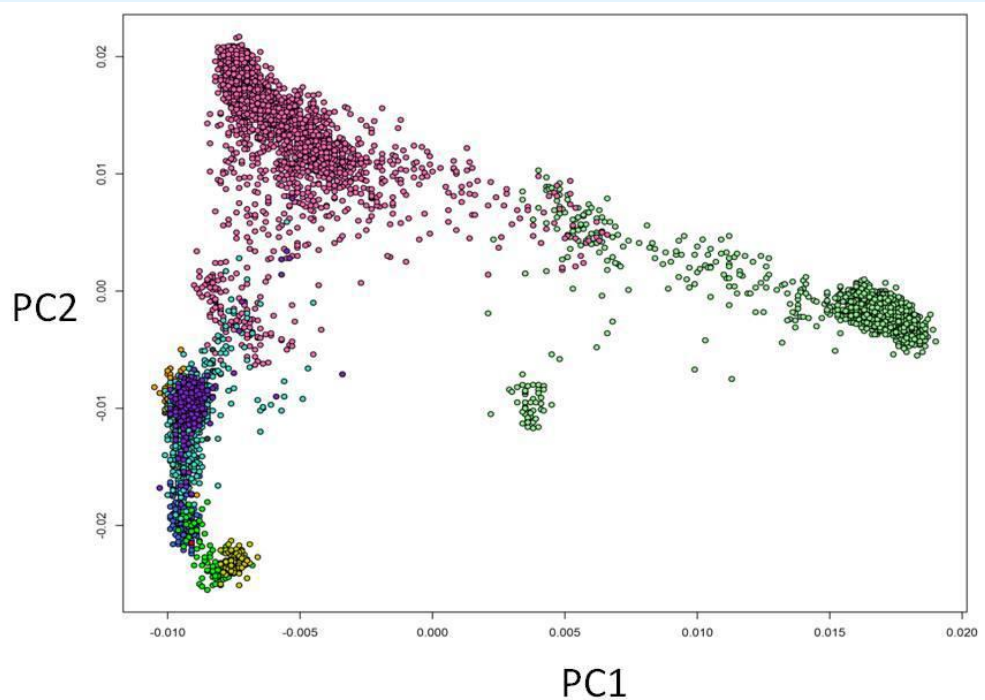
## Quantile-quantile plot of the association P values obtained



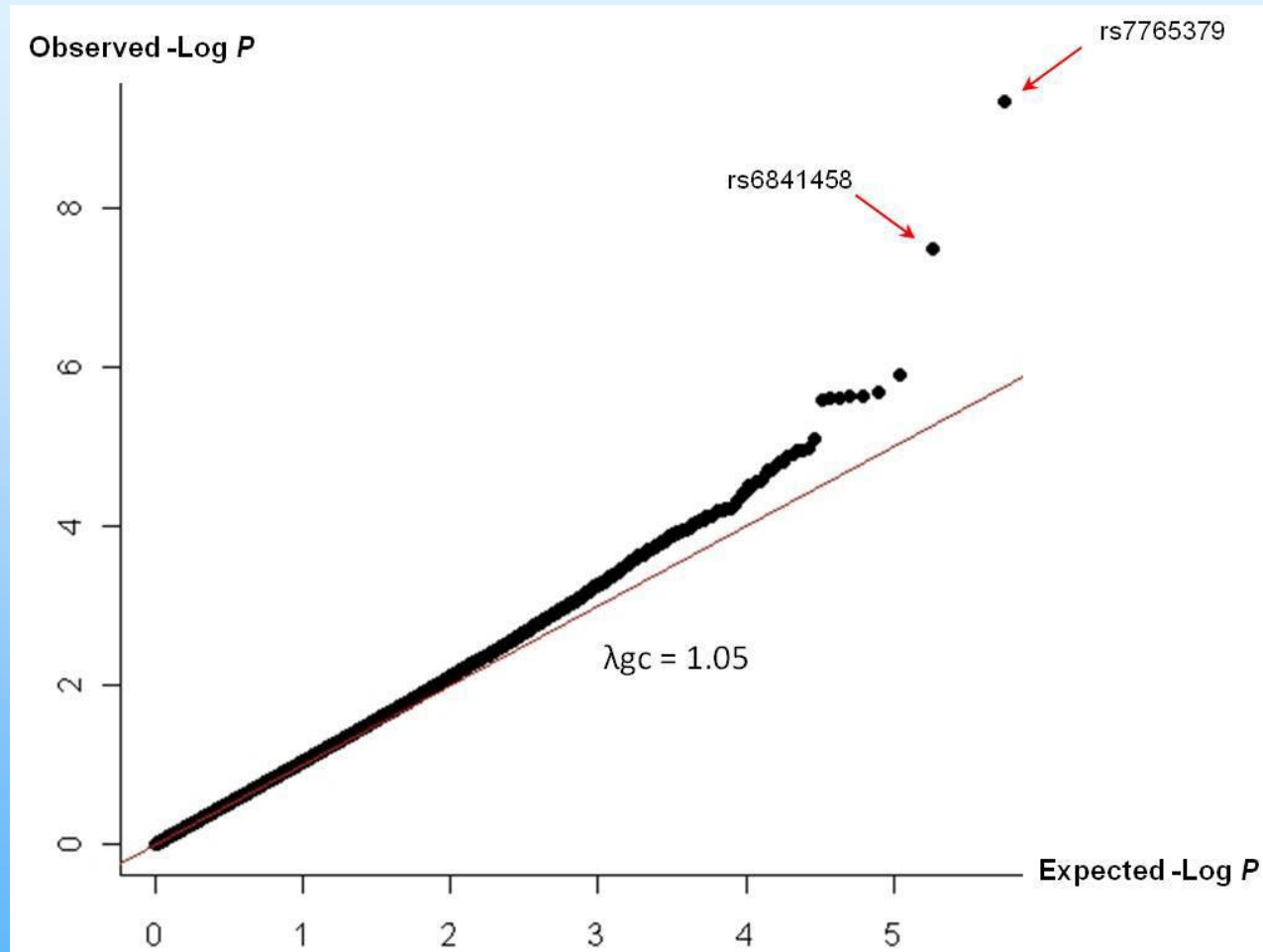
- Associated with typhoid?
- Associated by chance?
- Cases and controls not well matched?

## Principal-component analysis of the Vietnamese cases and controls in the context of Asian populations (1000 Genomes Project)

CDX Chinese Dai, Xishuangbanna, China  
CHB Chinese Han in Beijing  
CHD Chinese in Denver USA  
CHS Southern Han Chinese  
JPT Japanese  
KHV Vietnamese Kinh from HCMC  
SIMES Singaporean Malays  
SINDI South Indians in Singapore



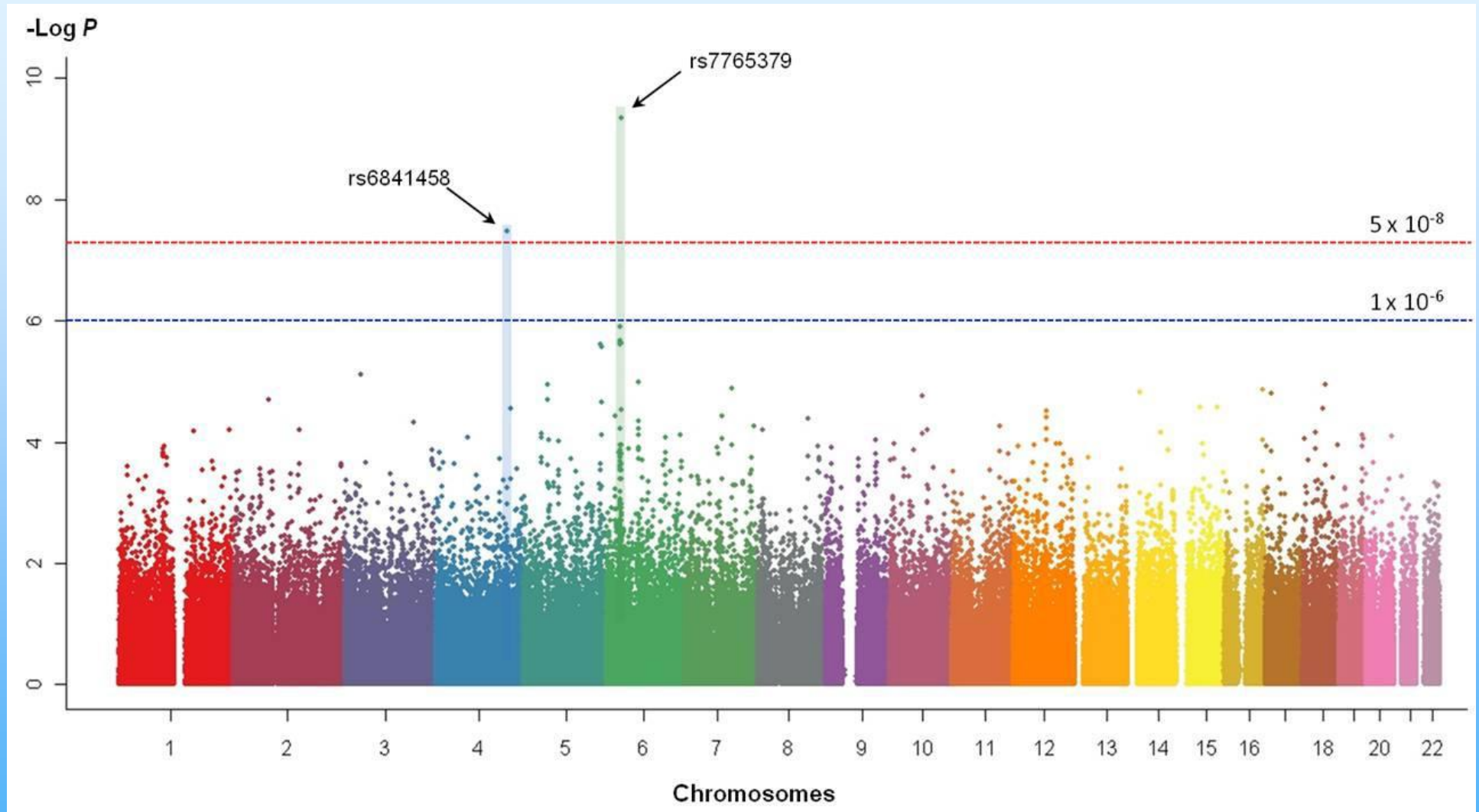
## Quantile-quantile plot of the association P values obtained



- Associated with typhoid?
- Associated by chance?
- Cases and controls are well matched

Two SNPs for replication

## Manhattan plot of the association P values obtained in the Vietnamese cases and controls





## SNP rs7765379 is associated with enteric fever in the Vietnamese and Nepalese

595 cases and 386 controls (Nepal); 151 cases and 668 controls (Vietnam)

SNP [gene]	Reference allele	Effect allele	Genotype count <sup>a</sup> in cases (freq.)	Genotype count <sup>a</sup> in controls (freq.)	<i>P</i> value <sup>b</sup>	OR	Collection
rs7765379	A	C	0/9/423 (0.0104)	6/208/1,797 (0.055)	$4.5 \times 10^{-10}$ ( $2.53 \times 10^{-8}$ )	0.18	Discovery (Vietnam) <sup>c</sup>
(HLA-DRB1-HLA-DQB1)	A	C	0/16/574 <sup>d</sup> (0.0136)	3/24/359 (0.039)	0.00070 (0.00062)	0.34	Replication (Nepal) <sup>e</sup>
	A	C	0/3/148 (0.010)	1/69/598 (0.053)	0.00013 (0.00098)	0.18	Replication (Vietnam) <sup>f</sup>
					$2.29 \times 10^{-13}$	0.22 (0.15–0.34)	Meta-analysis

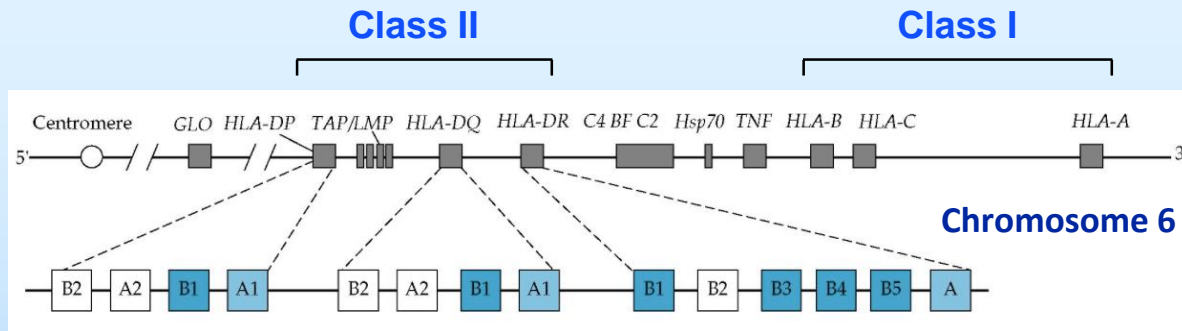
## Allele frequency distributions for rs7765379 in multiple Vietnamese collections

Under-representation of rs7765379 C allele appears specifically confined to enteric fever

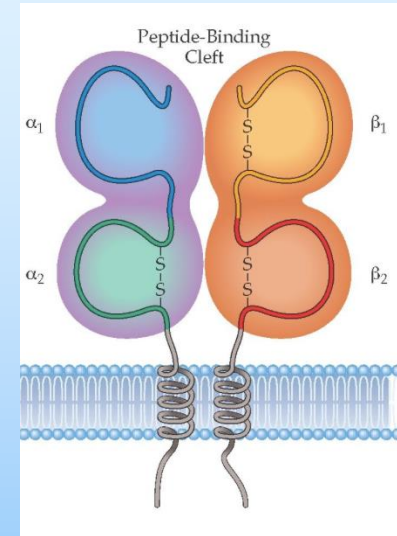
Stability of association across non-typhoid infectious and non-communicable diseases

	Vietnam discovery (Salmonella enteric fever)		Vietnam replication (Salmonella enteric fever)		Vietnam	Vietnam	Vietnam	Vietnam
	Case	Control	Case	Control	Dengue cases	Pulmonary TB cases	Meningeal TB cases	PACG cases
AA	423	1797	148	598	1795	1265	426	319
AC	9	208	3	69	208	147	50	35
CC	0	6	0	1	6	4	2	2
<b>Total</b>	<b>432</b>	<b>2011</b>	<b>151</b>	<b>668</b>	<b>2009</b>	<b>1416</b>	<b>478</b>	<b>356</b>
<b>Frequency</b>	<b>0.0104</b>	<b>0.055</b>	<b>0.01</b>	<b>0.053</b>	<b>0.055</b>	<b>0.055</b>	<b>0.056</b>	<b>0.055</b>

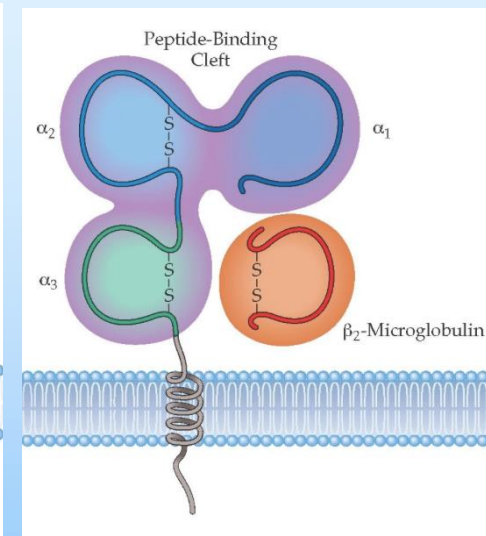
# rs7765379 maps to HLA class II, in proximity to HLA-DQB1 and HLA-DRB1



**MHC Class II**



**MHC Class I**



## Genes of the Class II and Class III Major Histocompatibility Complex Are Associated with Typhoid Fever in Vietnam

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### ORIGINAL INVESTIGATION

## A *TNF* region haplotype offers protection from typhoid fever in Vietnamese patients

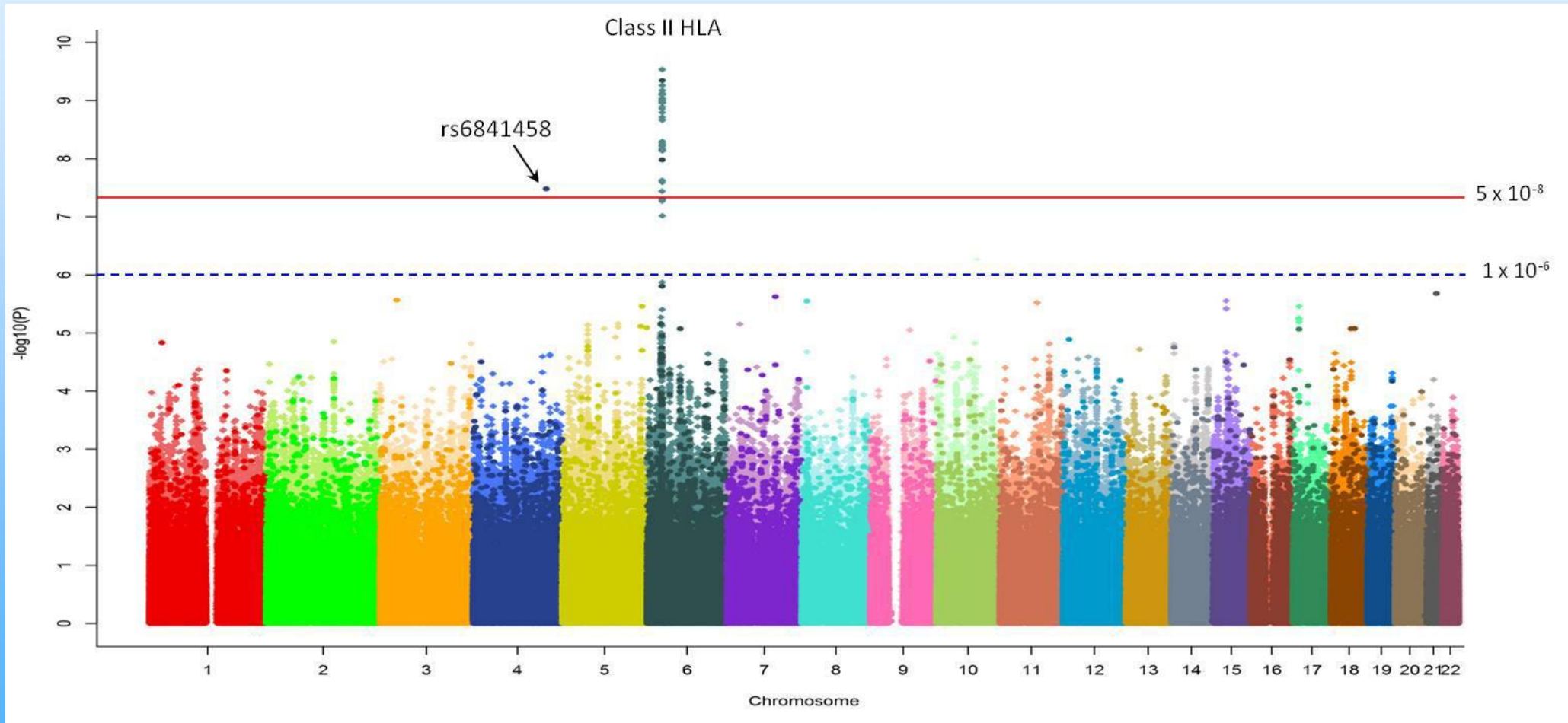
Sarah J. Dunstan · Nguyen Thi Hue · Kirk Rockett · Julian Forton · Andrew P. Morris ·  
Mahamadou Diakite · Mai Ngoc Lanh · Le Thi Phuong · Deborah House · Christopher M. Parry ·  
Ha Vinh · Nguyen T. Hieu · Gordon Dougan · Tran Tinh Hien · Dominic Kwiatowski · Jeremy J. Farrar

Extensive linkage disequilibrium in HLA region

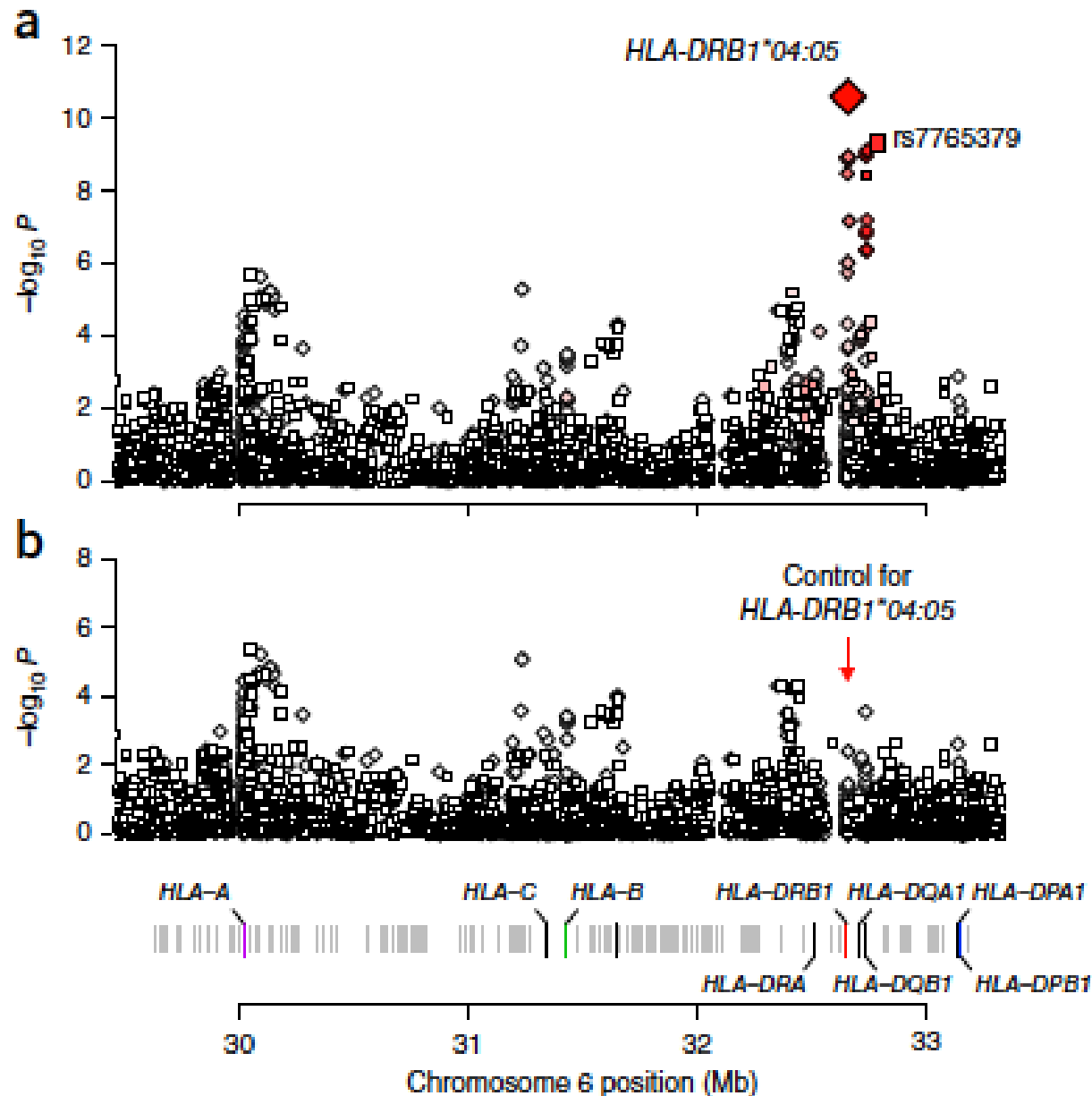
Sample size too small and density of SNPs typed too low

Current GWAS can confirm that the protective gene is HLA-DRB1

## Manhattan plot of the association P values obtained in the discovery sample collection after genome-wide imputation (1000 Genomes Project Asian reference panel)



## Enteric fever association within the broad HLA region



HLA-DRB1\*04:05  
(OR= 0.14,  $P=2.6 \times 10^{-11}$ )

rs7765379 and HLA-DRB1\*04:05  
 $r^2=0.83$

Squares directly typed SNPs  
Diamonds imputed markers

When conditioning the analysis on HLA-DRB1\*04:05, we no longer observed any convincing evidence of association ( $P > 4 \times 10^{-6}$ )

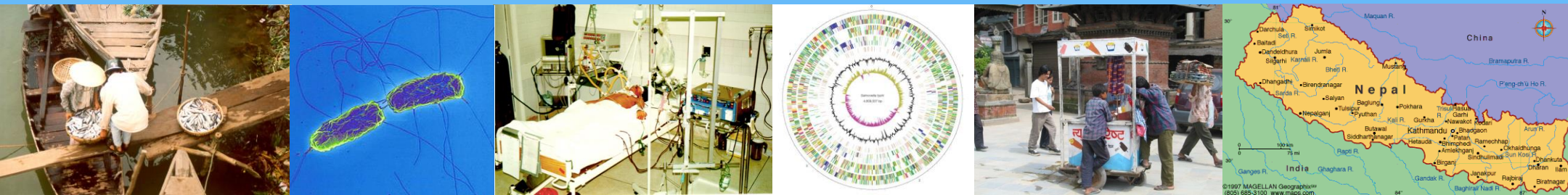
- Are there more susceptibility loci for enteric fever beyond HLA-DRB1?
- Are they of smaller effect size, and can we detect them?

## Expansion of GWAS

We genotyped a total of 200 new Vietnamese enteric fever cases

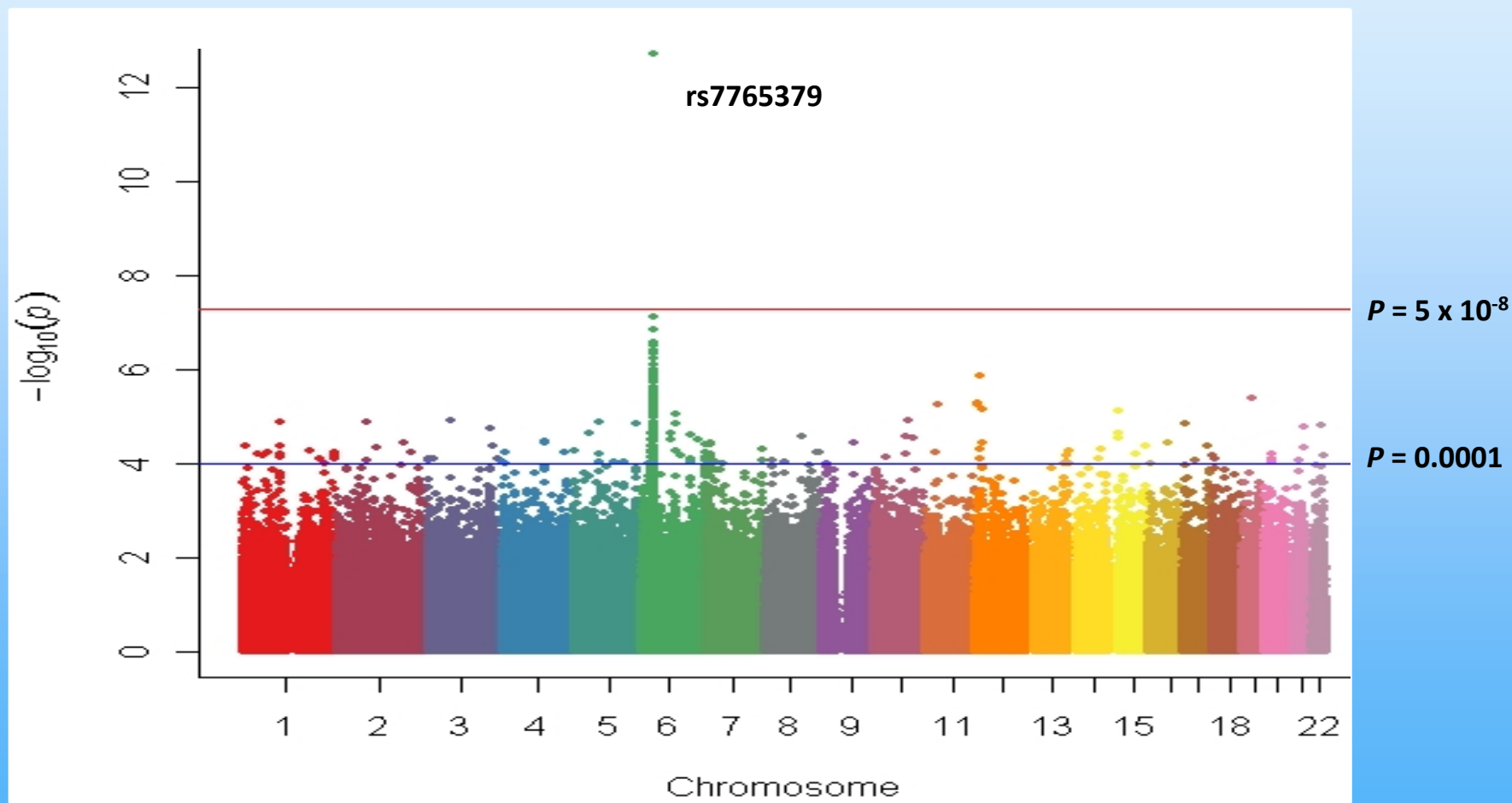
As well as 400 Nepal enteric fever cases and 200 cord blood controls which passed initial QC

1/3 sample drop out due to variable quality of archived samples

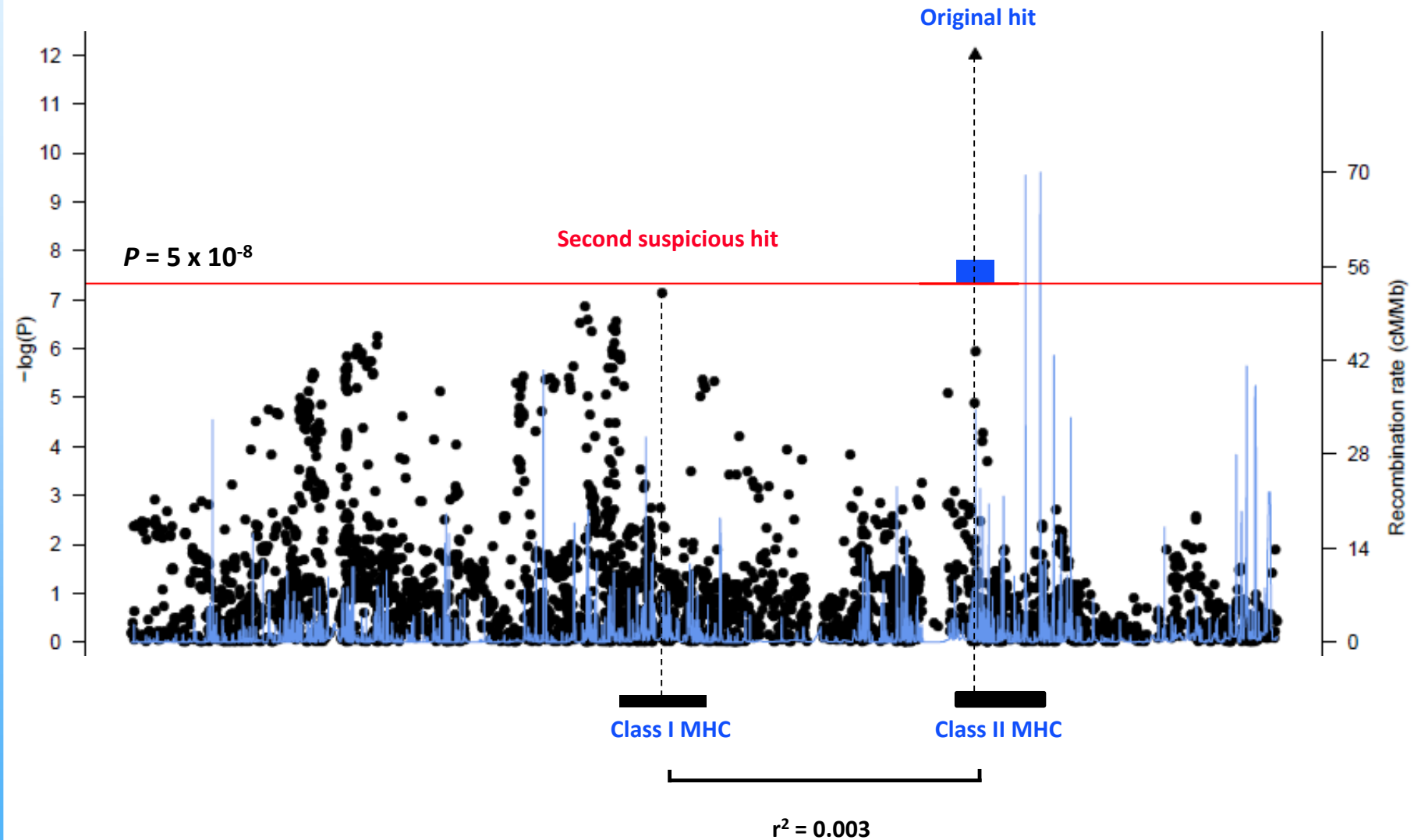




## Manhattan plot of the meta-GWAS (980 typhoid cases and 2200 controls)

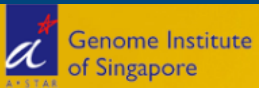


## Potential Class I hit is independent from rs7765379 and *HLA-DRB1*



# Summary

- HLA-DRB1\*0405 confers 5 fold protection ➡ a major contributor to resistance
- only malaria and HIV have larger gene effects (sickle cell and CCR5/HLA)
- sequence polymorphisms HLA-DRB1 ➡ functional differences MHC class II AA  
➡ enteric fever risk
- This could influence     *S.*Typhi epitope selection?  
                                      antigen presentation?  
                                      magnitude of T cell response?  
                                      type of T cell response?
- Studies to understand biological mechanism behind natural disease resistance
- Larger scale studies to verify and identify new disease genes to obtain a complete understanding of the impact of human genetic variation on enteric fever



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