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?

Dunstan et al, 2014, Nature Genetics, 122:51
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

Dunstan et al, 2014, Nature Genetics, 122:51
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

Dunstan et al, 2014, Nature Genetics, 122:51
Manhattan plot of the association $P$ values obtained in the Vietnamese cases and controls

Dunstan et al, 2014, Nature Genetics, 122:51
SNP rs7765379 is associated with enteric fever in the Vietnamese and Nepalese
595 cases and 386 controls (Nepal); 151 cases and 668 controls (Vietnam)

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

Dunstan et al, 2014, Nature Genetics, 122:51
rs7765379 maps to HLA class II, in proximity to HLA-DQB1 and HLA-DRB1

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

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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

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

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

Dunstan et al, 2014, Nature Genetics, 122:51
Enteric fever association within the broad HLA region

HLA-DRB1*04:05
(OR= 0.14, P=2.6x10^{-11})

rs7765379 and HLA-DRB1*04:05
r²=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>4x10^{-6})

Dunstan et al, 2014, Nature Genetics, 122:51
• 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)

rs7765379

$P = 5 \times 10^{-8}$

$P = 0.0001$
Potential Class I hit is independent from rs7765379 and \textit{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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