Host genetics of tuberculosis susceptibility

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But what exactly is a genetic disease?

Phenylketonuria (PKU) is a metabolic disease that is caused by phenylalanine:

No phenylalanine = No PKU!

Is PKU a genetic disease? YES!

Phenylalanine is an environmental factor that is NECESSARY but NOT SUFFICIENT for disease (PKU) expression.

*M. tuberculosis* is necessary, but is it sufficient?
Tuberculosis Pathogenesis

1. 20-50%
   - RESISTANCE to infection (No entry into latency)

2. 5%
   - PRIMARY TB
     - Clinical TB without latency

3. 5%
   - LATENT TB INFECTION (LTBI)
     - No overt clinical symptoms
     - 90%
   - REACTIVATION TB
     - Pulmonary symptoms

M. tuberculosis is not sufficient to cause TB
Three examples that support a role of genetic predisposition to clinical tuberculosis disease:

(i) the Lübeck accident
(ii) risk of tuberculosis recurrence
(iii) twin studies
## The Lübeck Accident

<table>
<thead>
<tr>
<th>Virulence level</th>
<th>Number</th>
<th>Disease severity</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>death</td>
<td>serious disease</td>
<td>mild symptoms</td>
<td>no symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>93</td>
<td>6 = 6.5%</td>
<td>9 = 9.7%</td>
<td>78 = 83.8%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>83</td>
<td>18 = 21.7%</td>
<td>34 = 41.0%</td>
<td>31 = 37.3%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>74</td>
<td>53 = 71.6%</td>
<td>18 = 24.3%</td>
<td>3 = 4.1%</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>251</td>
<td>77</td>
<td>61</td>
<td>112</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Risk of Recurrence of TB

Re-infection disease rate of 2.2/100 PYRS corresponds to 4 times the age-adjusted incidence

Verver et al AJRCCM 171: 1430, 2005
Twin studies

DZ TWINS

2 fertilizations

Share 50% of genetic background

MZ TWINS

1 fertilization

Share 100% of genetic background
# Twin studies

<table>
<thead>
<tr>
<th>Concordance</th>
<th>Monozygous twins</th>
<th>Dizygous twins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65%</td>
<td>25%</td>
<td>Diehl and Von Verschuer, Beitr. Klin Kunsch 92: 275, 1936</td>
</tr>
<tr>
<td></td>
<td>62%</td>
<td>18%</td>
<td>Kallmann and Reisner, Am Rev Respir Dis 47, 549, 1942</td>
</tr>
<tr>
<td></td>
<td>32%</td>
<td>14%</td>
<td>Comstock, Am Rev Respir Dis 117, 621, 1978</td>
</tr>
</tbody>
</table>

Significant excess of concordance among monozygous twins demonstrates the importance of host genetic factors.
Candidate gene approaches

Many examples - one selected gene: NRAMP1
Candidate NRAMP1: TB outbreak

- Entire pedigree: 85 individuals
- Genotypes available from 65 individuals
- Majority of cases occurred within 6 months of diagnosis of index case
- Last case was diagnosed 2 years after index case
- Case criteria: clinical sign of active disease PLUS culture OR response to anti-TB therapy

Greenwood et al AJHG 67:405,2000
### Candidate *NRAMP1*: TB outbreak

<table>
<thead>
<tr>
<th>Liability class</th>
<th>Penetrance of homozygous</th>
<th># individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low risk allele</td>
<td>High risk allele</td>
</tr>
<tr>
<td></td>
<td>RR=10</td>
<td>RR=100</td>
</tr>
<tr>
<td>Previously unexposed</td>
<td>0.085</td>
<td>0.0085</td>
</tr>
<tr>
<td>Previously exposed or vaccinated</td>
<td>0.037</td>
<td>0.0037</td>
</tr>
<tr>
<td>PPD negative during epidemic</td>
<td>0.010</td>
<td>0.0010</td>
</tr>
<tr>
<td>Age &lt;2 yrs, &gt;65yrs</td>
<td>0.425</td>
<td>0.2125</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Greenwood et al AJHG 2000
Candidate *NRAMP1*: TB outbreak

With liability classes: Strong evidence for strong genetic effect (NRAMP1)

\[ P = < 0.000001, \text{RR}=10 \]

Without liability classes: NO evidence for any genetic effect


Environmental setting defines the playing field for genetic factor
Positional cloning approaches: *TOX* gene
Positional cloning approaches: *TOX* gene

Positional cloning approaches: *TOX* gene

<table>
<thead>
<tr>
<th>Stratum</th>
<th>SNP</th>
<th>Minor Allele</th>
<th>Major Allele</th>
<th>MAP</th>
<th>Family-Based Study</th>
<th>Case-Control Study</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>p Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full</td>
<td>rs1568952</td>
<td>A</td>
<td>G</td>
<td>0.36</td>
<td>3.21 (1.41–7.35)</td>
<td>0.007</td>
<td>1.98 (1.33–2.94)</td>
</tr>
<tr>
<td></td>
<td>rs2726600</td>
<td>G</td>
<td>A</td>
<td>0.40</td>
<td>2.65 (1.27–5.56)</td>
<td>0.0093</td>
<td>1.61 (1.12–2.31)</td>
</tr>
<tr>
<td>&lt;25 Years</td>
<td>rs1568952</td>
<td>A</td>
<td>G</td>
<td>-</td>
<td>5.54 (1.97–15.53)</td>
<td>0.0003</td>
<td>2.86 (1.72–4.77)</td>
</tr>
<tr>
<td></td>
<td>rs2726600</td>
<td>G</td>
<td>A</td>
<td>-</td>
<td>2.56 (1.37–4.80)</td>
<td>0.0025</td>
<td>2.00 (1.24–3.23)</td>
</tr>
<tr>
<td>≥25 Years</td>
<td>rs1568952</td>
<td>A</td>
<td>G</td>
<td>-</td>
<td>0.65 (0.12–3.66)</td>
<td>0.62</td>
<td>1.52 (0.93–2.47)</td>
</tr>
<tr>
<td></td>
<td>rs2726600</td>
<td>G</td>
<td>A</td>
<td>-</td>
<td>1.73 (0.56–5.33)</td>
<td>0.33</td>
<td>1.38 (0.89–2.14)</td>
</tr>
</tbody>
</table>

The following abbreviations are used: MAF, minor allele frequency; OR, odds ratio; and CI, confidence interval.

*MAF was estimated from among 316 founders.

*All p values are two sided.*

Positional cloning approaches: *TOX* gene
Genome-wide association studies (GWAS)
Chromosome 18 locus not replicated outside of West Africa

Chromosome 11 locus replicated outside of West Africa

Thye et al. Nat Genet. 2010 Sep;42(9):739-41

Tuberculosis Pathogenesis

1. Exposure to *M. tuberculosis*
   - Susceptibility to infection
   - Resistance to infection
     (No entry into latency)

2. Primary TB
   - Clinical TB without latency

3. Latent TB Infection (LTBI)
   - No overt clinical symptoms

   Reversion to clinical disease
   - Reactivation TB
     - Pulmonary symptoms
Latent *M. tuberculosis* infection

How do we measure infection?

No “gold standard”

Three types of assays

*In vivo* tuberculin skin test (TST)

*In vitro* production of antigen-specific IFN\(\gamma\) production (ELISA)

*In vitro* determination of frequency/number of antigen-specific T-cells
  (ELISpot/FACS)
Tuberculin Skin Test

Detection of people infected by *M. tuberculosis*

**Public Health**
- ≥ 5 mm (Immuno-)
- ≥ 10 mm (no BCG)
- ≥ 15 mm (BCG)

**Infection**

Intrinsically a **quantitative measure**
Max Lurie’s Rabbits

Healthy

Infected

Cavitary disease
Mean survival 9.2 months

Disseminated disease
Mean survival 4.8 months

Innate resistance

20-40% no disease
85% Tuberculin test negative
11-19 months of exposure

Lurie M B.  *Am Rev Tuberc* 1941; 44 (suppl): 1–125

Lurie’s rabbits

Werneck-Barroso E. Int J Tuberc Lung Dis 1999;3:166-68
Genetics of LTBI: TST

✓ **Familial correlation studies**: heritability between 30 à 90%
  (Jepson et al., *Infect Immun*, 2001)

✓ **Molecular studies**:
  • linkage study in Uganda
    (Stein et al., *Plos One*, 2008)
  • Candidate gene *IL10* and binary Mantoux
  • *Il12RB1* and *TLR2* polymorphisms and persistent TST-negativity
    Stein et al Poster X7 4020, Keystone Meeting, Host Response in Tuberculosis
    Whistler, March 13-18, 2013
Extent of TST reactivity

Familial correlation compatible with a major gene effect?

⇒ Complex Segregation Analysis (CSA)
A major gene controls TST reactivity in Colombia

Codominant gene ($p<10^{-6}$), MAF: 0.41 (predisposing to high reactivity)

35% (17%) predisposed to low (high) values

⇒ explains 72% of TST residual variability!

Cobat et al. CID 54:968, 2012
A “what does it mean” example

Mean TST

16 years
BCG+

AA  aA  aa

3mm  12mm  22mm
Genetic linkage study of TST reactivity

Location: Cape Town, South Africa

- 128 nuclear families $\geq 2$ sibs
- 186 parents
- 350 children
- DNA
- Immune phenotypes
- Covariates
- 6,000 SNPs genotyped for linkage analysis
TST distribution is bimodal

TST = 0 vs. Non 0

TST-BIN

Pearson residuals

TST in mm

Major locus for TST negativity *per se* (*TST1*) maps to 11p14
Household contact study in Paris

Chromosomes

Information content

LOD score

significant

suggestive

Cobat et al submitted
ENDOPHENOTYPES

134 nuclear families [2-6 sibs]; 390 children; whole blood assays

TNF production by whole blood after stimulation by:

- BCG
- BCG + IFN-\(\gamma\)
Bivariate linkage analysis of TNF production

Cobat et al Clin Infect Dis. 2013 Oct;57(7):963-70
Major pleiotropic locus for BCG-triggered TNF overlaps innate resistance to *Mtb* infection locus!

Cobat et al submitted 2014
Take-home message I

- Host genetic background is a major confounder of TST reactivity
- A major locus on chromosome 11p controls TST = 0
How to interpret TST = 0?

- A false positive
- Anergy
- Lack of exposure
- Resistance to LTBI
Genetics suggests a connection between innate resistance to *Mtb* infection with innate efficiency to produce TNF
McGill Center for the Study of Host Resistance

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