Human Genetics of Tuberculosis

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Infectious diseases: the greatest killer
The microbial theory of Infectious diseases

“In the course of hereditary flacherie, it is not the microbe that is transmitted from the parents to the offspring, but the predisposition to disease”

L. Pasteur, Maladies des vers à soie (1865)
Variability of response to exposure and infection

The discovery of asymptomatic infections (1910s)
Tuberculosis (TB)
Individual variability in response to infection

- The Lübeck disaster in 1926

Accidental inoculation with *M. tuberculosis*
- 251 infants

Death by year 1
- 77 infants

Various signs of infection
- 127 infants

No sign of infection
- 47 infants
TB: Individual variability in clinical outcomes

Variability of response to exposure and infection

MYCOBACTERIA

EXPOSURE FACTORS

VIRULENCE FACTORS

M. tuberculosis → INFECTION

~90%

HOST

NON-GENETIC FACTORS

GENETIC FACTORS

PRIMARIES TB (Extra-pulmonary)

~5%

PRIMARIES TB (Pulmonary)

~5%
Inter-individual variability but familial aggregation

« The occurrence of tuberculosis in families led to the view that it was an inherited disease. The demonstration of a characteristic bacterium by Koch in 1882 disposed of this view »

T. Smith, Parasitism & Disease (1934)
Familial (twin) studies (1930s)

FAMILIAL SUSCEPTIBILITY TO TUBERCULOSIS
Its Importance as a Public Health Problem
BY RUTH RICE PUFFER, DR.P.H.
Tennessee Department of Public Health
CAMBRIDGE, MASSACHUSETTS
HARVARD UNIVERSITY PRESS
1944

Zwillingsstüberkulose
Zwillingsforschung
und
erbliche Tuberkulosedisposition

Von
Karl Diehl und Otmar Frhr. v. Verschuer

TWIN STUDIES ON GENETIC VARIATIONS IN RESISTANCE TO TUBERCULOSIS
FRANZ J. KALLMANN AND DAVID REISSNER

TUBERCULOSIS MORBIDITY RATES OF RELATIVES OF TWIN INDEX CASES
Human genetics in tuberculosis?

- Concept
- Epidemiological/familial observations
- Experimental models
- Human genetic studies
  - Genetic epidemiology
  - Mendelian genetics
- Proof of concept
Human genetics of tuberculosis

Why do some exposed individuals (and not others) get infected and develop tuberculosis?

What are the critical immunological pathways in natural conditions of infection?

→ Search of genetic variants that:
- may explain differences between individuals (in part)
- are influencing the immune response to *M. tuberculosis*
### Methods of investigation in humans

#### How to identify the causal genetic variant?

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Severe/disseminated TB (children)</th>
<th>Pulmonary TB (adults)</th>
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<tbody>
<tr>
<td><strong>Tools</strong></td>
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<td><strong>Complex Genetics</strong></td>
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- **Rare mutations**
  - Strong individual effect

- **Common polymorphisms**
  - Modest individual effect

*Using the considerable progress in genomics technology (ultra-high throughput genotyping, sequencing….*)*
Continuous spectrum of genetic predisposition according to individual effect and frequency of genetic variant

Relative risk (odds ratio, OR)

(Manolio et al, Nature, 2009)
Considerable number of genetic variants

Human genome is > 3000 millions base pairs (A, T, C, G)

> 40 millions reported variants in humans (most of them are <1%)
Most frequent variants are single nucleotide polymorphisms (SNPs)
(simple change of one base to another, eg from A to G)
How to identify the causal genetic variant in appropriate samples?

MENDELIAN AND COMPLEX INHERITANCE

HYPOTHESIS TESTING
(Candidate gene/pathway approach)

HYPOTHESIS GENERATING
(Genome-wide approach)

ANIMAL
HUMAN

LINKAGE
ASSOCIATION
SEQUENCE

CANDIDATE GENES/VARIANTS

‘RARE’ MUTATIONS
‘COMMON’ POLYMORPHISMS

ASSOCIATION STUDIES
(Replications)

FUNCTIONAL STUDIES
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Mendelian susceptibility to mycobacterial diseases (MSMD)

* Infections by BCG and environmental mycobacteria mostly in young children

- Limited disease
  - Granuloma

- Disseminated disease
  - Lepromatous like
* Otherwise healthy individuals

* Very rare (10^-5 – 10^-6) but often familial (consanguinity and/or multiplex)
MSMD: 7 genes, 14 genetic diseases

Mycobacteria

Macrophage/Dendritic Cell

T Lymphocyte

→ Specific antimycobacterial pathway *in natura* (IL12/IFN-γ)

→ Medical implications (IFN-γ treatment)

→ From BCG/EM to *M. tuberculosis*
IL-12Rβ1 deficiency: the most common disorder → >100 kindreds from 30 countries
IL-12Rβ1 deficiency → Allelic heterogeneity

Autosomal recessive (54 different mutations):
Patients were homozygous (87%) or compound heterozygous (13%)
Functional homogeneity: Complete IL-12Rβ1 deficiency

Abolished response to IL-12
IL12R-β1 deficiency and Tuberculosis

Complete IL12R-β1 deficiency: No cellular responses to IL-12
→ Mendelian tuberculosis
IL-12Rβ1 deficiency and Tuberculosis

Systematic sequencing of *IL12RB1* in a sample of 50 children (<15 yrs) with severe TB from Morocco, Turkey and Iran.

→ 2 patients with complete IL12-Rβ1 deficiency

\[ \text{Kindred A (Morocco)} \]

→ Proportion of Mendelian TB could be far from negligible
  (4% in a small sample by testing a single gene)

→ Identification of mutations in *IL12RB2* in patients with severe TB

→ Genome-wide approach: Investigation of a larger sample of patients by Whole exome sequencing
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TB: Major public health problem

~ 1/3 world’s population exposed to *M. tuberculosis*
~ 9.2 million new cases/year and ~ 1.7 million deaths/year
HYPOTHESIS TESTING  
(Candidate gene/pathway approach)

HYPOTHESIS GENERATING  
(Genome-wide approach)

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FUNCTIONAL STUDIES

Huge number of candidate gene studies: very few replicated and convincing
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(Candidate gene/pathway approach)

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PULMONARY TB
Genome-wide linkage screen in Morocco

96 multiplex families

<table>
<thead>
<tr>
<th># affected offspring</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
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<tr>
<td># families</td>
<td>68</td>
<td>21</td>
<td>7</td>
</tr>
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Total of 227 affected offspring (92%>15 years, 90%<40 years) with positive pulmonary TB

Linkage to chromosome 8q12-q13

39 families with affected parent

All 96 families

→ Refined mapping by association studies
Refined mapping (association study) in Morocco

PRIMARY ASSOCIATION SAMPLE
• 203 families including 285 offspring with pulmonary TB
  → family-based association analysis

REPLICATION ASSOCIATION SAMPLE
• 317 pulmonary TB patients and 650 healthy controls
  → Case/control association of the best signals found in primary analysis

GENETIC VARIANTS
→ ~ 3000 SNPs to account for genetic variability in polymorphisms > 0.05 of the target region
Chr8 Family-based association P-values

Chromosome 8q12-13

55.09 → 61.20 Mb

Two replicated SNPs in TOX

44 SNPs with $p<0.01$ tested for replication
→ Only two replicated SNPs
## Association with TOX SNPs in Morocco

<table>
<thead>
<tr>
<th>Marker</th>
<th>Family-based</th>
<th></th>
<th></th>
<th></th>
<th>Case-control</th>
<th></th>
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<th>Combined</th>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>P</td>
<td>OR</td>
<td>P</td>
<td>OR</td>
<td>P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full population</td>
<td>rs1568952</td>
<td>3.2</td>
<td>0.007</td>
<td>2</td>
<td>6.10^{-4}</td>
<td>2.12</td>
<td>1.14x10^{-5}</td>
<td></td>
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<tr>
<td></td>
<td>rs2726600</td>
<td>2.3</td>
<td>0.009</td>
<td>1.6</td>
<td>0.0092</td>
<td>1.8</td>
<td>9.2x10^{-5}</td>
<td></td>
</tr>
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### Stronger effect in early-onset TB

**Strong age effect**

**No gender effect**

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<tr>
<th>Marker</th>
<th>OR</th>
<th>P</th>
<th>OR</th>
<th>P</th>
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<tr>
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<tr>
<td>rs2726600</td>
<td>2.3</td>
<td>0.04</td>
<td>1.6</td>
<td>0.0092</td>
<td>1.8</td>
<td>9.2x10^{-5}</td>
</tr>
<tr>
<td><strong>Under 25</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1568952</td>
<td>5.5</td>
<td>0.0003</td>
<td>2.8</td>
<td>2.9x10^{-5}</td>
<td>3.3</td>
<td>4.4x10^{-8}</td>
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<tr>
<td>rs2726600</td>
<td>2.6</td>
<td>0.0025</td>
<td>2</td>
<td>0.0039</td>
<td>2.2</td>
<td>3.2x10^{-5}</td>
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<tr>
<td><strong>Over 25</strong></td>
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<td></td>
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<tr>
<td>rs1568952</td>
<td>0.65</td>
<td>0.62</td>
<td>1.5</td>
<td>0.094</td>
<td>1.4</td>
<td>0.15</td>
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<tr>
<td>rs2726600</td>
<td>1.7</td>
<td>0.33</td>
<td>1.4</td>
<td>0.15</td>
<td>1.4</td>
<td>0.09</td>
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**Mean:** 26.1 yrs

**Median:** 24
Validation in Madagascar

Madagascar family-based study:
257 affected offspring

Mean: 26.3 yrs
Median: 25

Under 25 years

<table>
<thead>
<tr>
<th>Marker</th>
<th>Risk allele</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Risk allele</th>
<th>OR (95% CI)</th>
<th>P</th>
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<tr>
<td>rs2726600</td>
<td>G (0.44)</td>
<td>2.2 (1.5-3.2)</td>
<td>3.2x10^{-5}</td>
<td>G (0.15)</td>
<td>1.77 (1.0-3.17)</td>
<td>0.04</td>
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TOX
Thymocyte selection-associated high mobility group box protein

TOX is involved in the development of CD4 T cells.

CD4 T cells are of major importance to maintain latent infection as shown by high incidence of pulmonary TB in HIV+ subjects.
rs2726600 is located in an important regulatory region

Functional studies investigating the expression of TOX in T cells according to rs276600 genotypes
COMPLEX PREDISPOSITION TO PULMONARY TB

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Genome-wide association studies (GWAS) in TB

Genome-wide association analyses identifies a susceptibility locus for tuberculosis on chromosome 18q11.2

OR=1.19 (1.12-1.26)

Total of ~3500 patients and ~7500 controls from The Gambia and Ghana
GWAS: Preliminary results in Morocco

12 SNPs <\(10^{-5}\)
1520 SNPs P<0.001

→ No strong signals in pulmonary TB with common polymorphisms

→ Refined analysis
→ Replication
**Summary for TB – General Implications**

*M. tuberculosis* → Infection

- **Latency**: ~95%
- **Primary Extrapulmonary**: ~5%

Mendelian component *(IL12/IFNγ pathway...)*
- Some BCG protection

TOX SNPs in early-onset PTB
- Limited role of common SNPs
- Known role of HIV infection, anti-TNF

What are the critical pathways *in natural conditions of infection*?
→ Understanding pathogenesis through human genetics

Implications for
→ Prevention (vaccination, target populations...)
→ Treatment (restore deficient immunity, e.g. IFN-γ treatment)
Proven role in severe TB of children
Proportion of Mendelian TB?
To be investigated especially in early-onset and familial forms of PTB
Limited role in PTB
Important in early-onset TB
Mendelian
Intermediate
Multigenic

Genetic cases

80%

Primary infection
Reinfection/reactivation

Age

Genetic spectrum depends on age
Fits pretty well for young cases
Additional factors especially in adults
Laboratory of Human Genetics of Infectious Diseases

Jean-Laurent Casanova and Laurent Abel
Huge number of collaborators

Medical clinicians around the world