Human Genetics of Tuberculosis

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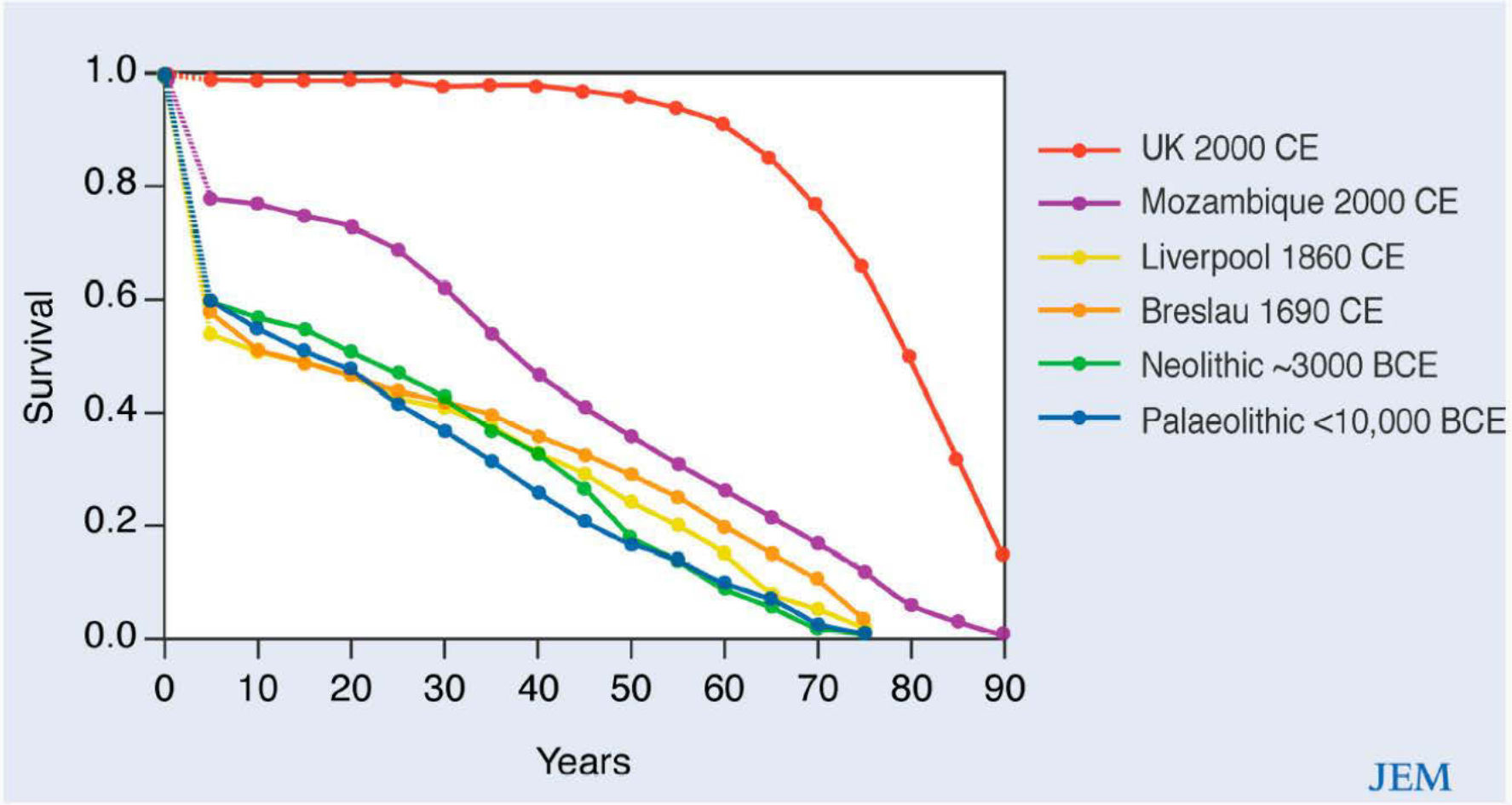
Infectious diseases: the greatest killer

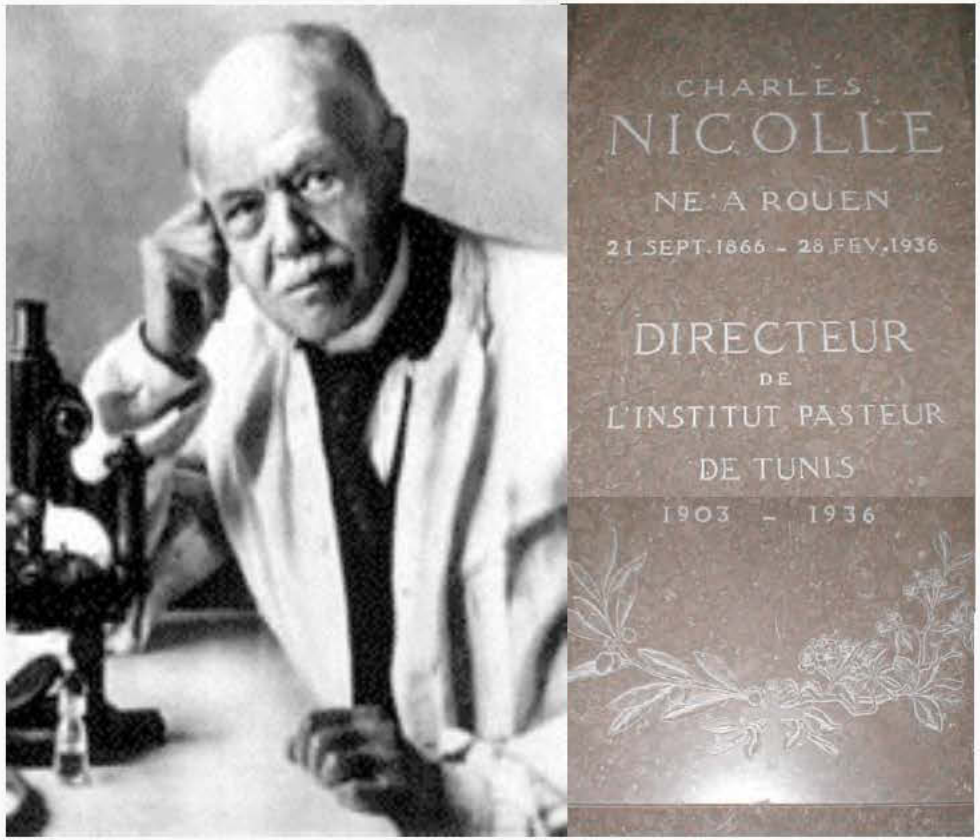


The microbial theory of Infectious diseases

"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

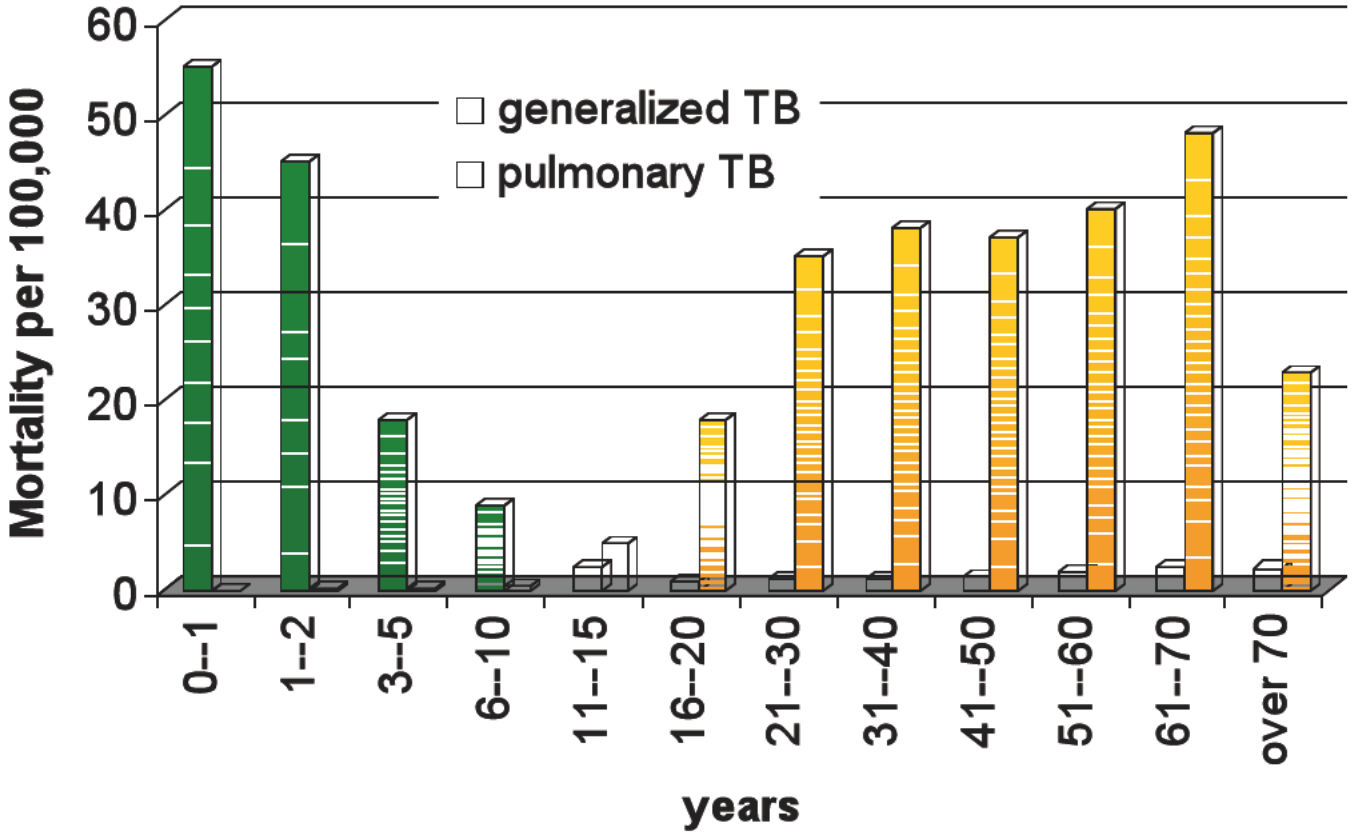
Accidental inoculation with *M. tuberculosis* 251 infants

Death by year 1
77 infants

Various signs of infection
127 infants

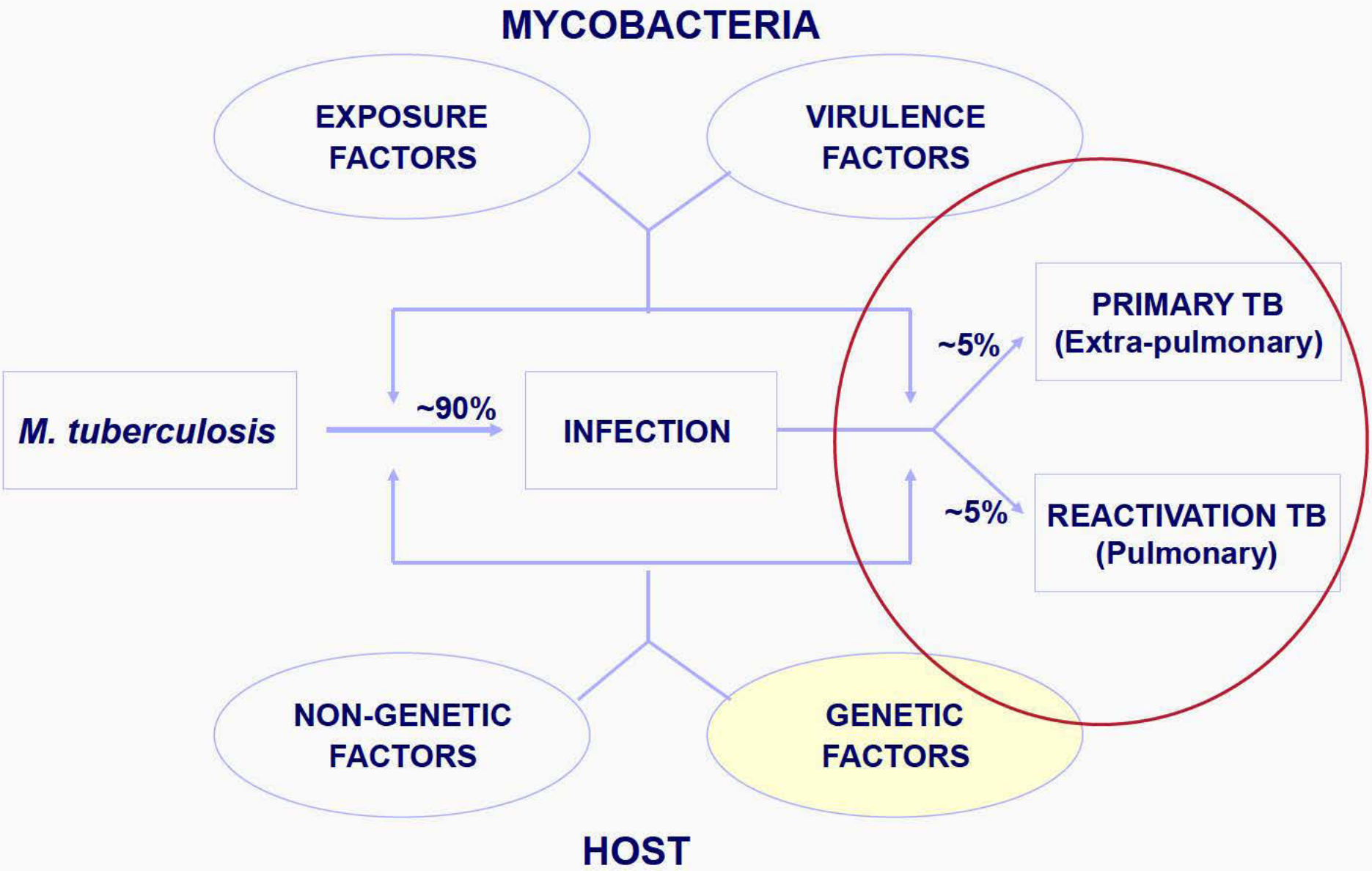
No sign of infection
47 infants

TB: Individual variability in clinical outcomes

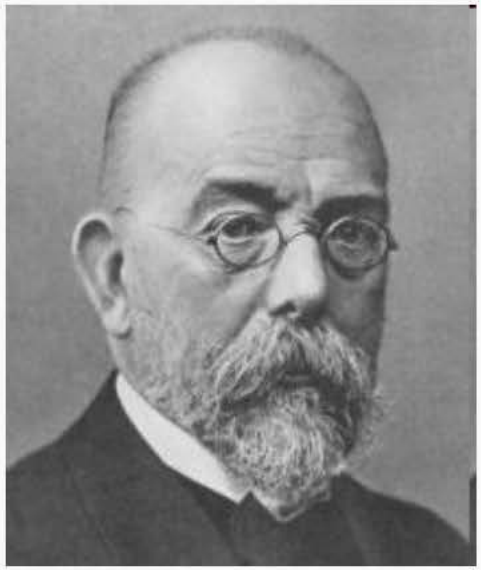


Ranke, K. 1910. Diagnose und Epidemiologie der Lungentuberculose des Kindes. Archiv für Kinderheilkunde 54:279-306.

Variability of response to exposure and infection



Inter-individual variability but familial aggregation



Robert Koch

Theobald Smith

« 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

Zwillingstuberkulose

Zwillingsforschung
und
erbliche Tuberkulosedisposition

Von

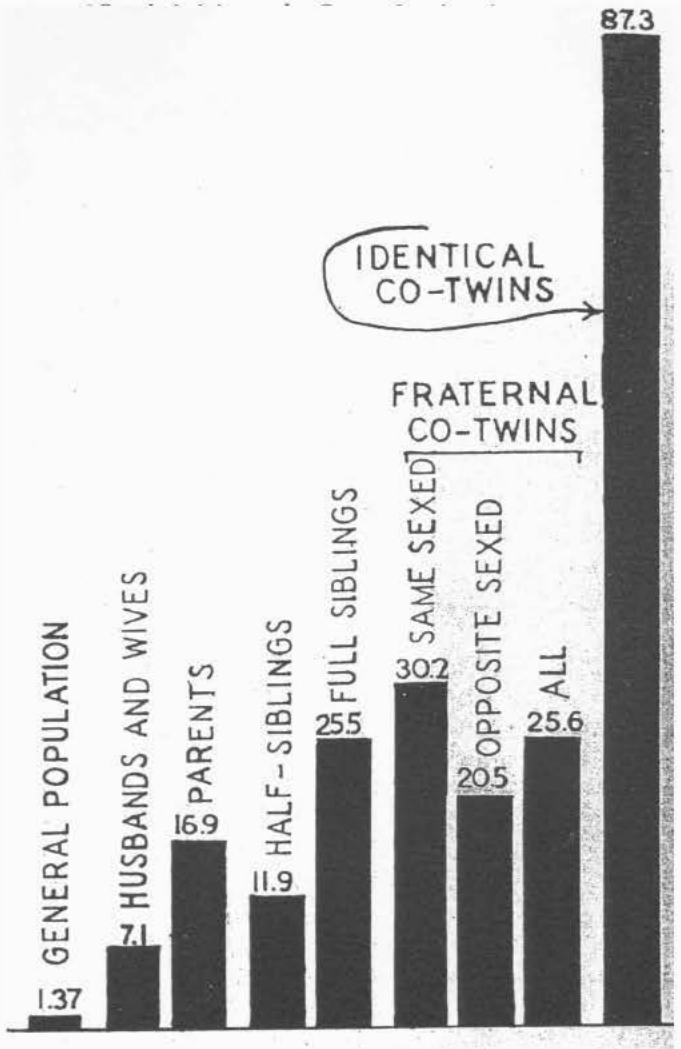
Karl Diehl und Otmar Frhr. v. Verschuer

[illegible] d. Tuberkulose-Kranken- [illegible] d. Stadt Berlin „Waldhaus Charlottenburg" in [illegible] (Osthavelland). Leiter d. Tuberkulose-Fürsorgestelle d. Kr. Osthavelland

Privatdozent und Leiter der Abteilung für menschliche Erblehre des Kaiser-Wilhelm-Instituts für Anthropologie, menschliche Erblehre und Eugenik in Berlin-Dahlem

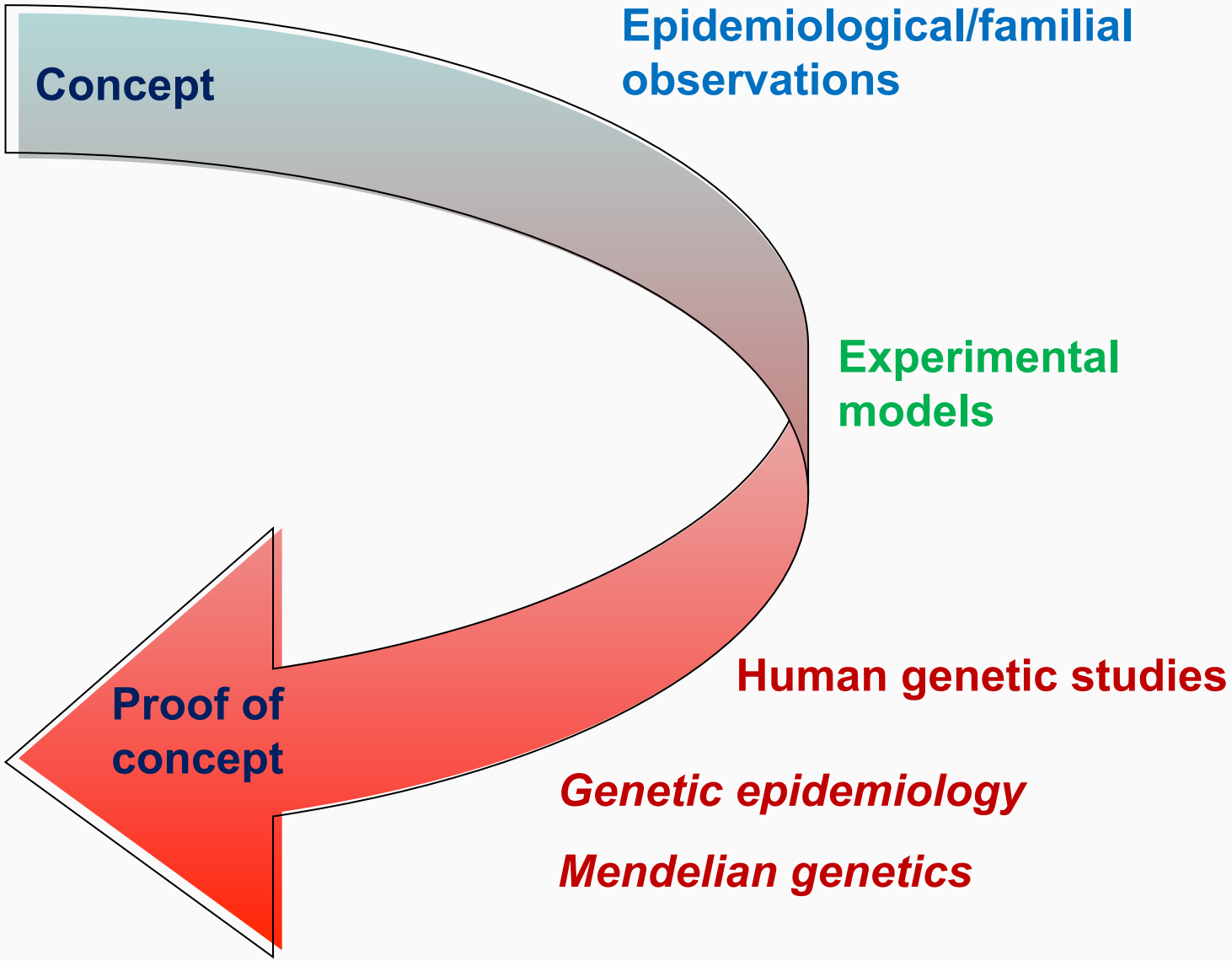
TWIN STUDIES ON GENETIC VARIATIONS IN RESISTANCE TO TUBERCULOSIS

FRANZ J. KALLMANN AND DAVID REISNER



TUBERCULOSIS MORBIDITY RATES OF RELATIVES OF TWIN INDEX CASES

Human genetics in tuberculosis?



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

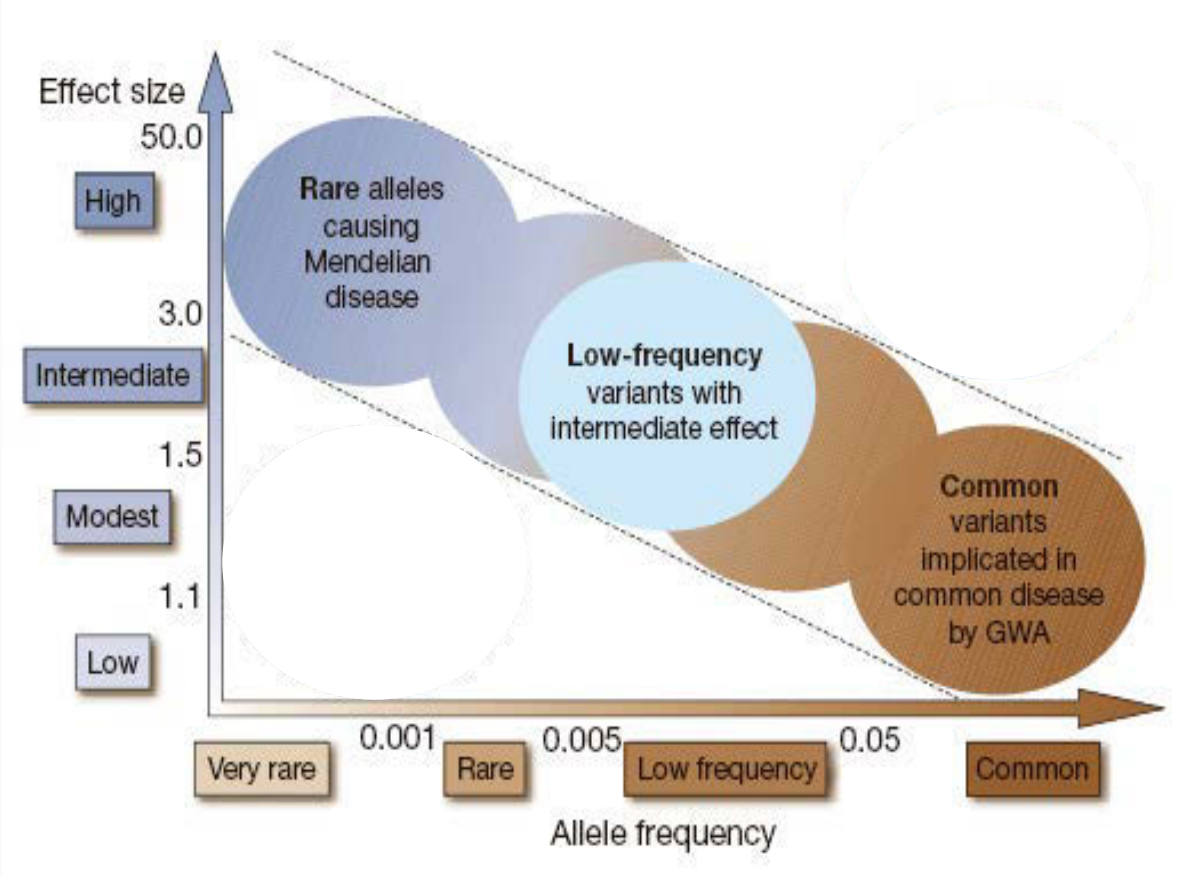
How to identify the causal genetic variant?

Phenotype	Severe/disseminated TB (children)	Pulmonary TB (adults)
Tools	**Mendelian Genetics**	**Complex Genetics**
Sample	Small	Large
	⇩	⇩
	Rare mutations **Strong individual effect**	**Common polymorphisms** **Modest individual effect**

Using the considerable progress in genomics technology (ultra-high throughput genotyping, sequencing….)

Continous spectrum of genetic predisposition according to individual effect and frequency of genetic variant

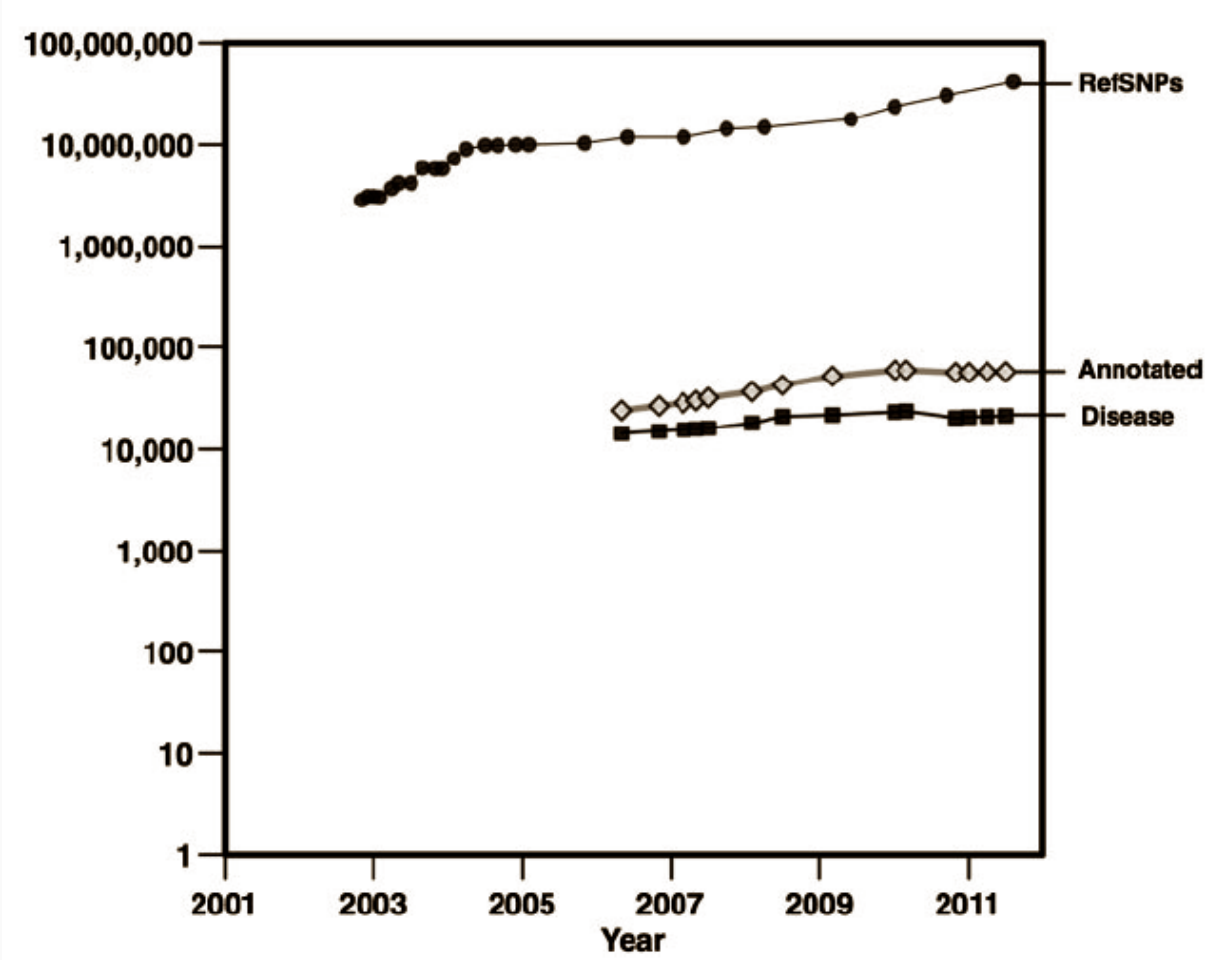
Relative risk (odds ratio, OR)



(Manolio et al, Nature, 2009)

Considerable number of genetic variants

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

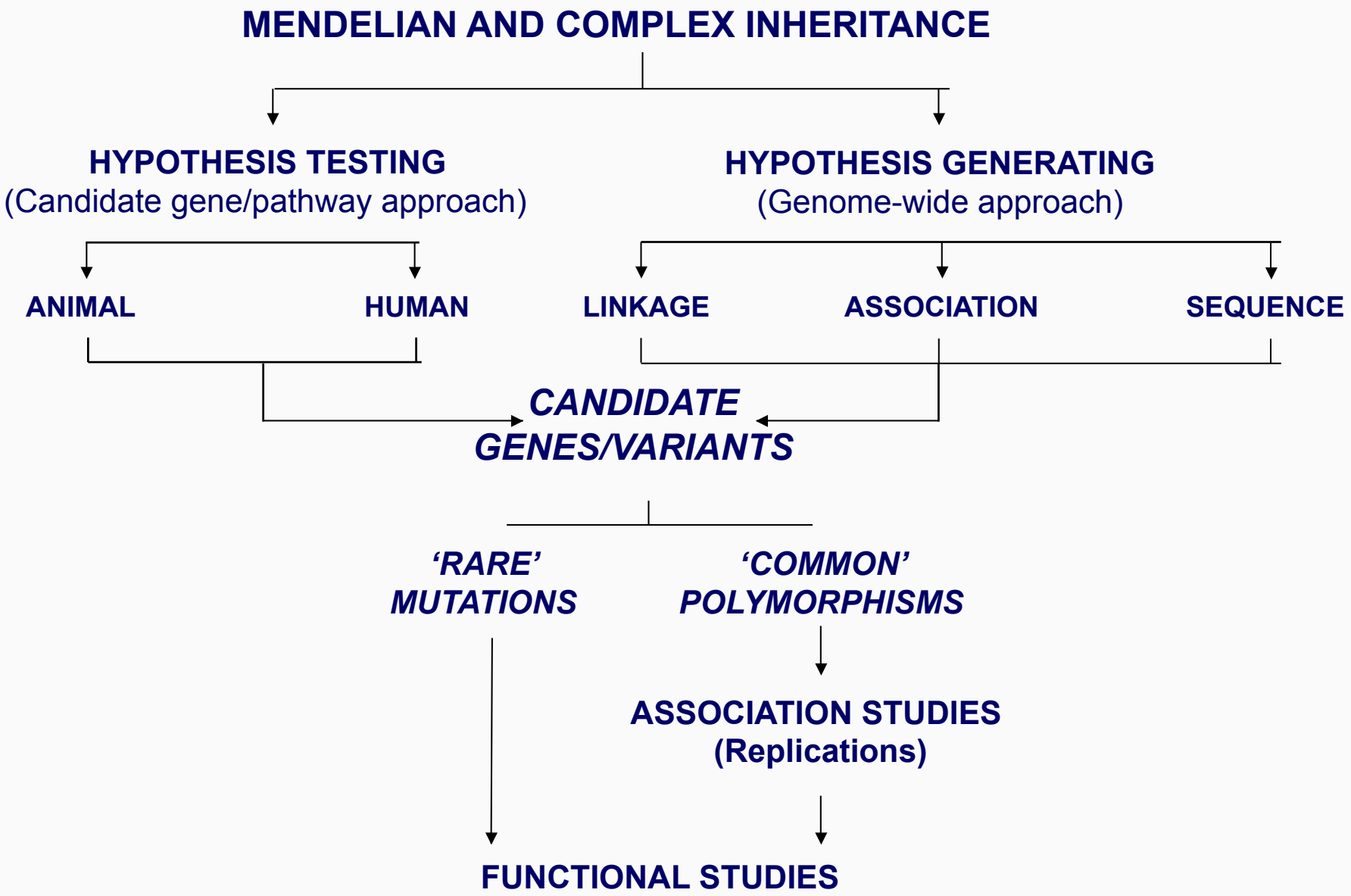


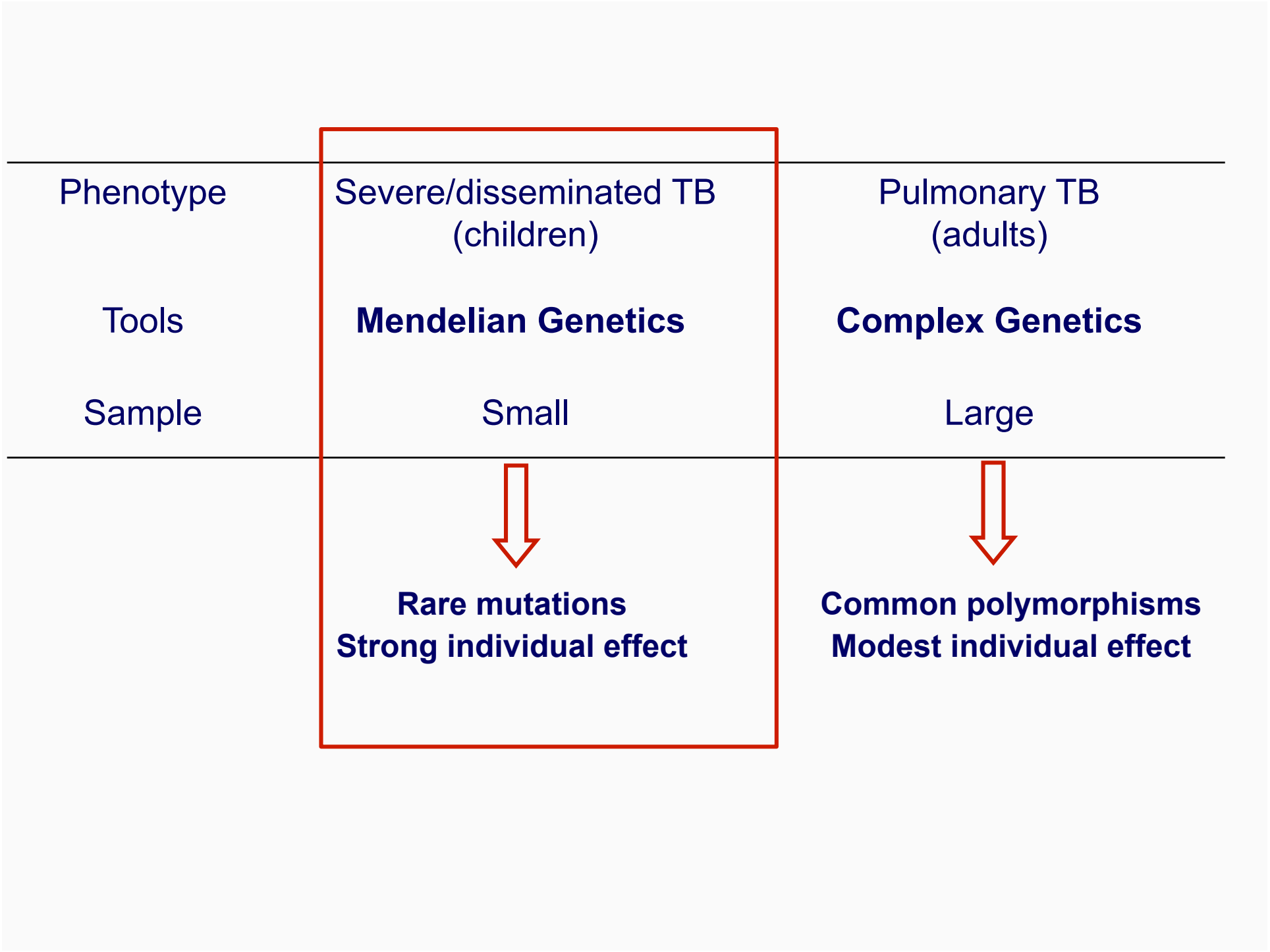
> 40 millions reported variants in humans (most of them are <1%)

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?

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Severe/disseminated TB (children)
Pulmonary TB (adults)
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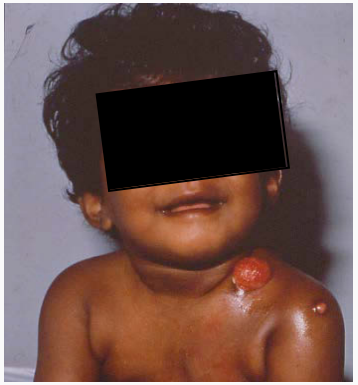
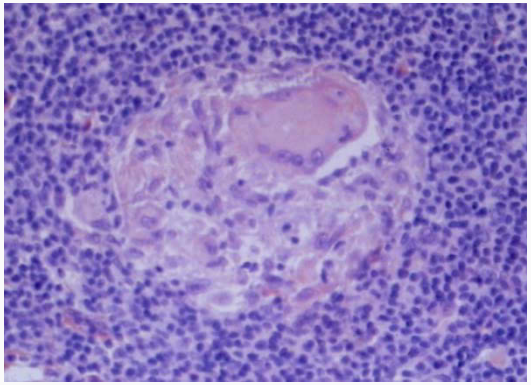
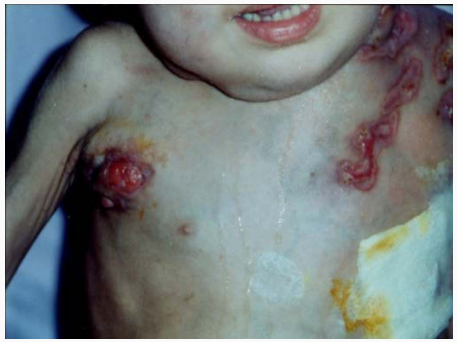
Mendelian susceptibility to mycobacterial diseases (MSMD)

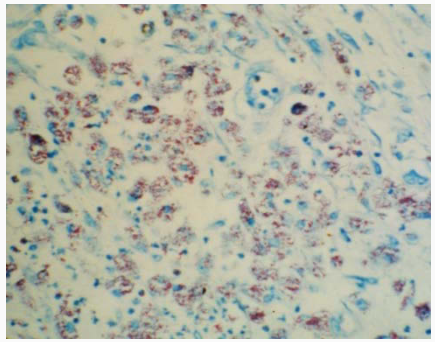
* Infections by BCG and environmental mycobacteria mostly in young children





Limited disease
Granuloma



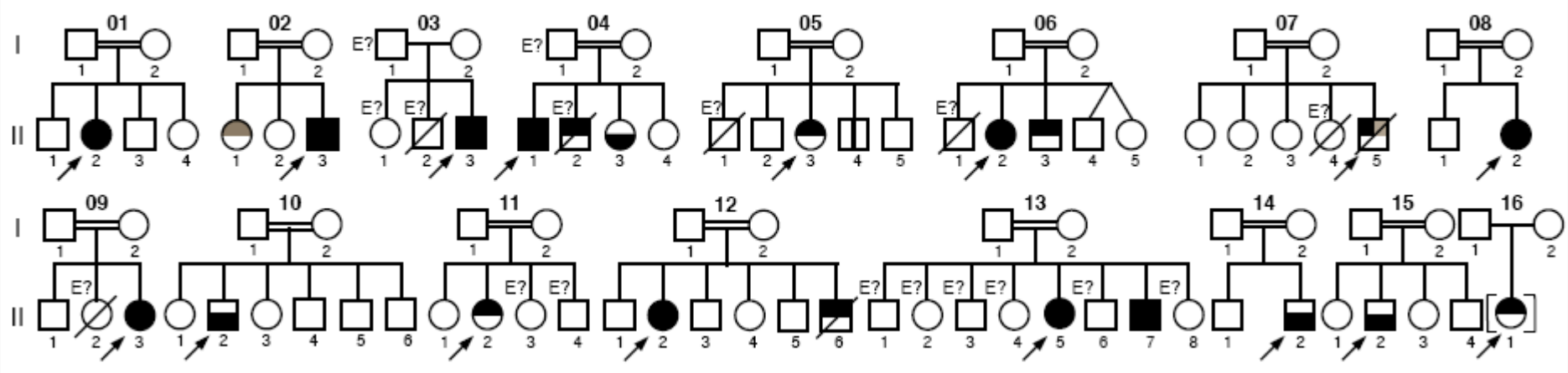


Disseminated disease
Lepromatous like

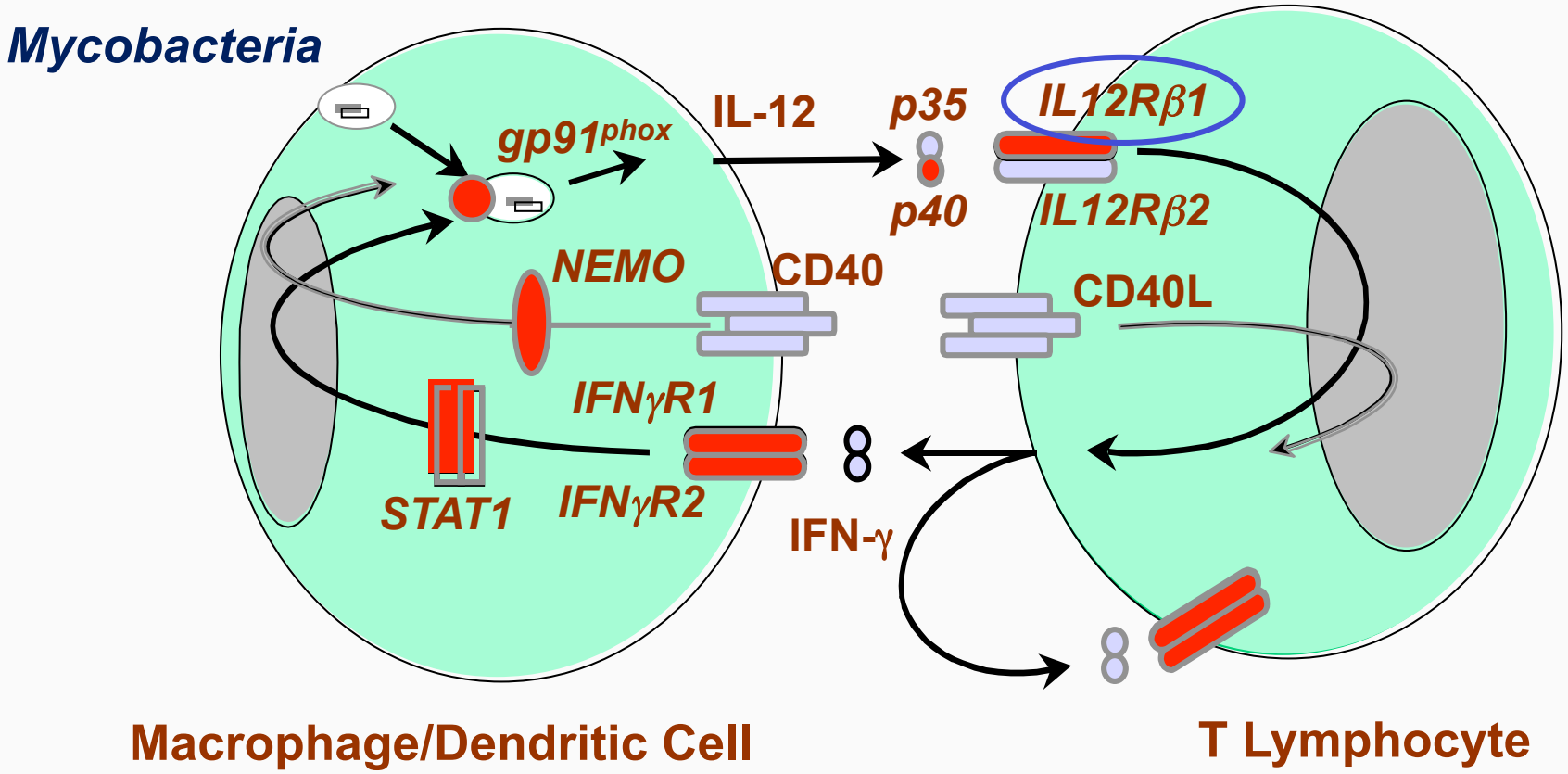
MSMD

* Otherwise healthy individuals

* Very rare (10^{-5} – 10^{-6}) but often familial (consanguinity and/or multiplex)



MSMD: 7 genes, 14 genetic diseases

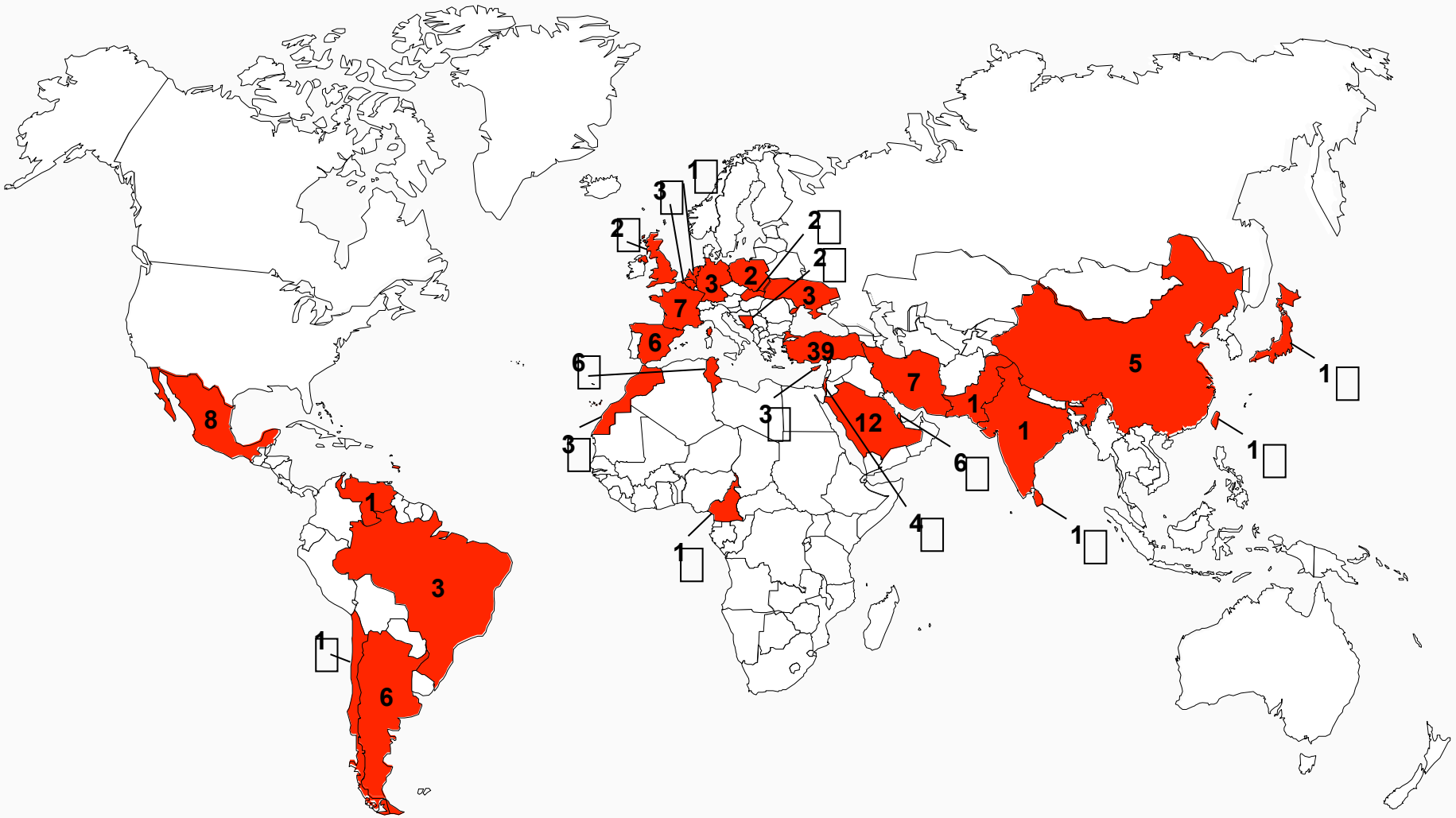


→ Specific antimycobacterial pathway *in natura* (IL12/IFN-γ)

→ Medical implications (IFN-γ treatment)

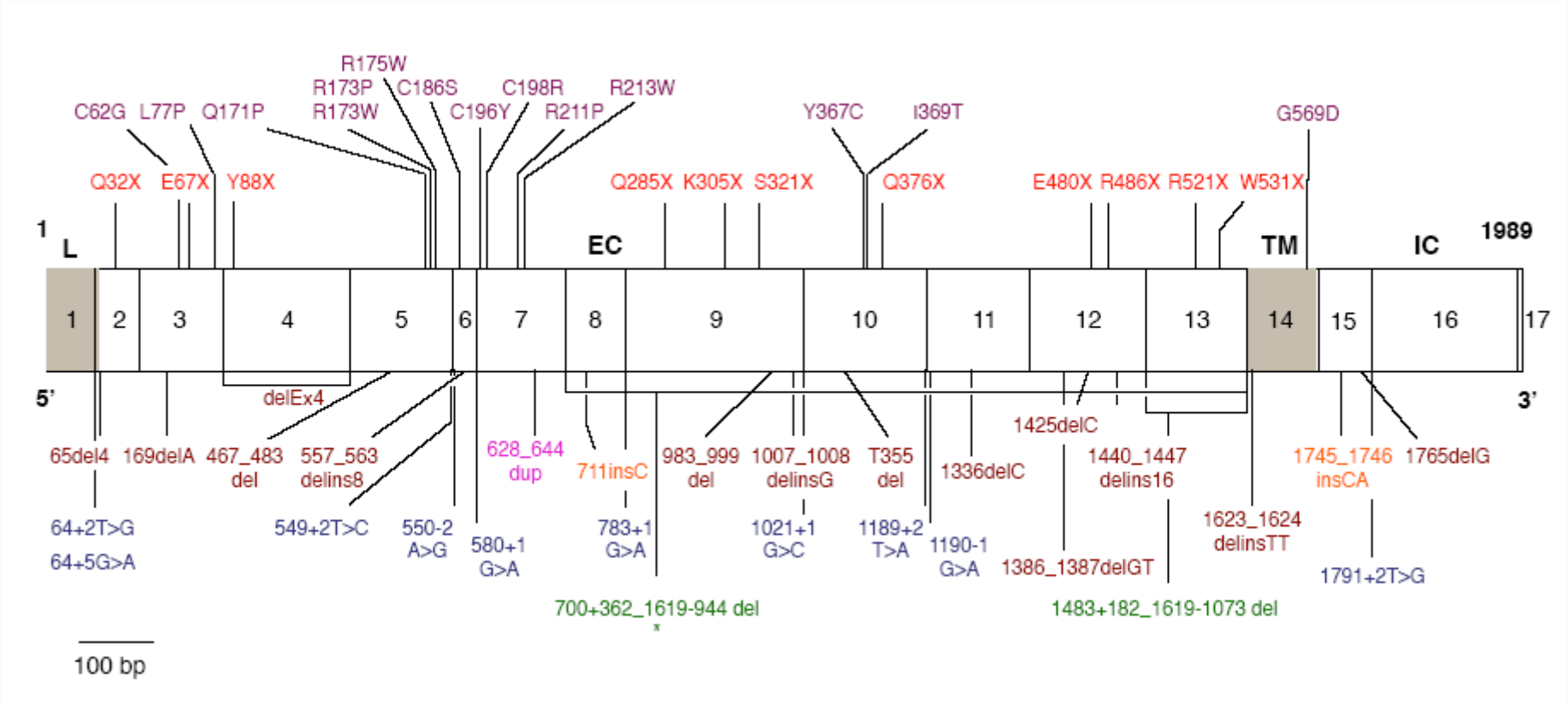
→ From BCG/EM to *M. tuberculosis*

IL-12Rβ1 deficiency: the most common disorder
→ >100 kindreds from 30 countries



IL-12Rβ1 deficiency→ Allelic heterogeneity

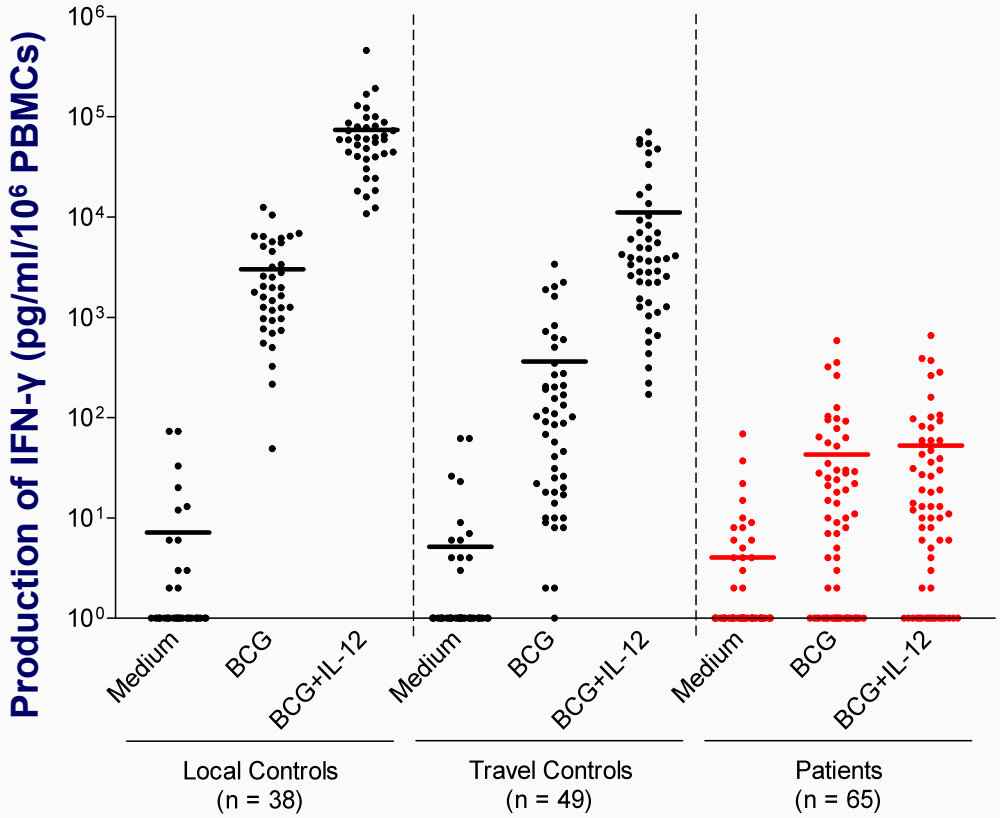
Autosomal recessive (54 different mutations):
Patients were homozygous (87%) or compound heterozygous (13%)



11 nonsense
14 missense
10 splice
1 duplication

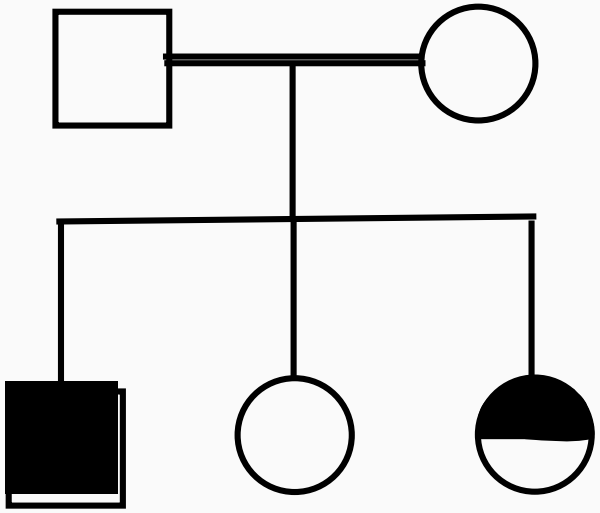
2 small insertions
9 small deletions
3 large deletions
4 insertions/deletions

Functional homogeneity: Complete IL-12Rβ1 deficiency



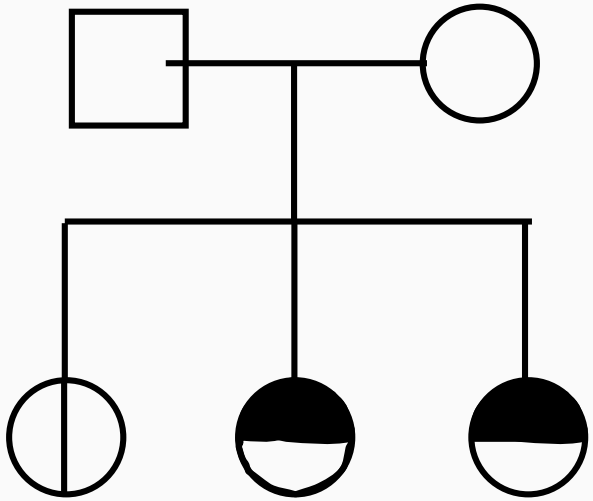
Abolished response to IL-12

IL12R-β1 deficiency and Tuberculosis



MSMD Abdominal TB

IL12RB1 mutation: R213W



Severe TB

IL12RB1 mutation: 1721+2T→G

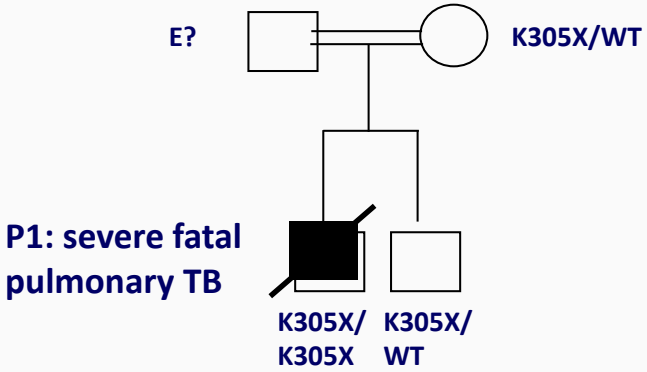
Complete IL12R-β1 deficiency : No cellular responses to IL-12

→ **Mendelian tuberculosis**

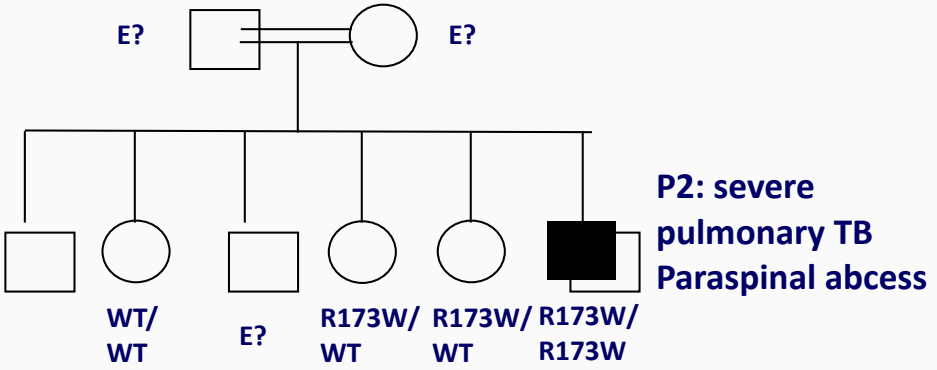
IL-12Rβ1 deficiency and Tuberculosis

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



Kindred A (Morocco)

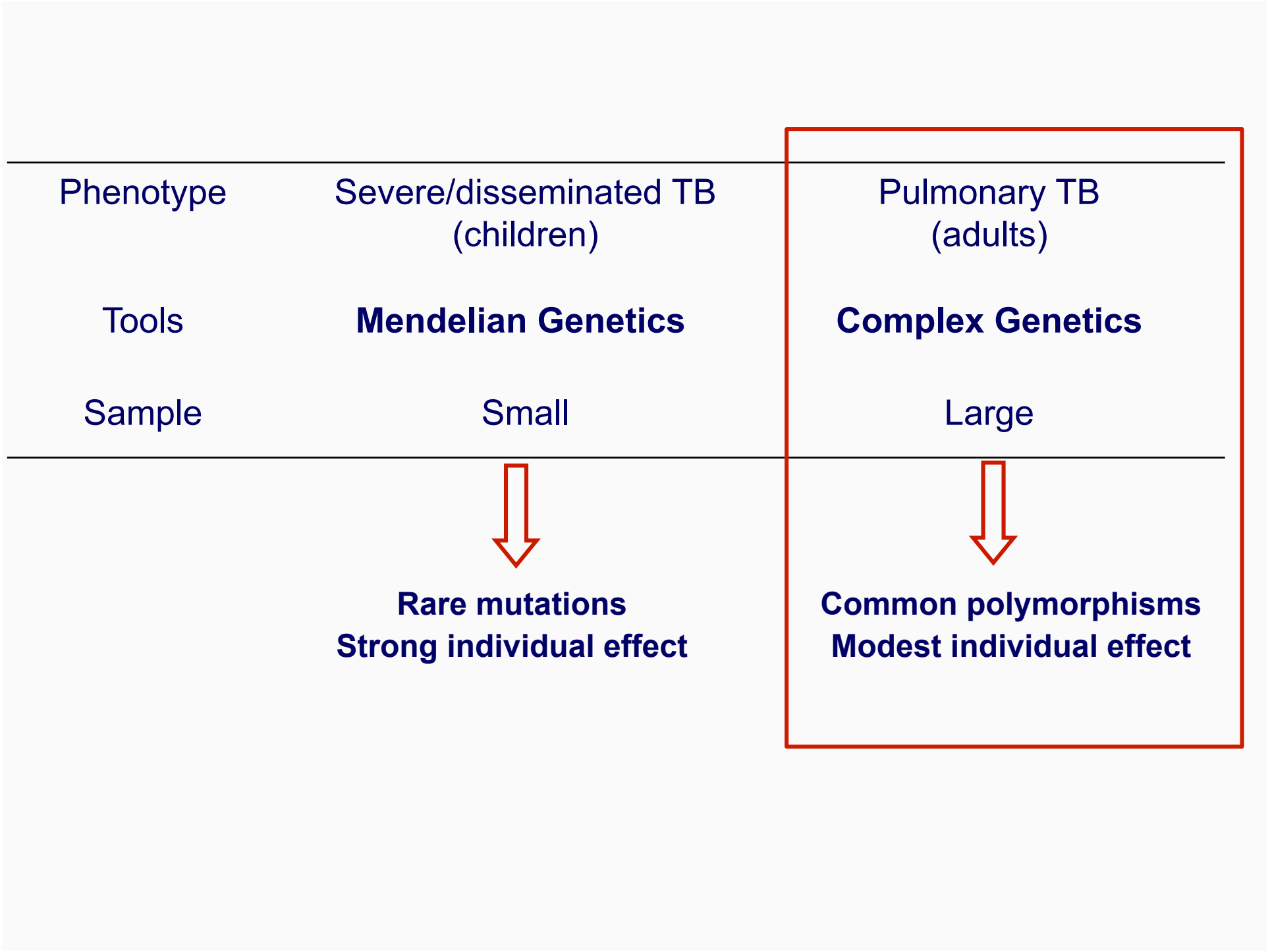


Kindred B (Iran)

→ 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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Rare mutations
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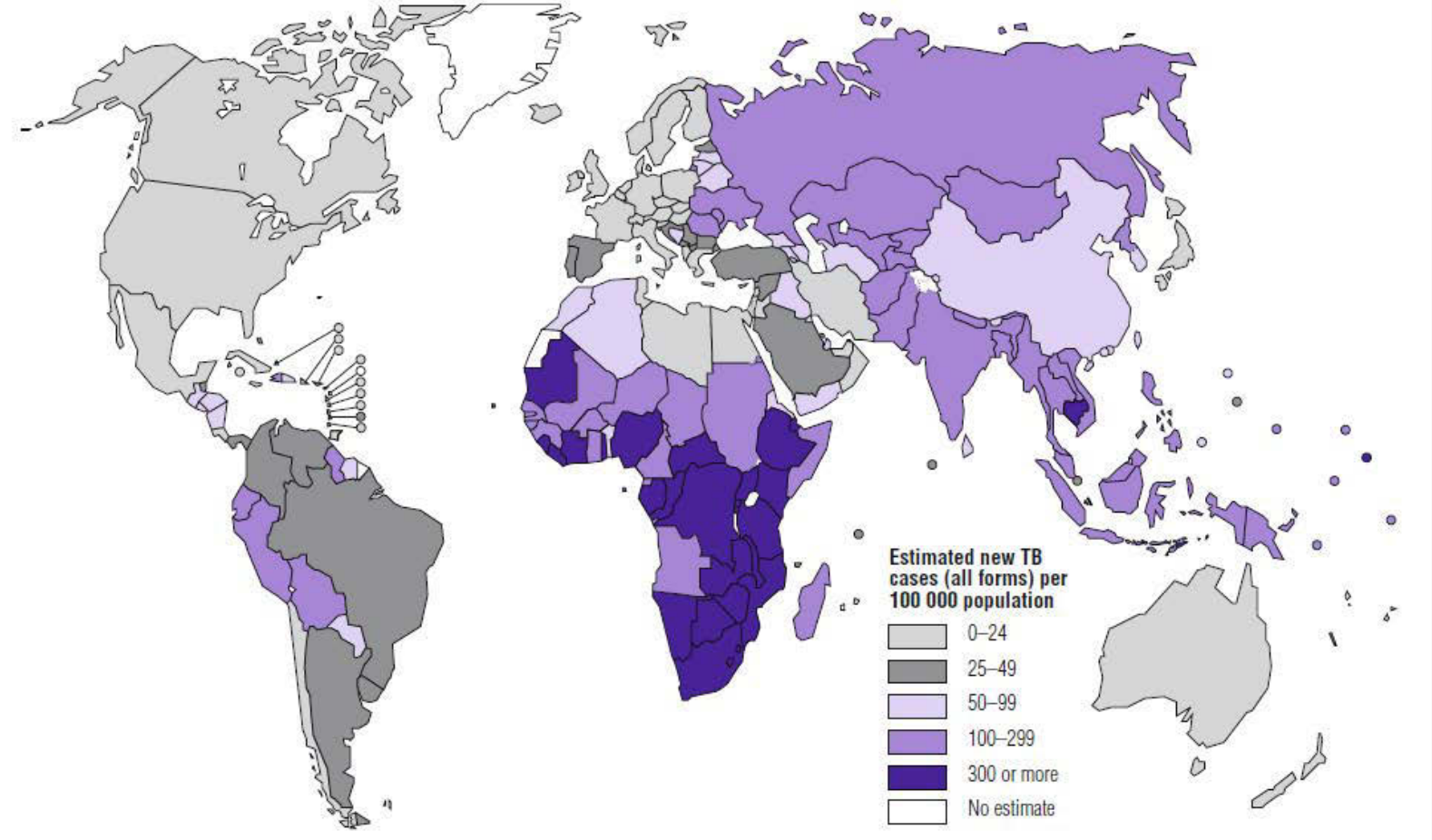
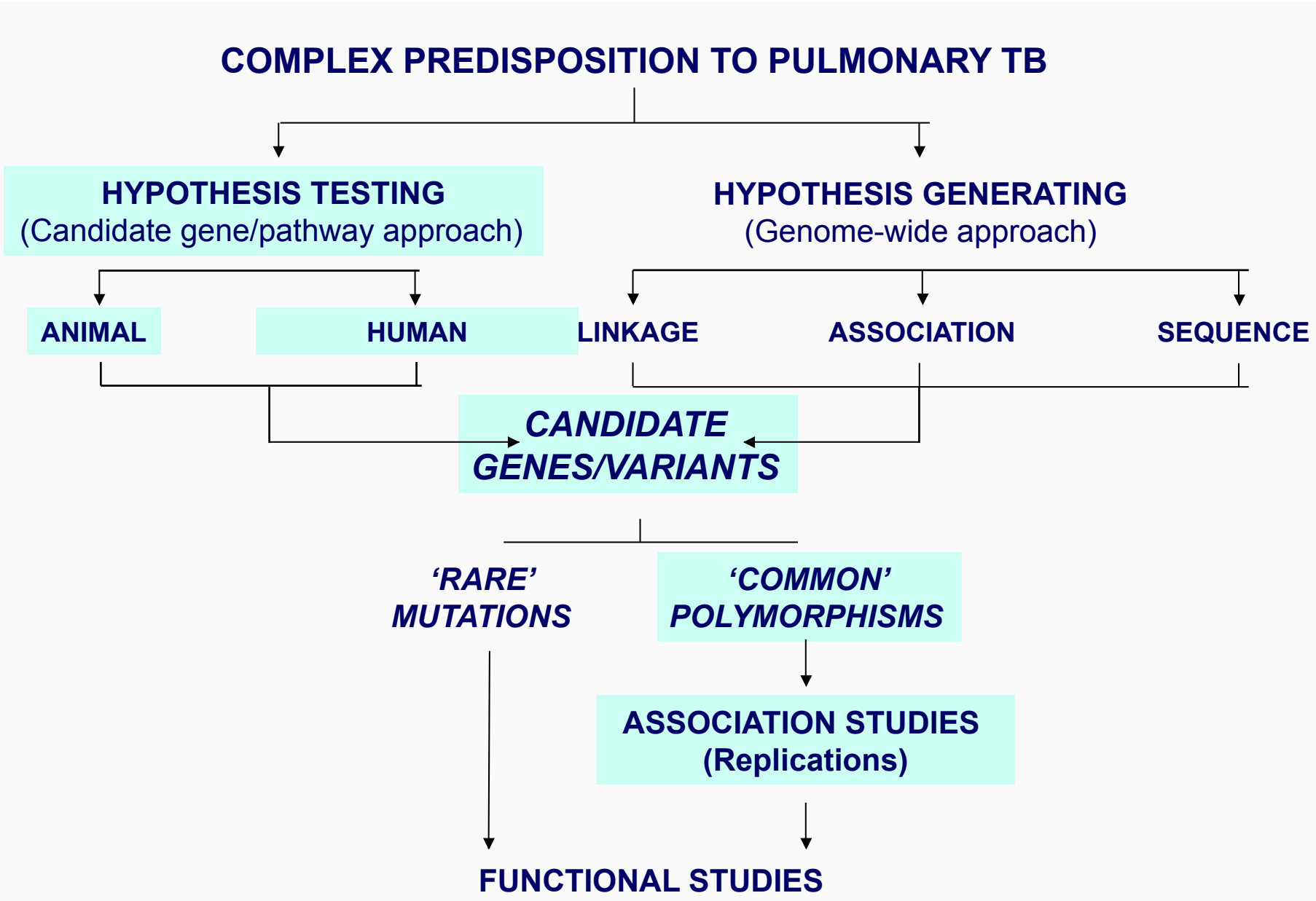
TB: Major public health problem

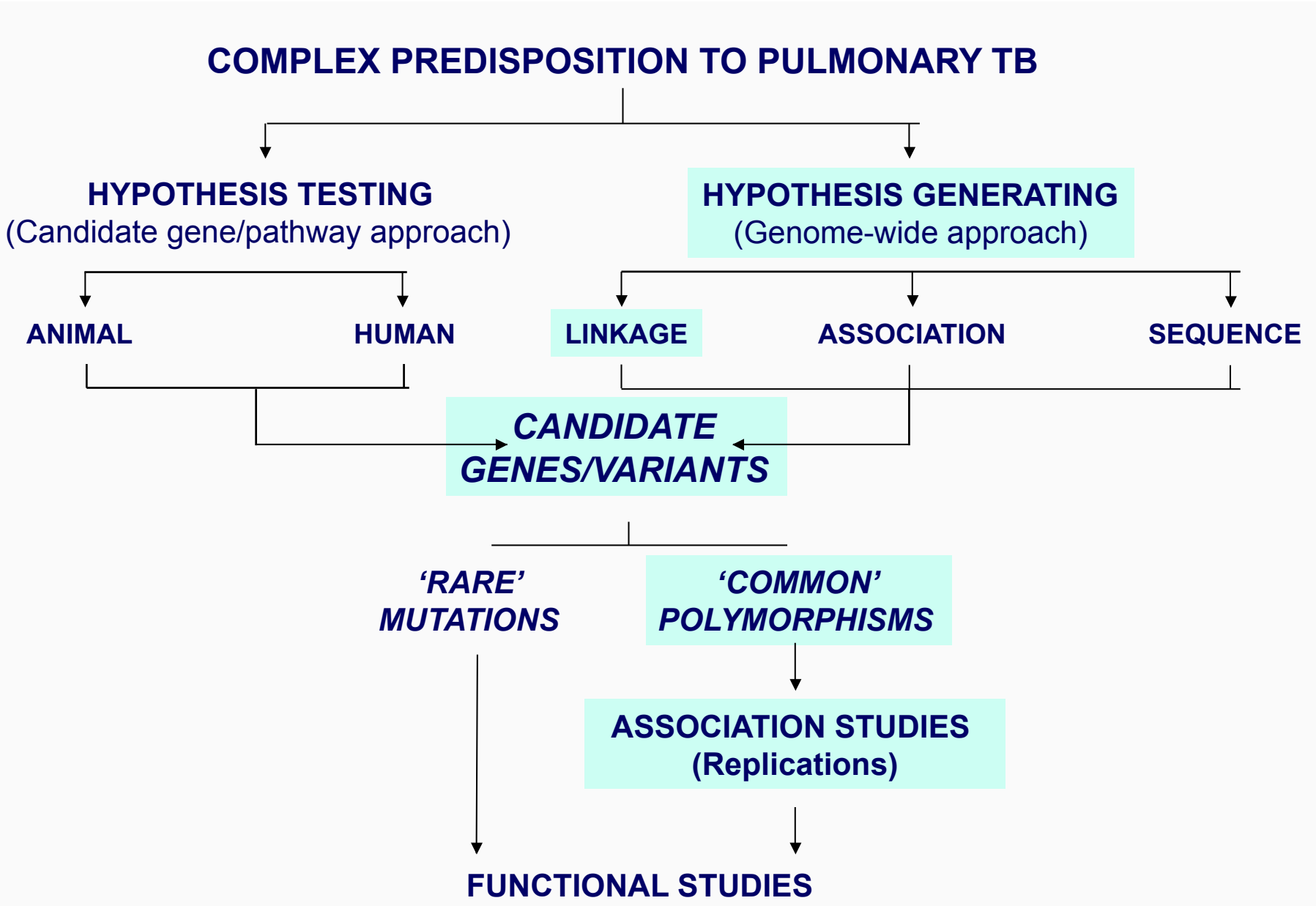


~ 1/3 world's population exposed to *M. tuberculosis*

~ 9.2 million new cases/year and ~ 1.7 million deaths/year



Huge number of candidate gene studies: very few replicated and convincing


COMPLEX PREDISPOSITION TO PULMONARY TB
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

PULMONARY TB

Genome-wide linkage screen in Morocco

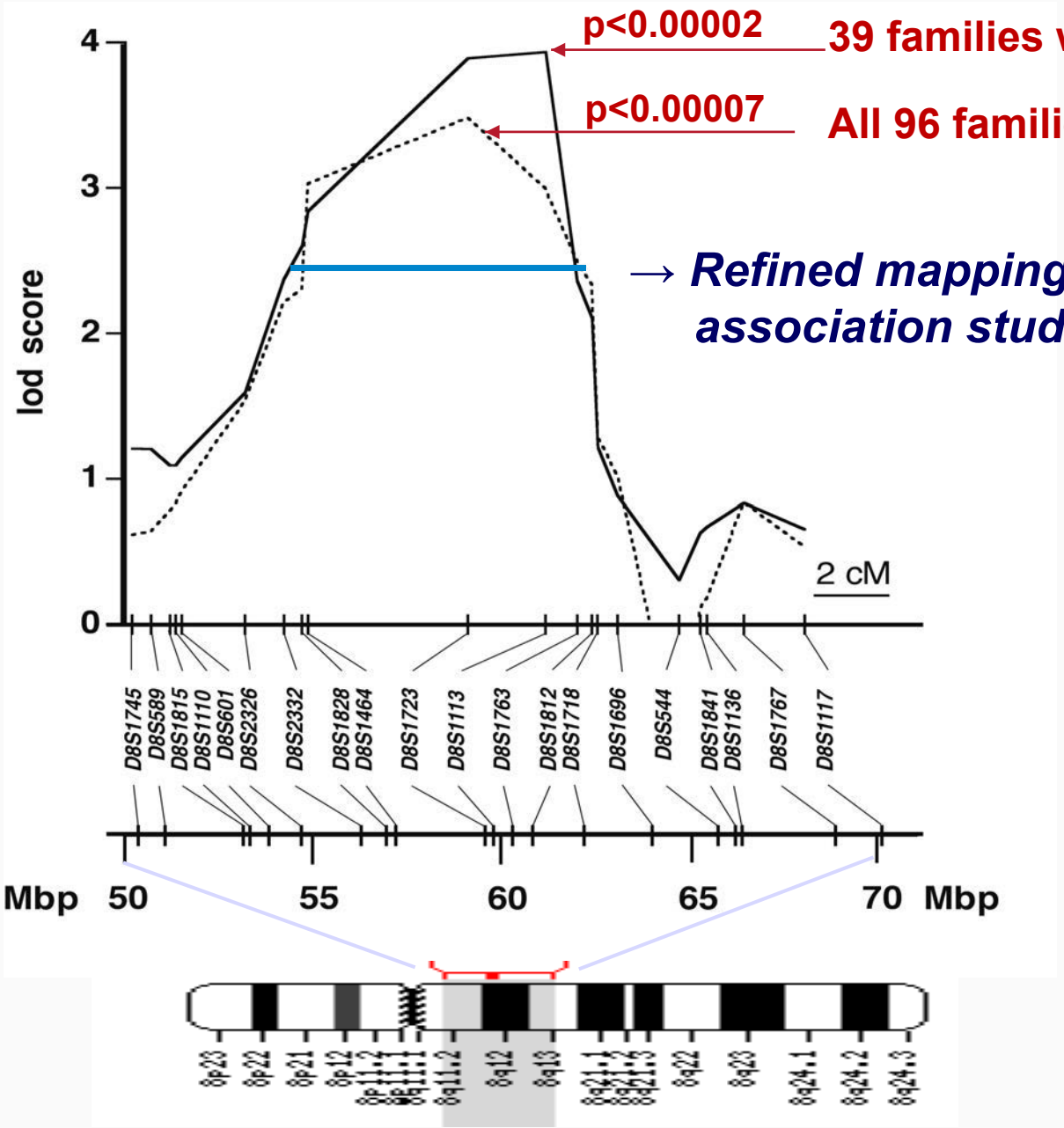
96 multiplex families

# affected offspring	2	3	4
# families	68	21	7

Total of 227 affected offspring (92%>15 years, 90%<40 years) with positive pulmonary TB

El Baghdadi et al, J Exp Med, 2006

Linkage to chromosome 8q12-q13



Refined mapping (association study) in Morocco

PRIMARY ASSOCIATION SAMPLE

- **203 families including 285 offspring with pulmonary TB**

→ family-based association analysis

REPLICATION ASSOCIATION SAMPLE

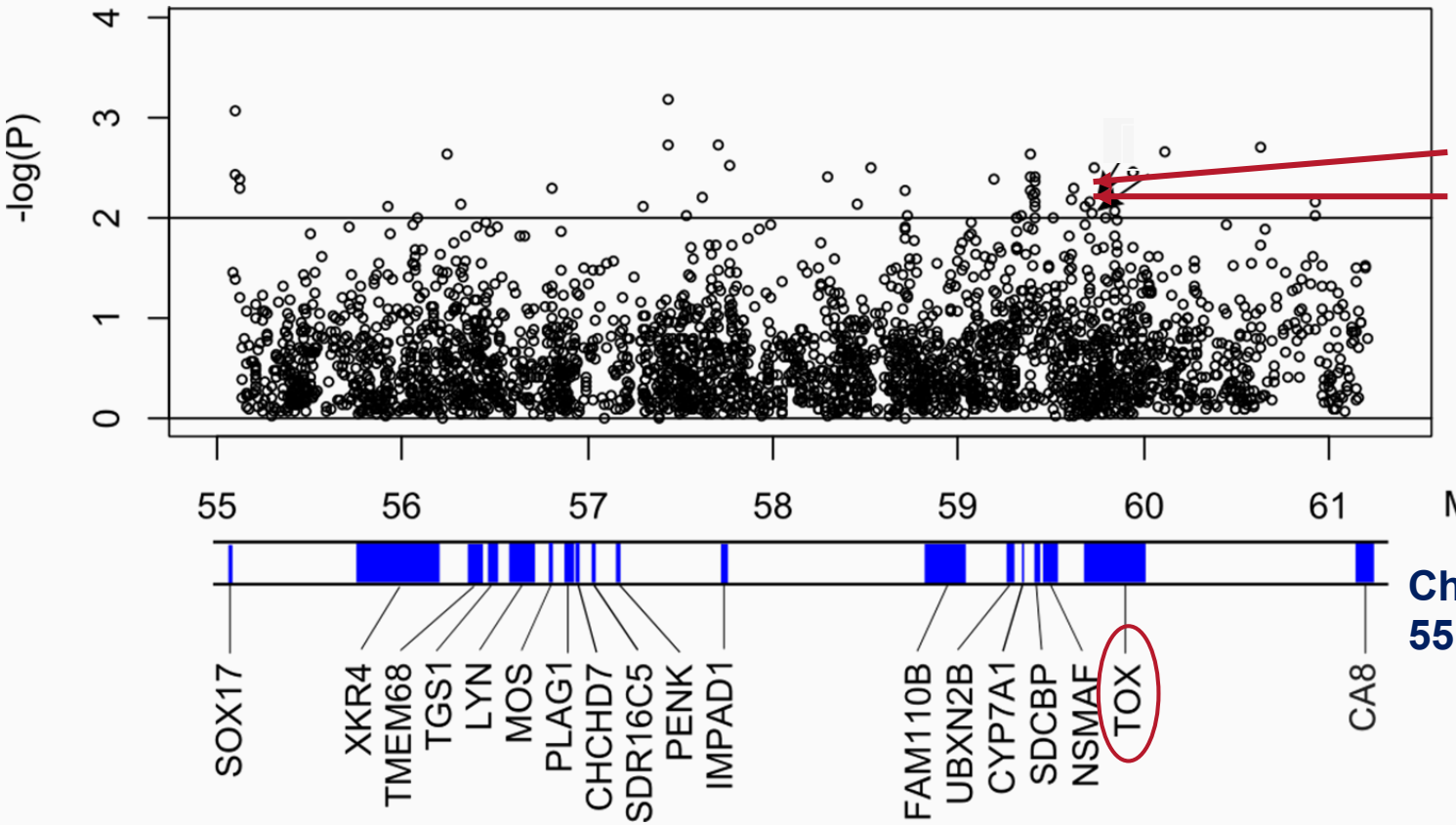
- **317 pulmonary TB patients and 650 healthy controls**

→ 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



Two replicated SNPs in *TOX*

Chromosome 8q12-13
55.09 → 61.20 Mb

44 SNPs with p<0.01 tested for replication
→ Only two replicated SNPs

Association with *TOX* SNPs in Morocco

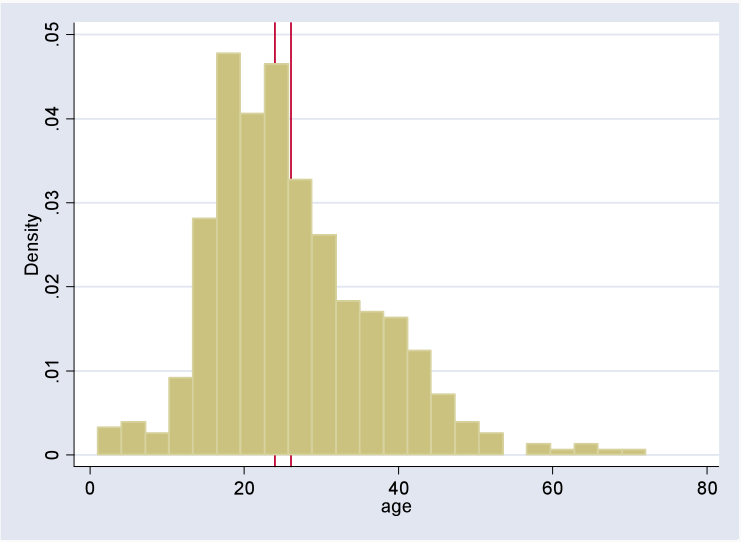
		Family-based		Case-control		Combined	
	Marker	OR	P	OR	P	OR	P
Full population	rs1568952	3,2	0,007	2	$6x10^{-4}$	2,12	$1.14x10^{-5}$
	rs2726600	2,3	0,009	1,6	0,0092	1,8	$9.2x10^{-5}$

Stronger effect in early-onset TB

		Family-based		Case-control		Combined	
	Marker	OR	P	OR	P	OR	P
Full population	rs1568952	3,2	0,007	2	$6x10^{-4}$	2,12	$1.14x10^{-5}$
	rs2726600	2,3	0,04	1,6	0,0092	1,8	$9.2x10^{-5}$
Under 25	rs1568952	5,5	0,0003	2,8	$2.9x10^{-5}$	3,3	$4.4x10^{-8}$
	rs2726600	2,6	0,0025	2	0,0039	2,2	$3.2x10^{-5}$
Over 25	rs1568952	0,65	0,62	1,5	0,094	1,4	0,15
	rs2726600	1,7	0,33	1,4	0,15	1,4	0,09

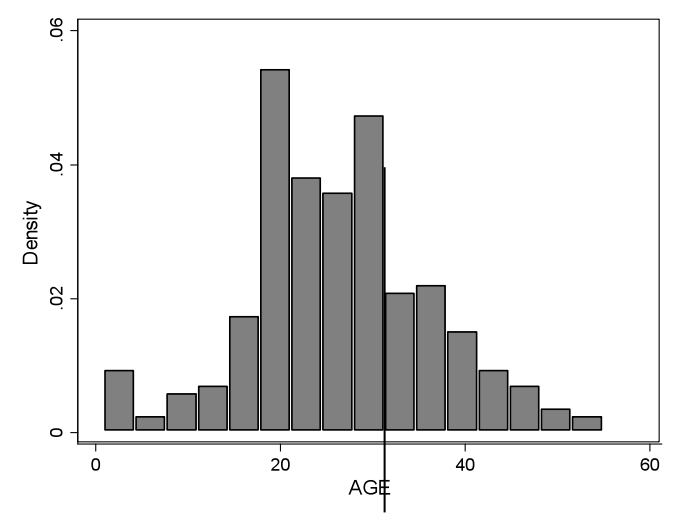
Strong age effect

No gender effect



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

	Morocco			*Madagascar*		
Marker	**Risk allele (Freq)**	**OR (95% CI)**	**P**	**Risk allele (Freq)**	**OR (95% CI)**	**P**
rs2726600	**G (0.44)**	**2.2 (1.5-3.2)**	**3.2×10^{-5}**	**G (0.15)**	**1.77 (1.0-3.17)**	**0.04**

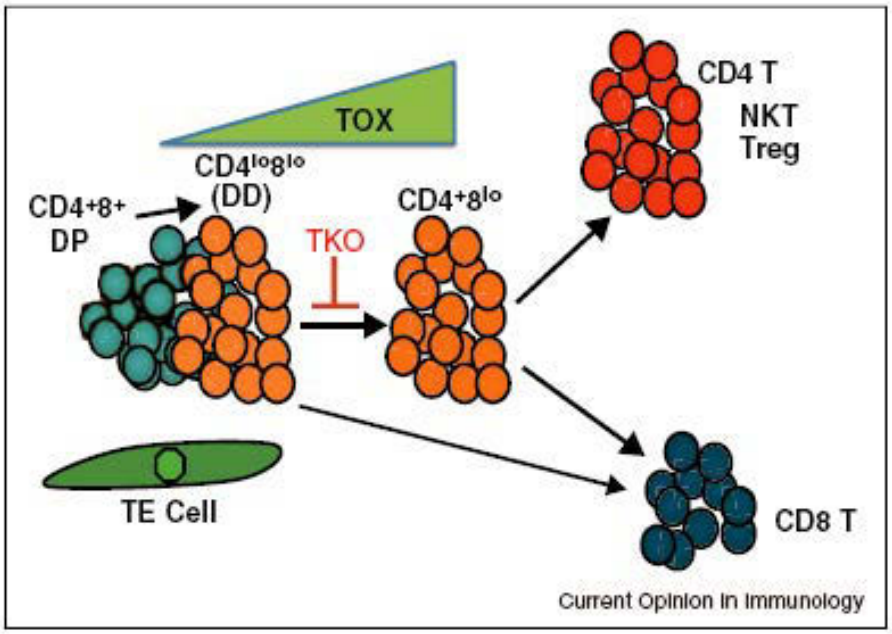
TOX

Thymocyte selection-associated high mobility group box protein

The many roles of TOX in the immune system

Parinaz Aliahmad[1], Akop Seksenyan[1] and Jonathan Kaye[1,2]

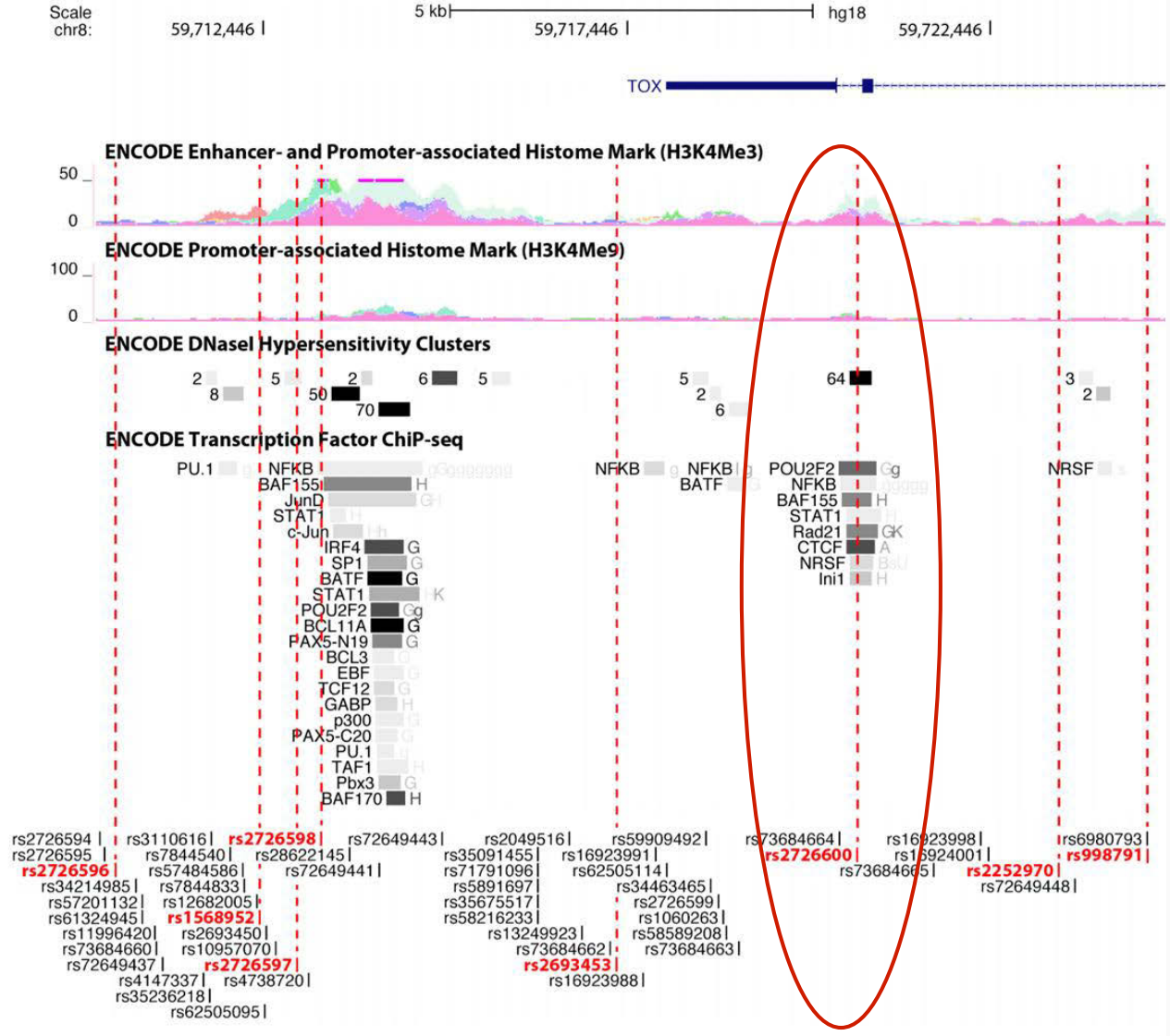
Current Opinion in Immunology 2011, 24:1–5



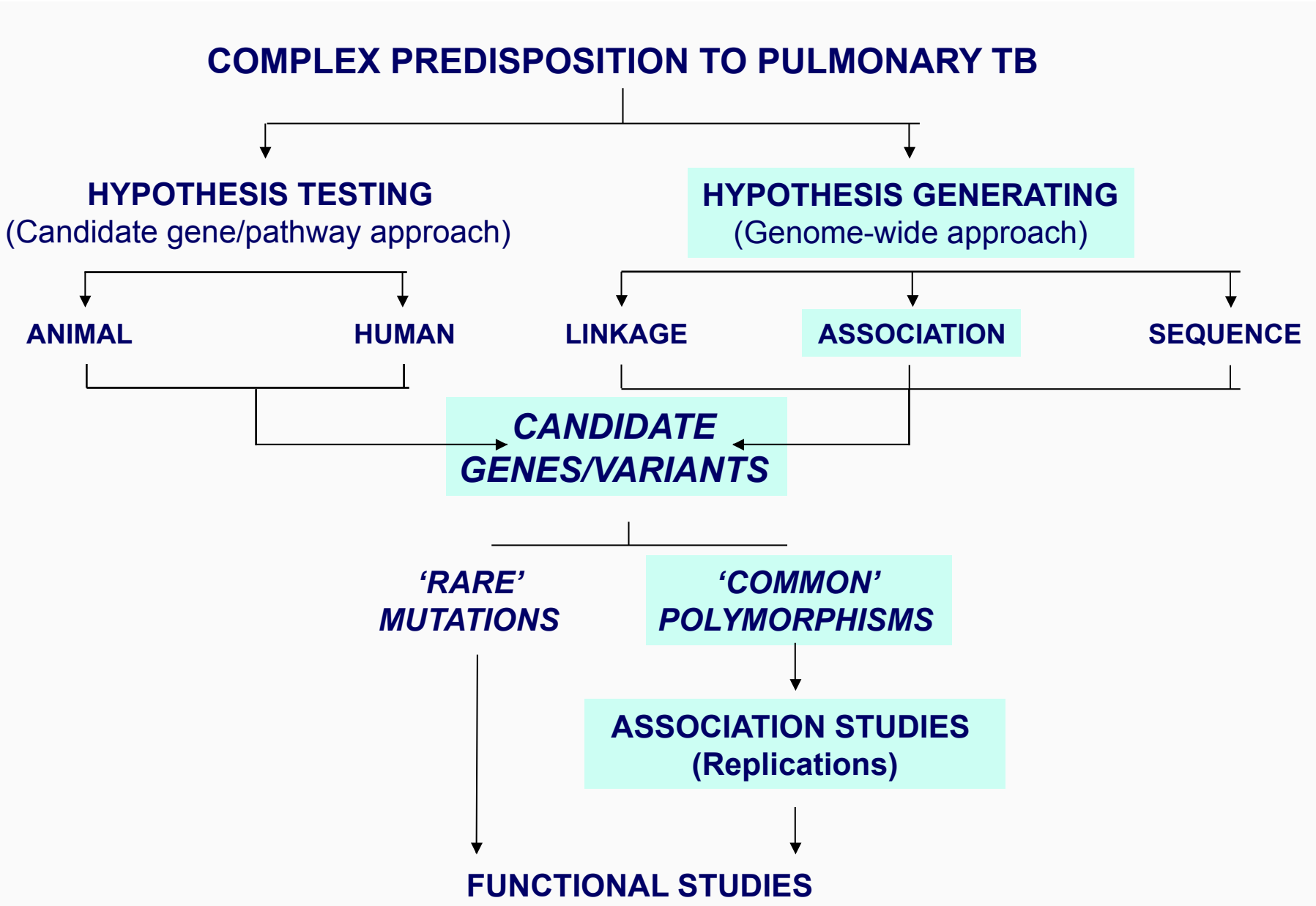
TOX is involved in the development of CD4 T cells.

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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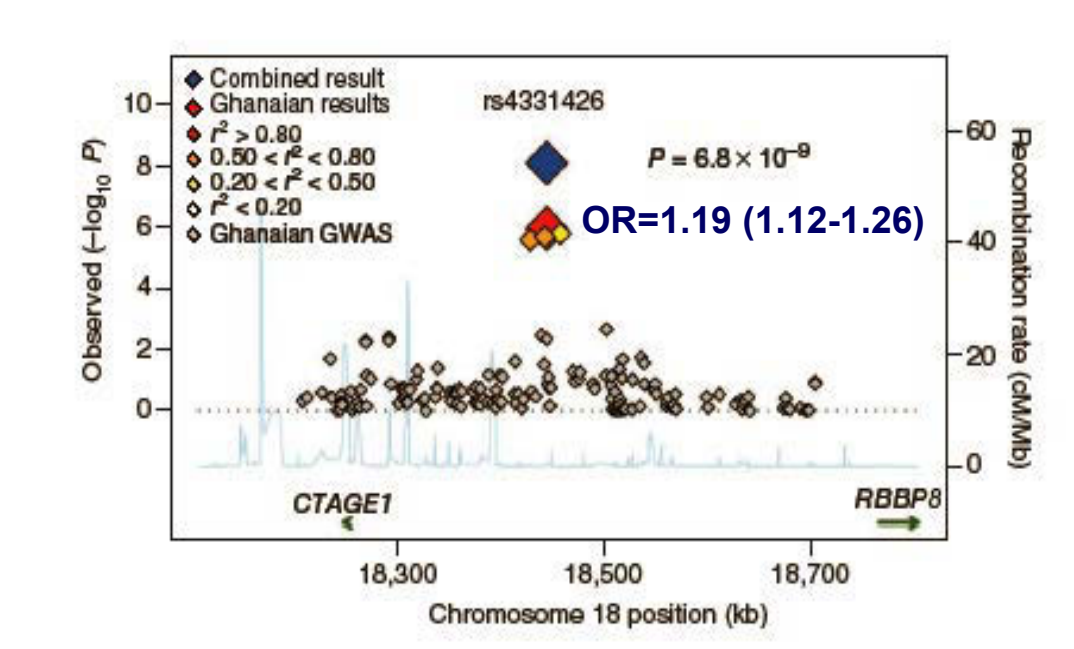
Genome-wide association studies (GWAS) in TB

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

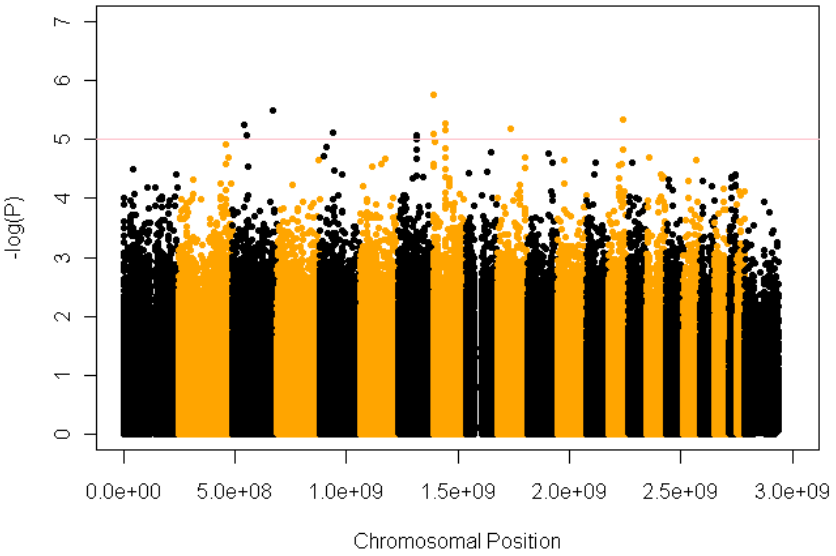
NATURE GENETICS VOLUME 42 | NUMBER 9 | SEPTEMBER 2010



Total of ~3500 patients and ~7500 controls from The Gambia and Ghana

GWAS: Preliminary results in Morocco

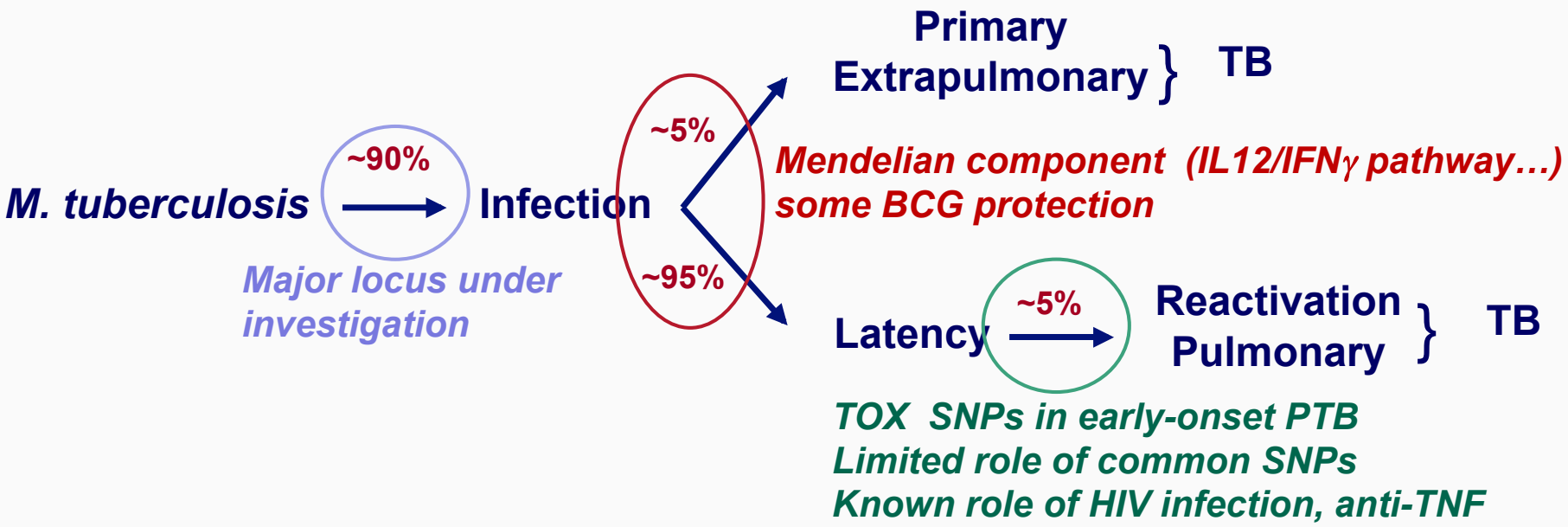
12 SNPs $<10^{-5}$
1520 SNPs P<0.001



→ Refined analysis
→ Replication

→ No strong signals in pulmonary TB with common polymorphisms

Summary for TB – General Implications



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

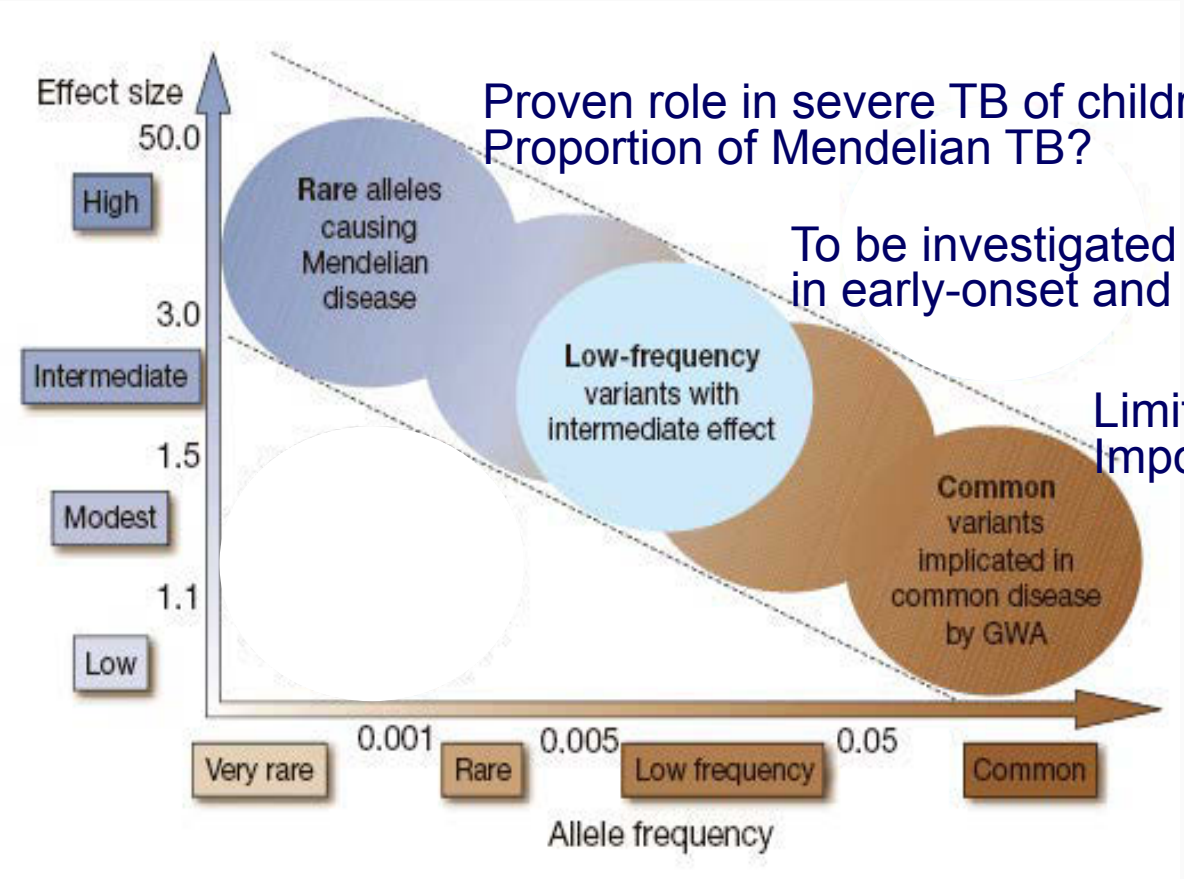
→ Understanding pathogenesis through human genetics

Implications for

→ prevention (vaccination, target populations…)

→ treatment (restore deficient immunity, e.g. IFN-γ treatment)

Genetic predisposition to tuberculosis
→ continuous spectrum

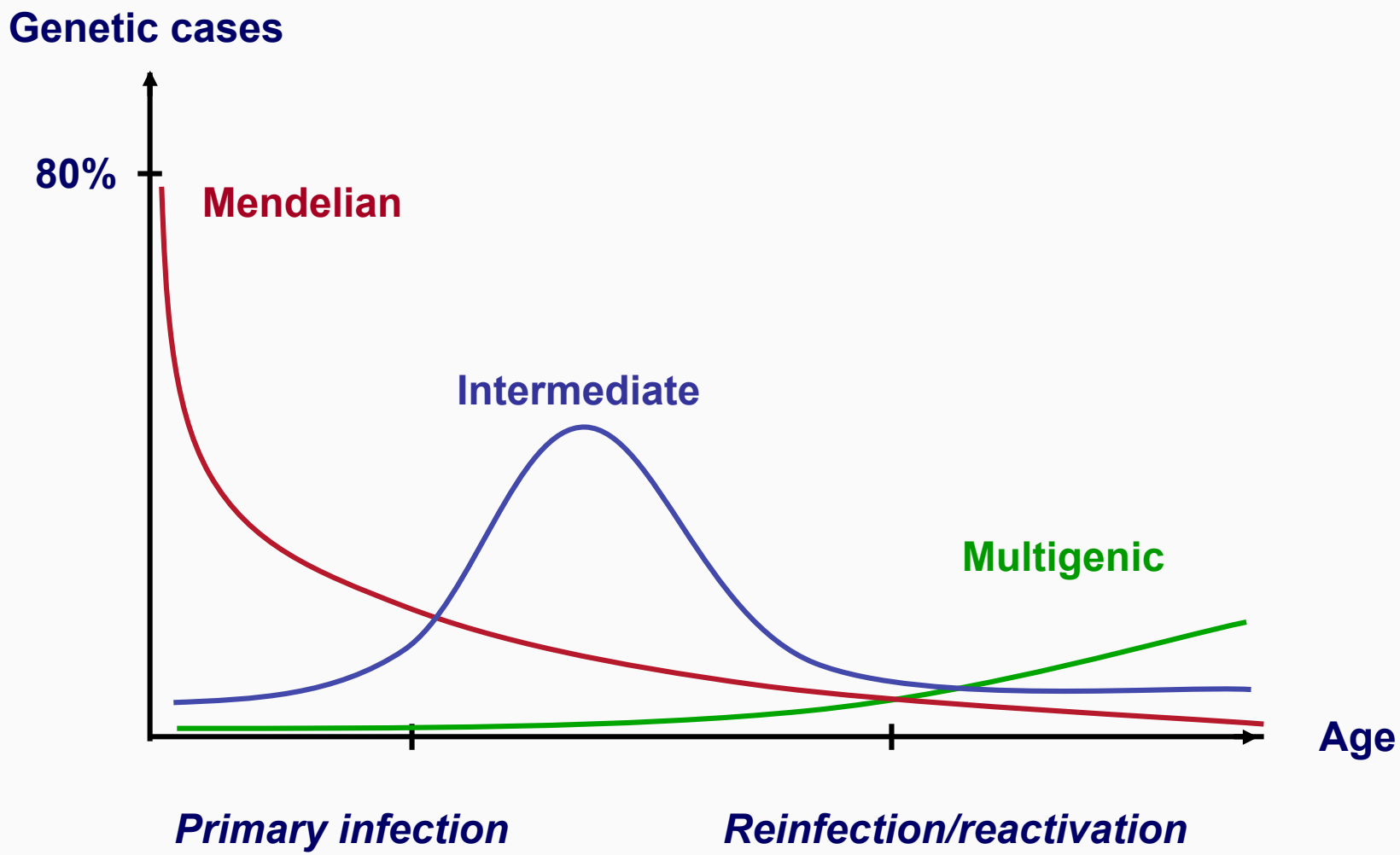


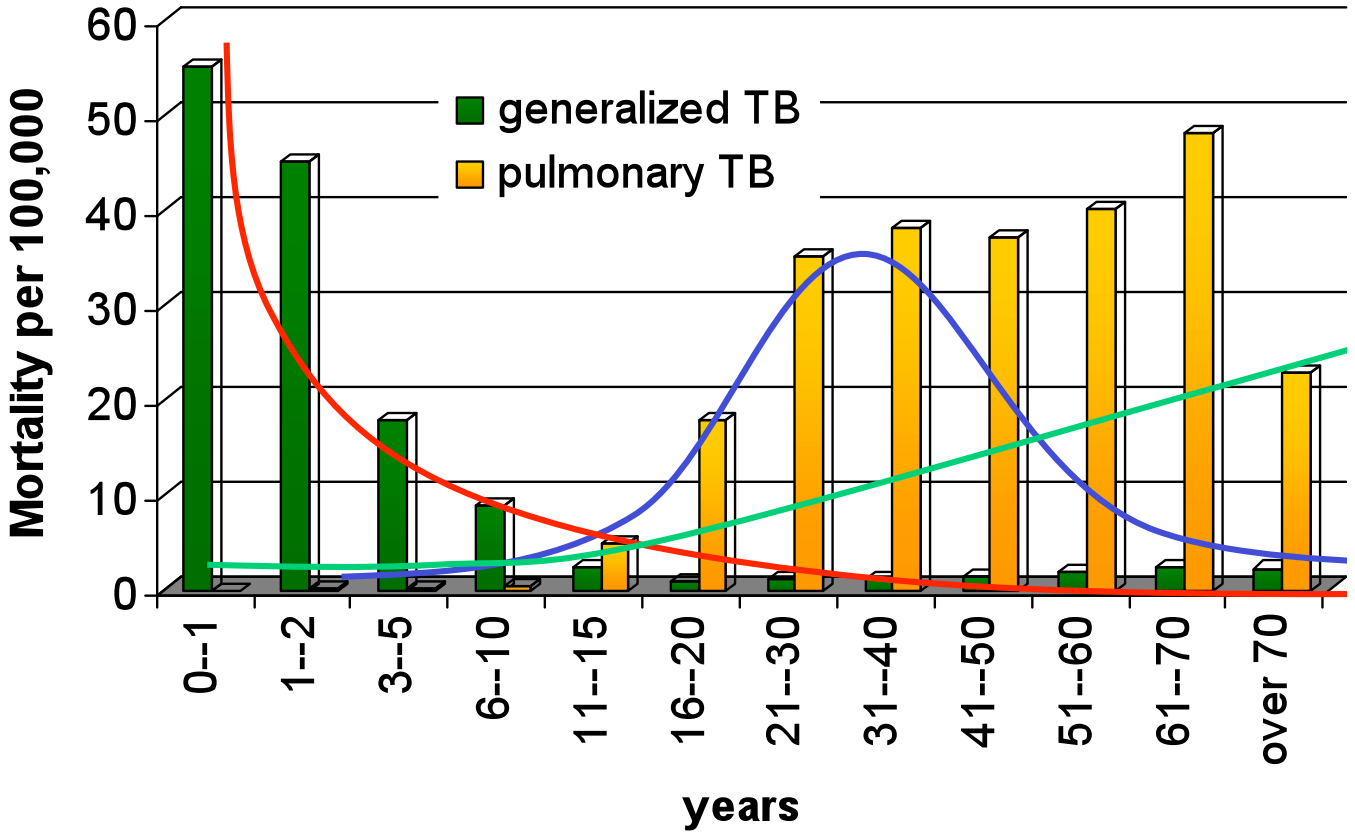
Proven role in severe TB of children
Proportion of Mendelian TB?

To be investigated especially
in early-onset and familial forms of PTB

Limited role in PTB
Important in early-onset TB

Genetic spectrum depends on age





Fits pretty well for young cases
Additional factors especially in adults

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