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:

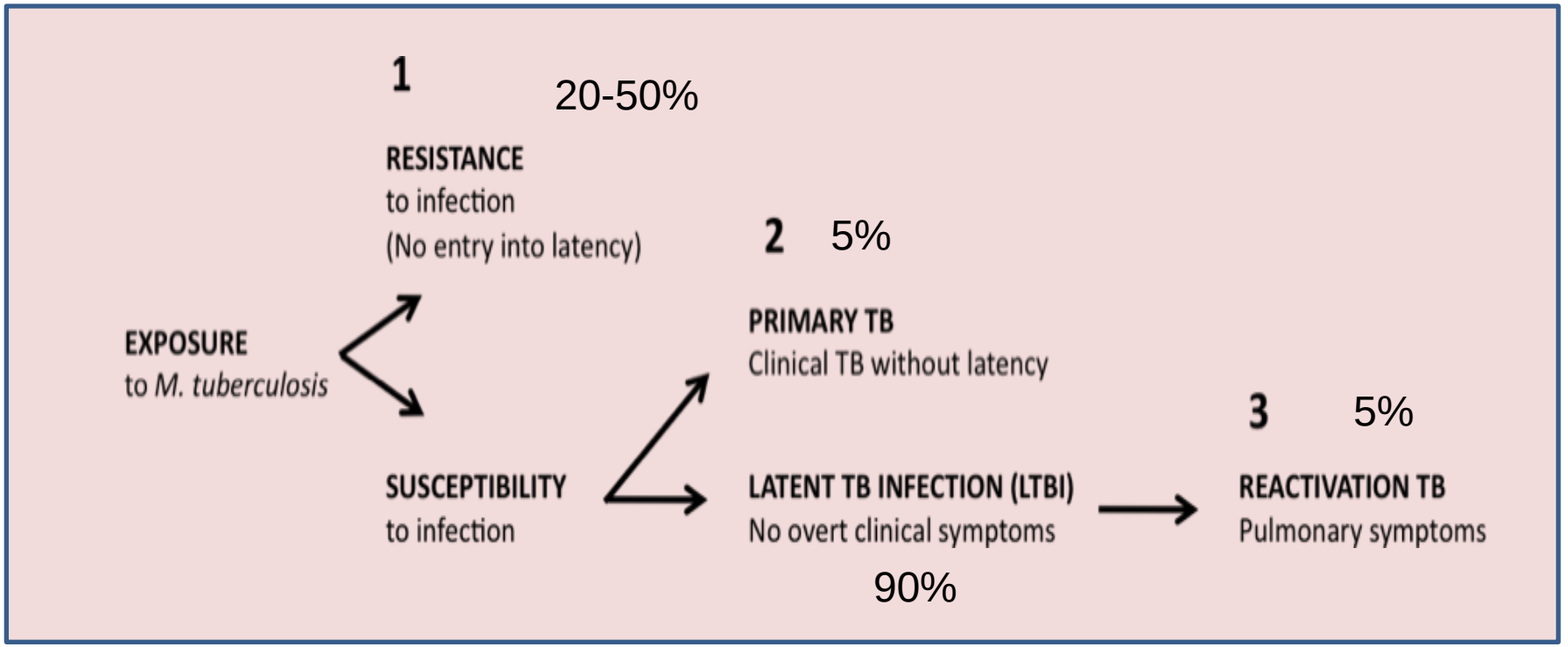
No phenylalanine = No PKU!

Is PKU a genetic disease? YES!

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

M. tuberculosis is necessary, but is it sufficient ?

Tuberculosis Pathogenesis



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

Virulence level	Number	Disease severity			
		death	serious disease	mild symptoms	no symptoms
1	1	-	-	-	1
2	93	6 = 6.5%	9 = 9.7%	78 = 83.8%	-
3	83	18 = 21.7%	34 = 41.0%	31 = 37.3%	-
4	74	53 = 71,6%	18 = 24.3%	3 = 4.1%	-
Totals	**251**	**77**	**61**	**112**	**1**

Moegling A. Die Epidemiologie der Lübecker Säuglingstuberkulose, Arbeiten a. d. Reichsges-Amt 69:1-24, 1931

Risk of Recurrence of TB

TABLE 3. PROPORTION AND RATE OF RECURRENCES AND REINFECTION DISEASE IN ENROLLED PATIENTS BY OUTCOME OF FIRST DISEASE EPISODE

Outcome of First Episode with DNA FP	No. Patients	PYRS Follow-up	No. Recurrences	Recurrence Rate/ 100 PYRS	No. DNA FP in Second Episode	No. Confirmed Reinfections (%)	Confirmed Reinfection Disease Rate/100 PYRS *(95% CI)*	Likely Reinfection Disease Rate/100 PYRS† *(95% CI)*
Cure*	358	1,794	48	2.7	21	19 (90)	1.1 (0.7–1.7)	2.4 (1.4–3.8)
TC*	89	466	13	2.8	10	5 (50)	1.1 (0.3–2.5)	1.4 (0.5–3.3)
Default	165	725	47	6.5	37	4 (11)	0.6 (0.2–1.4)	0.7 (0.2–1.6)

Definition of abbreviations: CI = confidence interval; FP = fingerprint; PYRS = person-years; TC = treatment completed.

* For successful treatment (either cure or TC) confirmed reinfections are 24 of 31 (77%), confirmed reinfection disease rate is 1.1 (0.7–1.6) per 100 PYRS, and likely reinfection disease rate is 2.2 (1.6–2.9) per 100 PYRS.

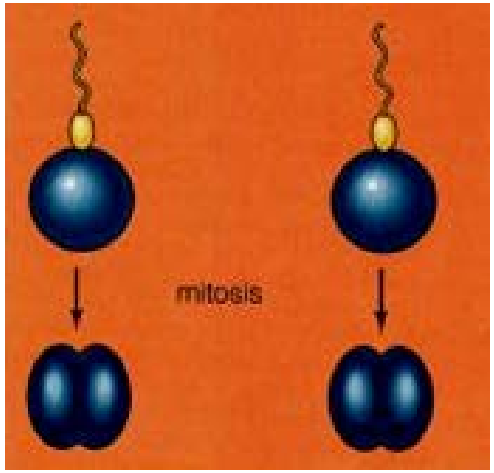
† The likely reinfection disease rate is the recurrence rate multiplied with the proportion confirmed reinfections among recurrences with a DNA FP available.

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

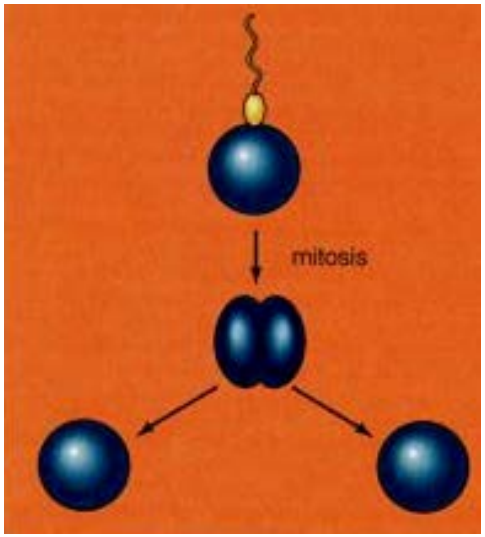
DZ TWINS



2 fertilizations

Share 50% of genetic background

MZ TWINS



1 fertilization

Share 100% of genetic background

Twin studies

Concordance

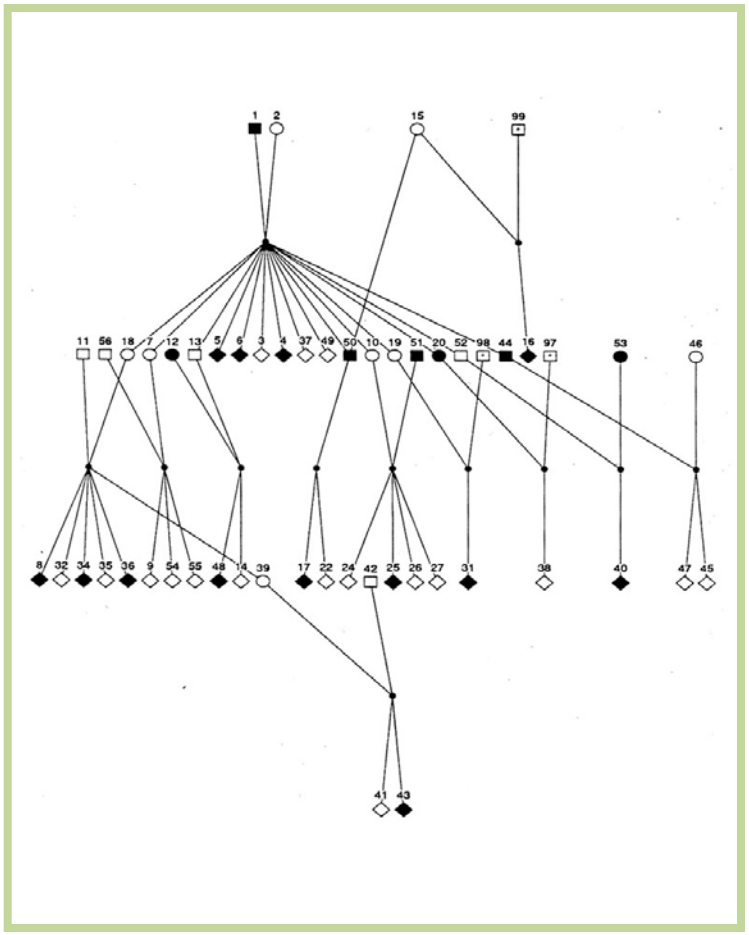
Monozygous twins		Dizygous twins	Reference
65%	⟷	**25%**	Diehl and Von Verschuer Beitr. Klin Kunsch 92: 275, 1936
62%	⟷	**18%**	Kallmann and Reisner, Am Rev Respir Dis 47, 549, 1942
32%	⟷	**14%**	Comstock, Am Rev Respir Dis 117, 621, 1978

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

Liability class	Penetrance of homozygous			# individuals
	Low risk allele		High risk allele	
	RR=10	RR=100		
Previously unexposed	0.085	0.0085	0.85	42
Previously exposed or vaccinated	0.037	0.0037	0.37	11
PPD negative during epidemic	0.010	0.0010	0.10	7
Age <2 yrs, >65yrs	0.425	0.2125	0.85	7
Unknown				14

Greenwood et al AJHG 2000

Candidate *NRAMP1*: TB outbreak

With liability classes: Strong evidence for strong genetic effect (NRAMP1)
P = < 0.000001, RR=10

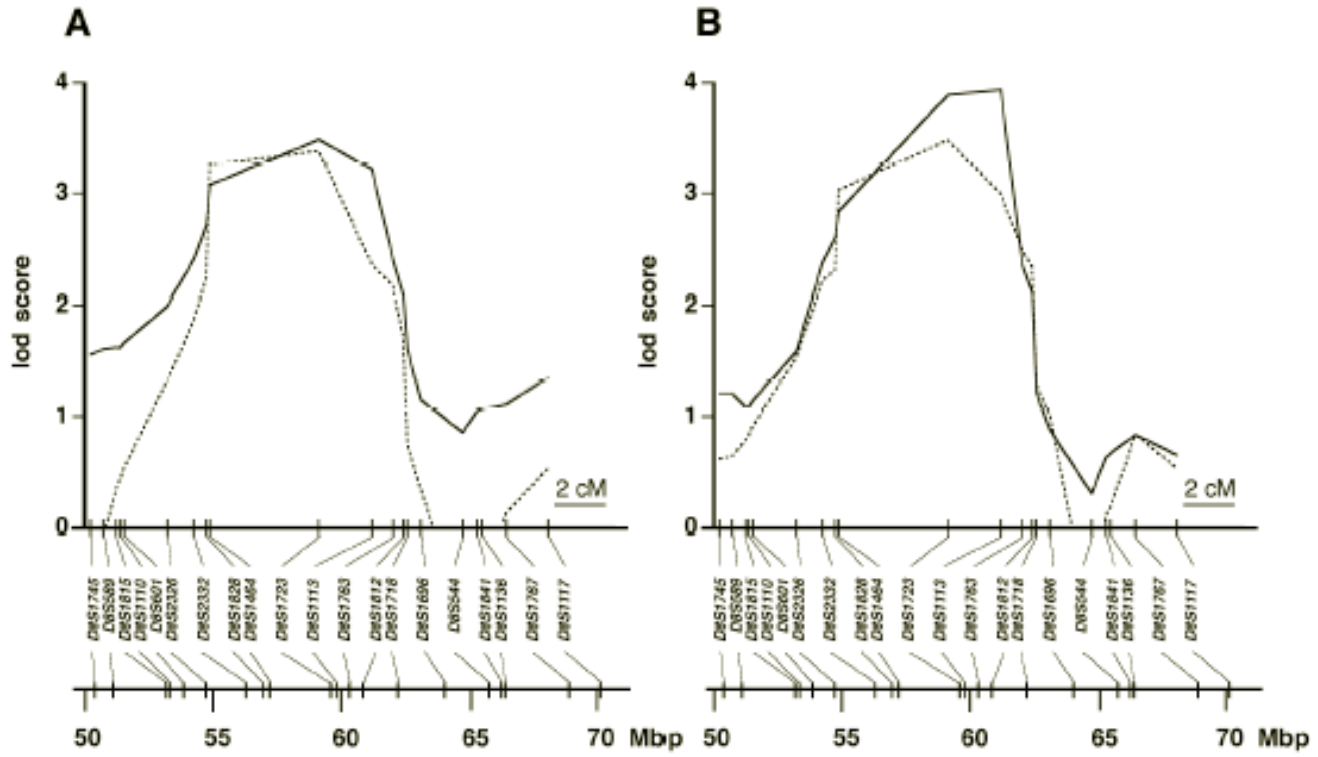
Without liability classes: NO evidence for any genetic effect

Greenwood et al. Am J Hum Genet 67:405, 2000

Environmental setting defines the playing field for genetic factor

Positional cloning approaches: *TOX* gene

Positional cloning approaches: *TOX* gene



Baghdadi et al. J Exp Med. 2006 Jul 10;203(7):1679-84.

Positional cloning approaches: *TOX* gene

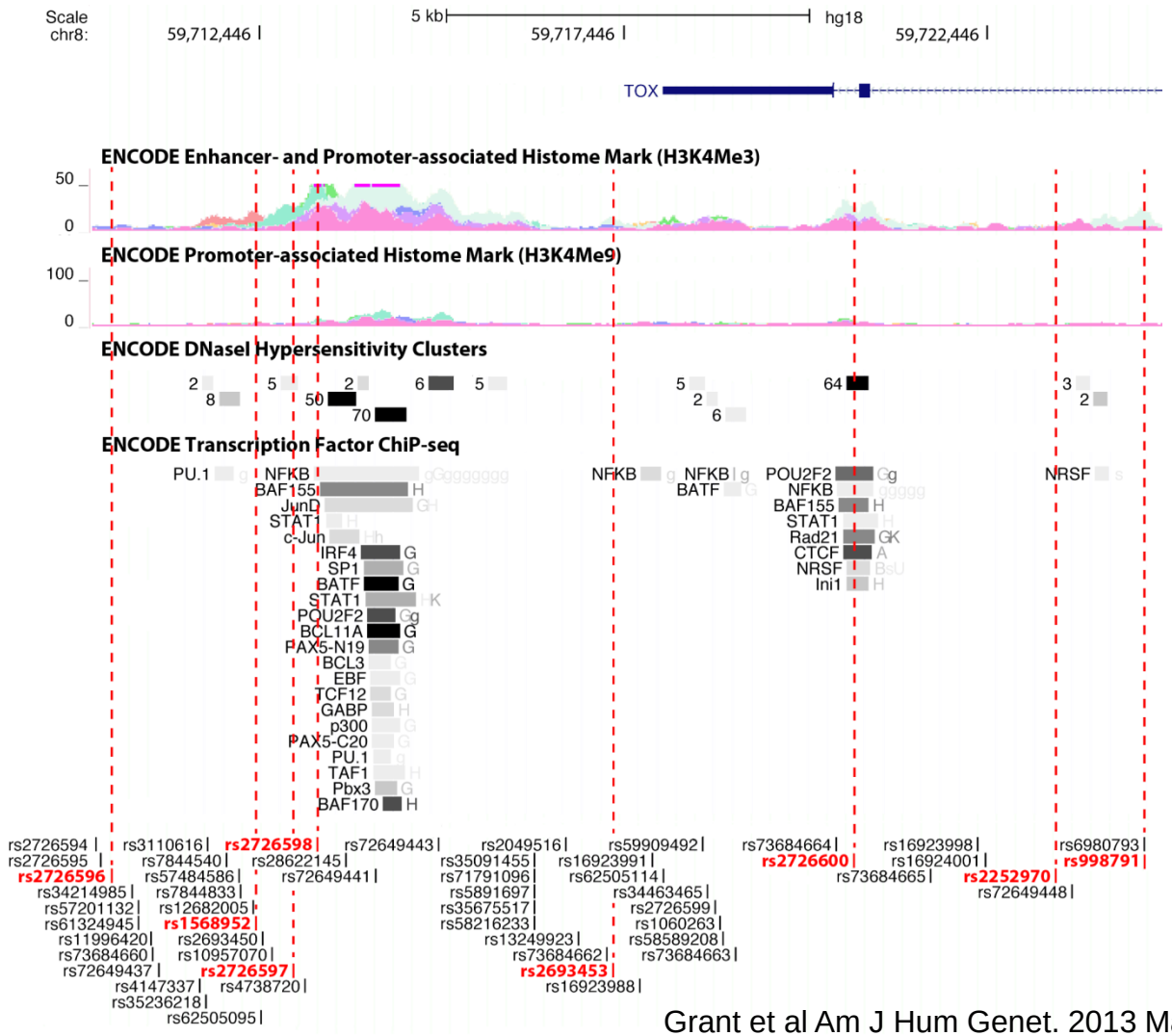
Table 1. Genetic Association Results for Replicated SNPs rs1568952 and rs2726600 in the Primary Moroccan Family-Based Study, the Moroccan Case-Control Replication Study, and Combined Analyses under the Recessive Model for the Minor Allele

					Family-Based Study		Case-Control Study		Combined	
Stratum	SNP	Minor Allele	Major Allele	MAF[a]	OR (95% CI)	p Value[b]	OR (95% CI)	p Value[b]	OR (95% CI)	p Value[b]
Full	rs1568952	A	G	0.36	3.21 (1.41–7.35)	0.007	1.98 (1.33–2.94)	6×10^{-4}	2.18 (1.53–3.10)	1.1×10^{-5}
	rs2726600	G	A	0.40	2.65 (1.27–5.56)	0.0093	1.61 (1.12–2.31)	0.0092	1.81 (1.34–2.43)	9.2×10^{-5}
<25 Years	rs1568952	A	G	-	5.54 (1.97–15.53)	0.0003	2.86 (1.72–4.77)	2.9×10^{-5}	3.09 (1.99–4.78)	4.4×10^{-8}
	rs2726600	G	A	-	2.56 (1.37–4.80)	0.0025	2.00 (1.24–3.23)	0.0039	2.19 (1.52–3.14)	3.2×10^{-5}
≥25 Years	rs1568952	A	G	-	0.65 (0.12–3.66)	0.62	1.52 (0.93–2.47)	0.094	1.42 (0.88–2.27)	0.15
	rs2726600	G	A	-	1.73 (0.56–5.33)	0.33	1.38 (0.89–2.14)	0.15	1.42 (0.95–2.13)	0.09

The following abbreviations are used: MAF, minor allele frequency; OR, odds ratio; and CI, confidence interval.
[a]MAF was estimated from among 316 founders.
[b]All p values are two sided.

Grant et al Am J Hum Genet. 2013 Mar 7;92(3):407-14

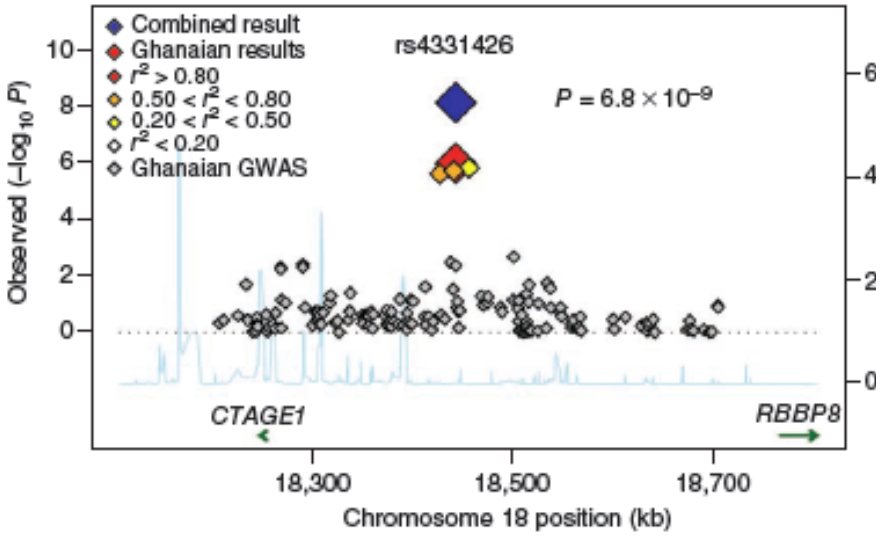
Positional cloning approaches: *TOX* gene



Grant et al Am J Hum Genet. 2013 Mar 7;92(3):407-14

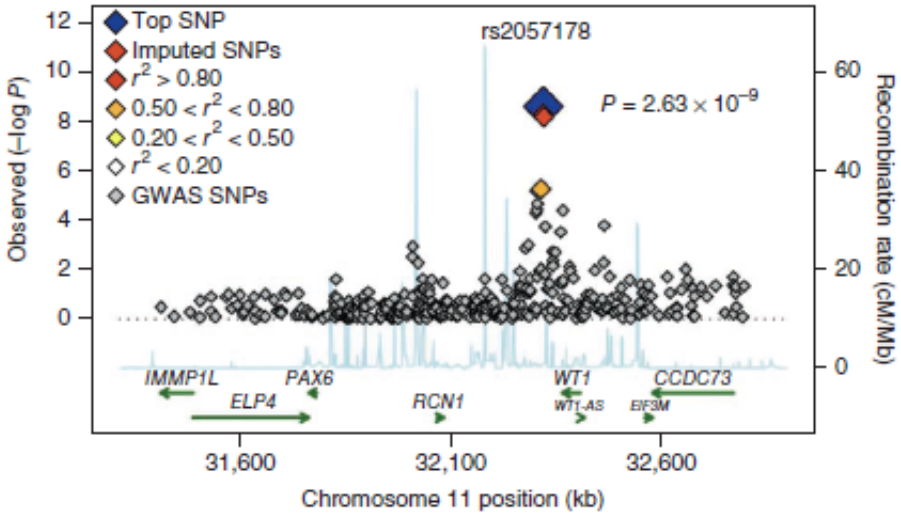
Genome-wide association studies (GWAS)

GWAS TB



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

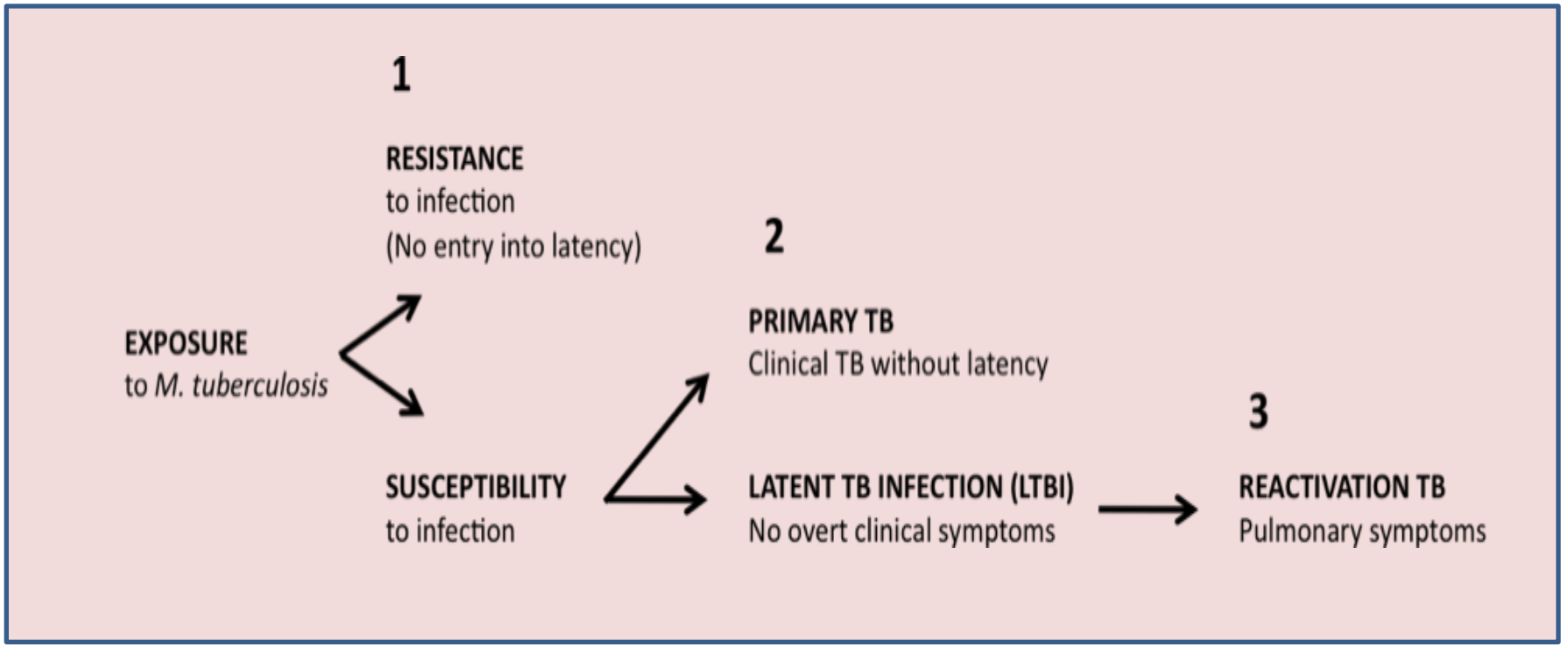
Chromosome 18 locus not replicated outside of West Africa



Thye et al. Nat Genet. 2012 Feb 5;44(3):257-9

Chromosome 11 locus replicated outside of West Africa

Tuberculosis Pathogenesis



Latent *M. tuberculosis* infection

How do we measure infection?

No “gold standard”

Three types of assays

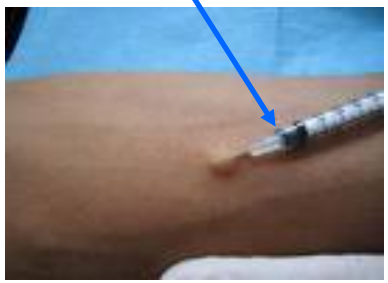
***In vivo* tuberculin skin test (TST)**

***In vitro* production of antigen-specific IFNγ production (ELISA)**

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

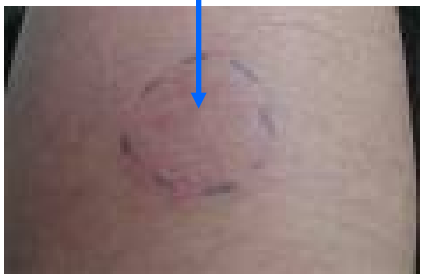
Tuberculin Skin Test

Tuberculin (PPD)



72hs

Delayed Type Hypersensitivity



Detection of people infected by *M. tuberculosis*

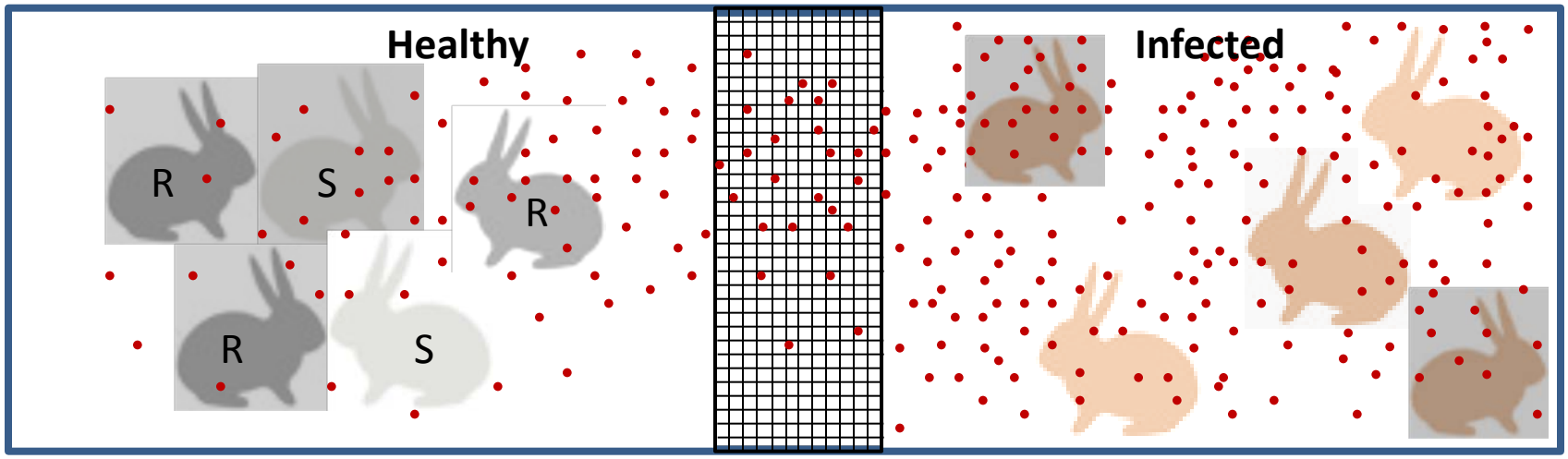
Public Health

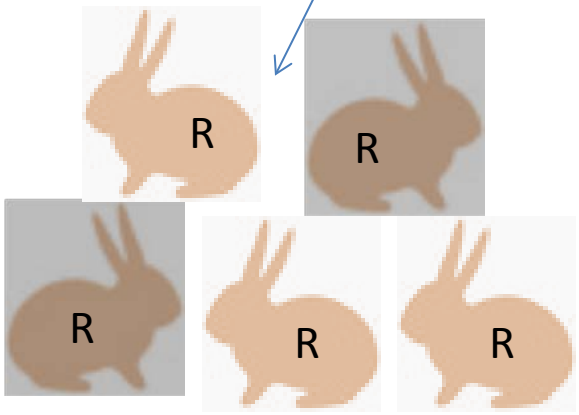
- **≥ 5 mm (Immuno-)**
- **≥ 10 mm (no BCG)**
- **≥ 15 mm (BCG)**

→ **Infection**

Intrinsically a quantitative measure

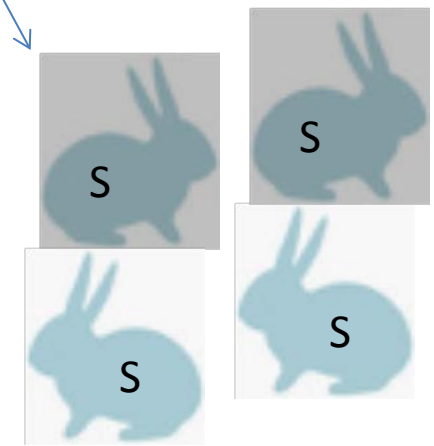
Max Lurie's Rabbits





Cavitary disease

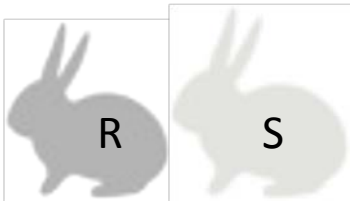
Mean survival 9.2 months



Disseminated disease

Mean survival 4.8 months

Innate resistance



20-40% no disease

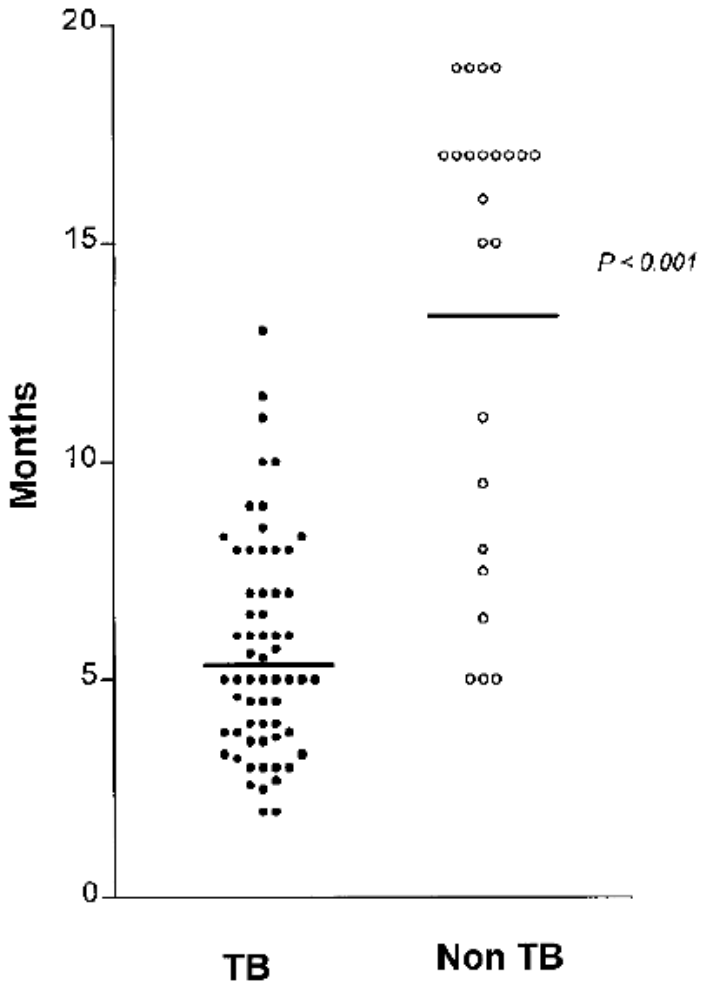
85% Tuberculin test negative

11-19 months of exposure

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

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

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%

(Sepulveda et al., Am J Respir Crit Care Med, 1994 / Tuber Lung Dis, 1994)

(Jepson et al., Infect Immun, 2001)

✓ **Molecular studies** :

- linkage study in Uganda

  (Stein et al., Plos One, 2008)

- Candidate gene *IL10* and binary Mantoux

  (Thye et al., Plos One, 2009 ; Zembrzuski et al., Tuberculosis, 2010)

- *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?

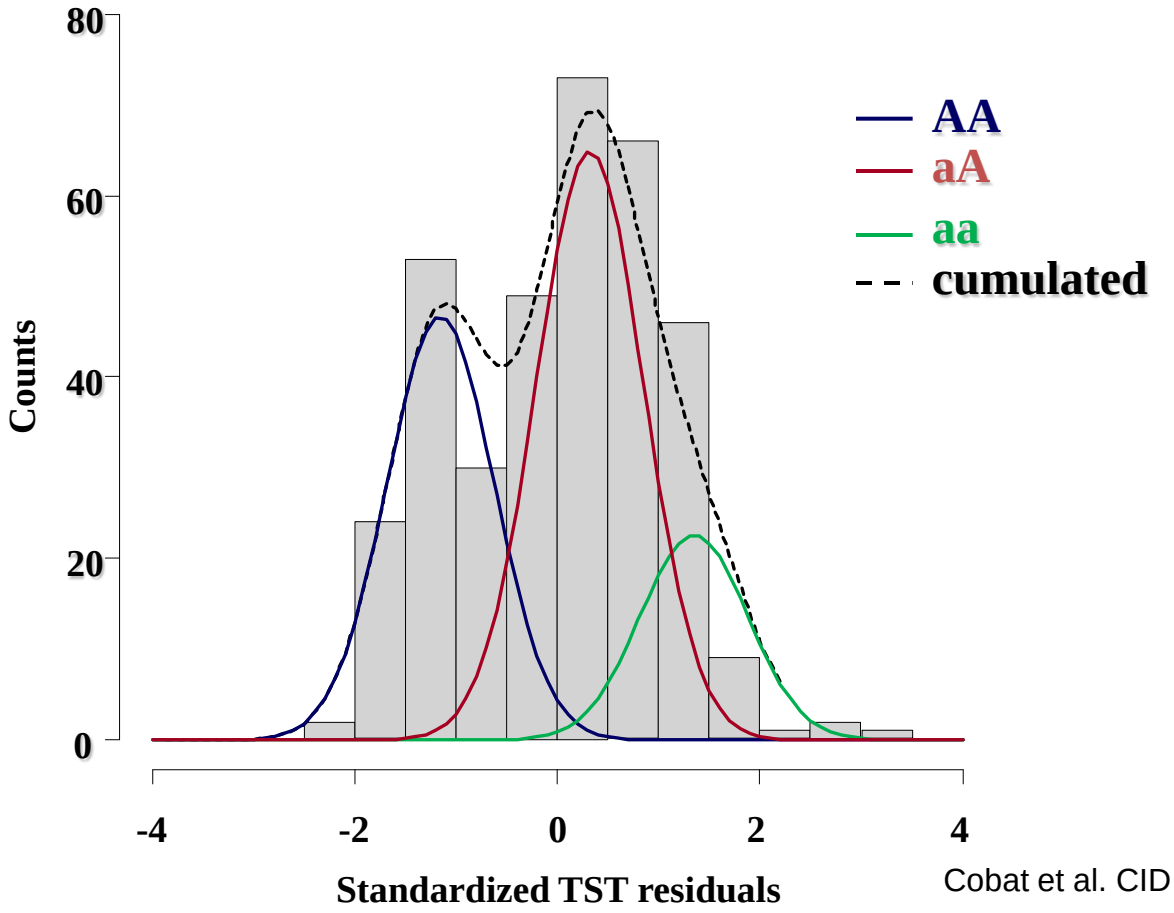
⇒ Complex Segregation Analysis (CSA)

A major gene controls TST reactivity in Colombia

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

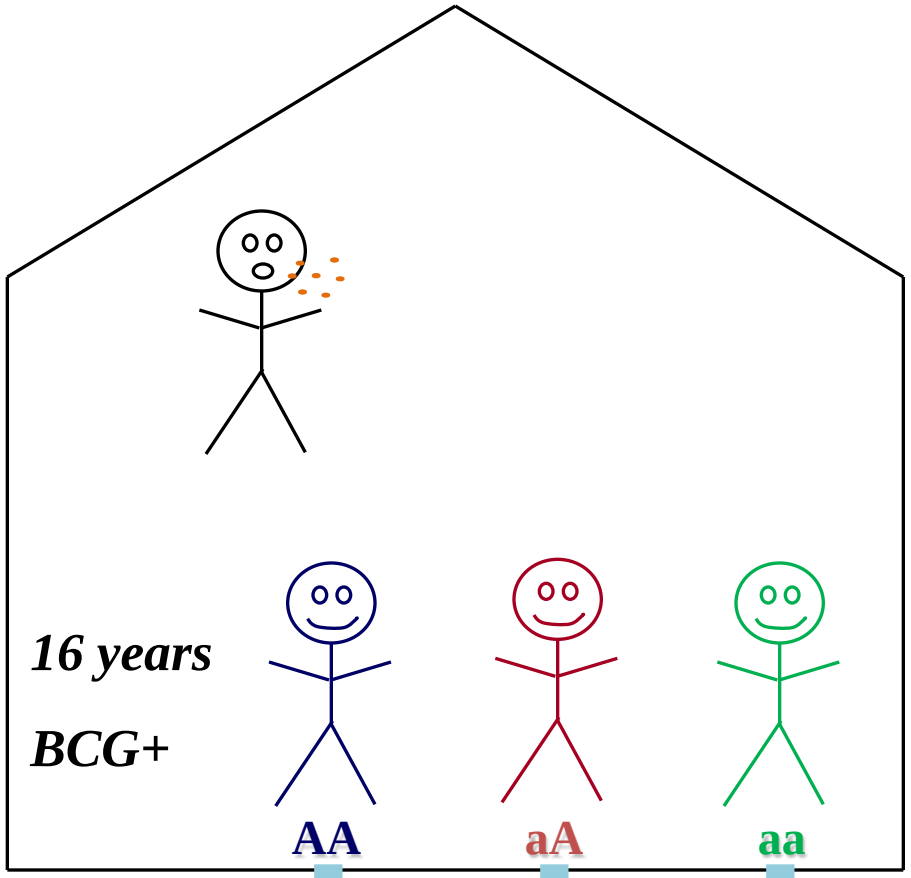
35% (17%) predisposed to low (high) values

$\Rightarrow$ explains 72% of TST residual variability!



Cobat et al. CID 54:968, 2012

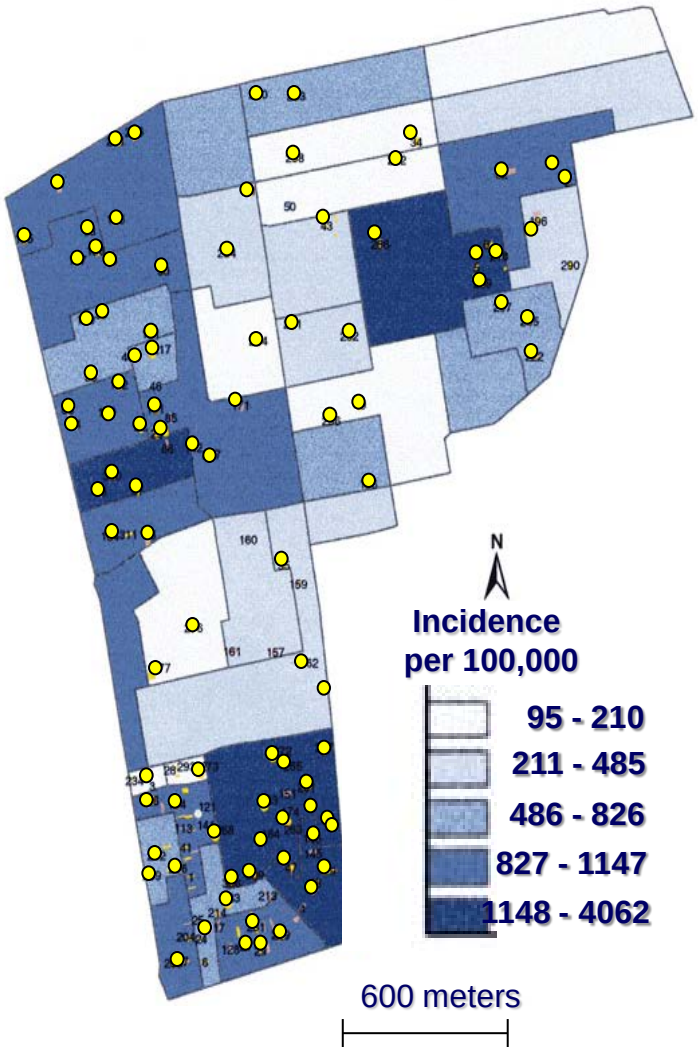
A “what does it mean” example



Mean TST **3mm** **12mm** **22mm**

Genetic linkage study of TST reactivity

Location: Cape Town, South Africa



- **128 nuclear families ≥ 2 sibs**

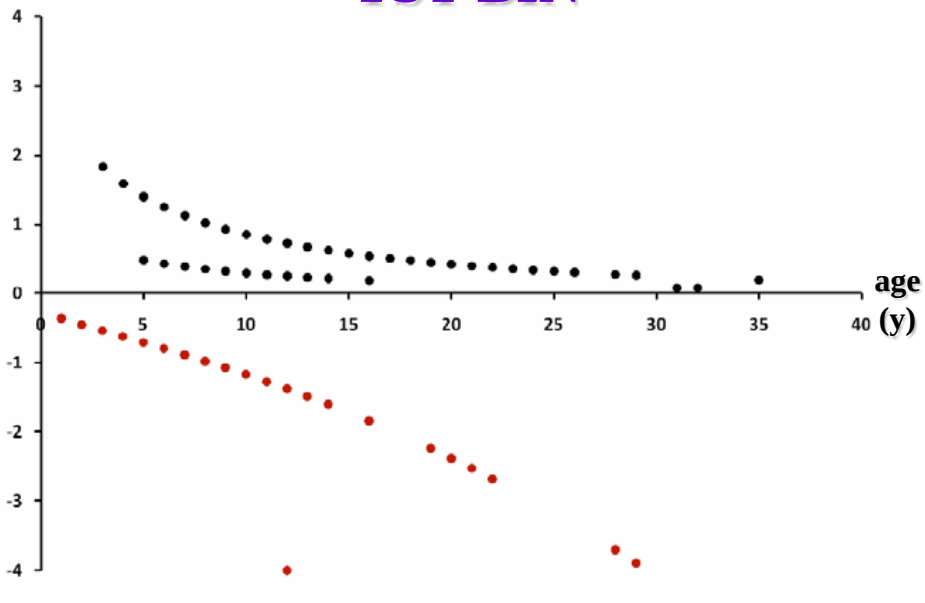
186 parents	350 children
DNA	DNA
	Immune phenotypes
	Covariates

6,000 SNPs genotyped for linkage analysis

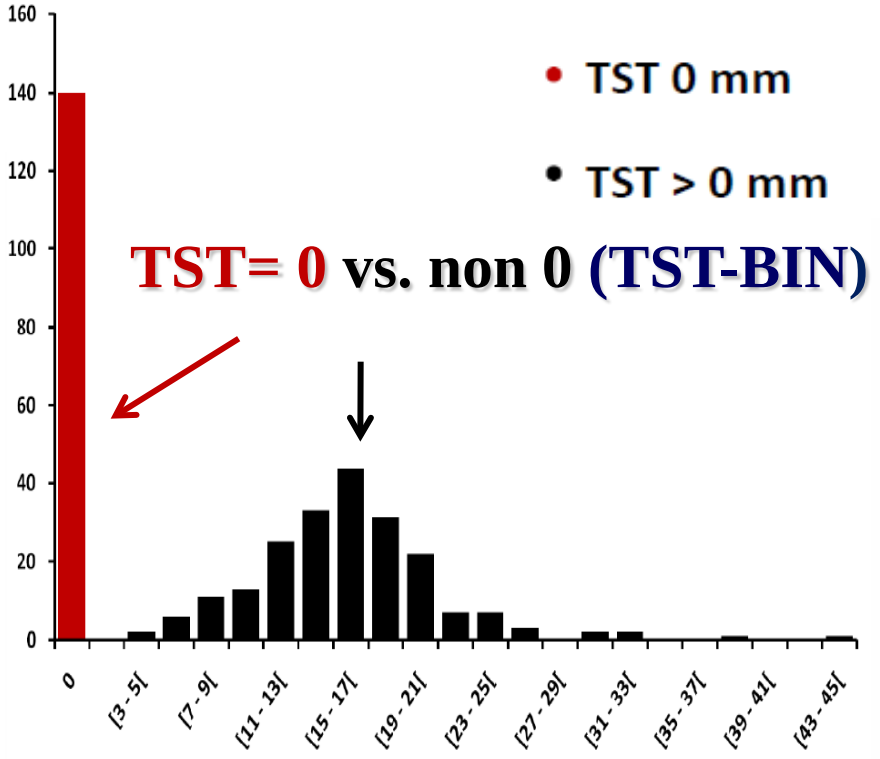
TST distribution is bimodal

TST= 0 vs. Non 0

TST-BIN



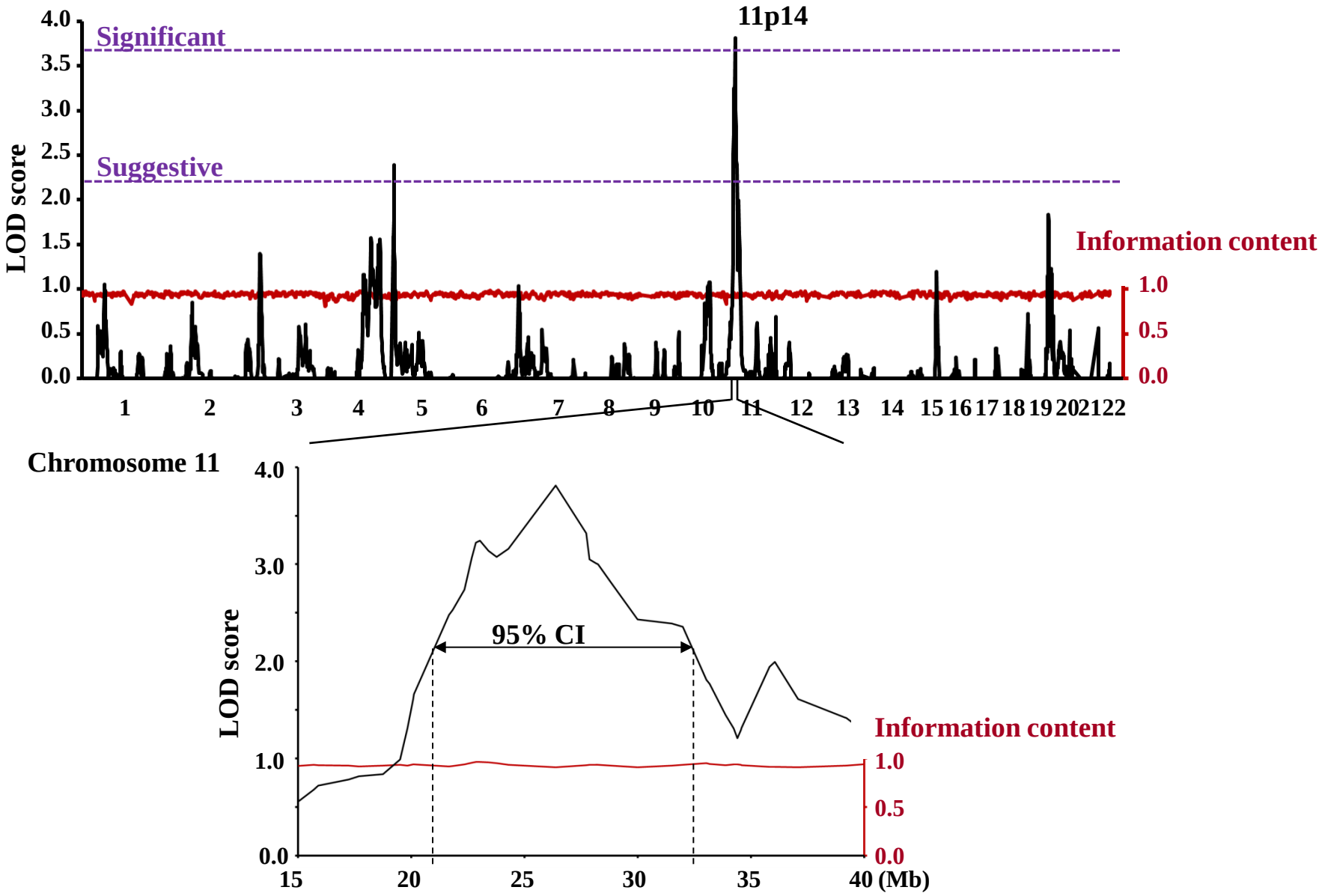
Pearson residuals



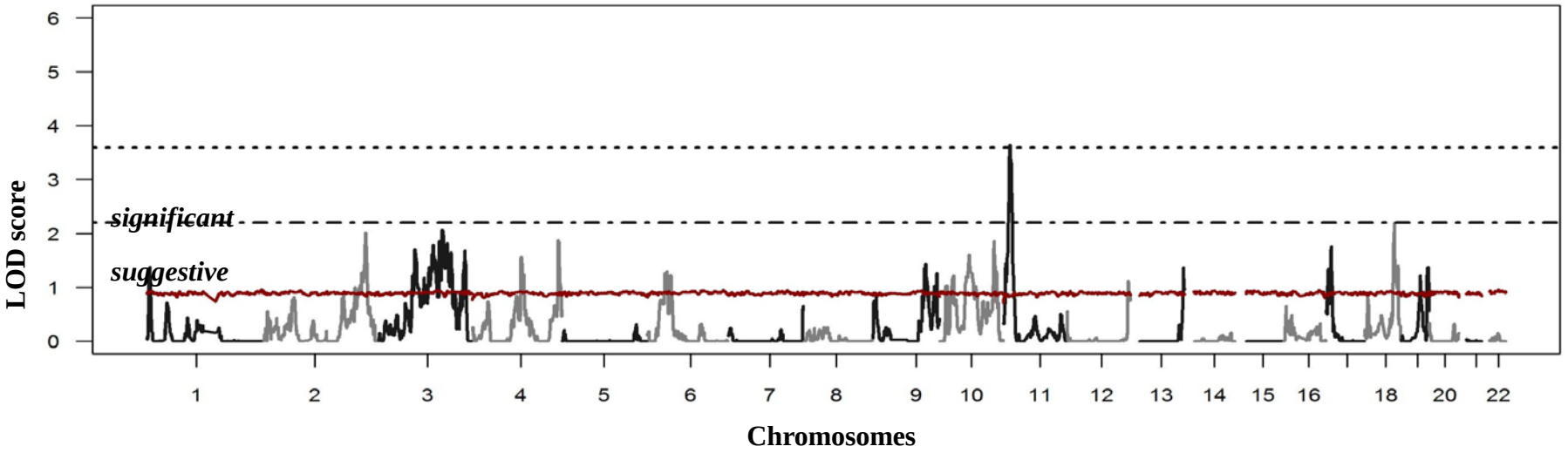
TST in mm

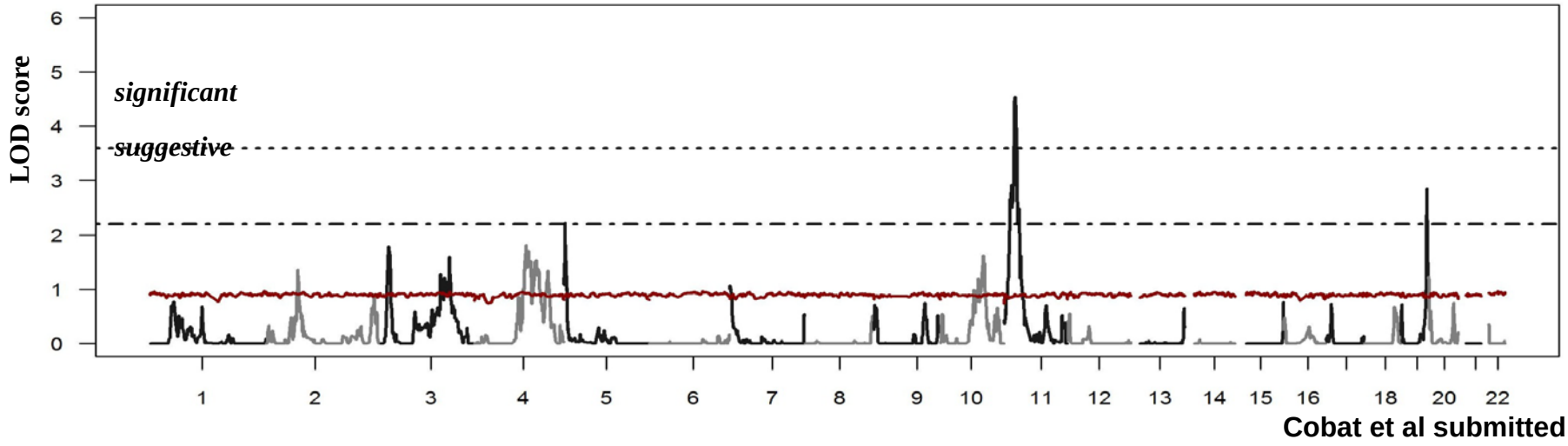
Cobat et al. *J Exp Med* 206:2583, 2009

Major locus for TST negativity *per se (TST1)* maps to 11p14



Household contact study in Paris





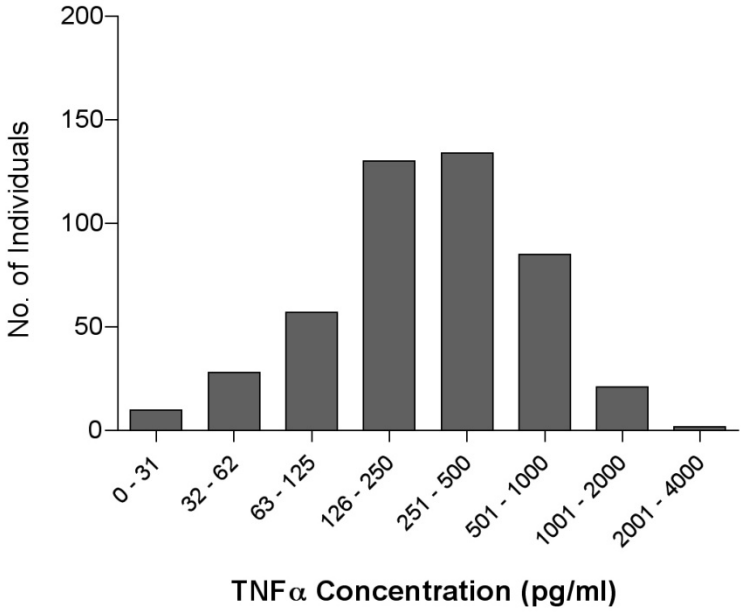
Cobat et al submitted

ENDOPHENOTYPES

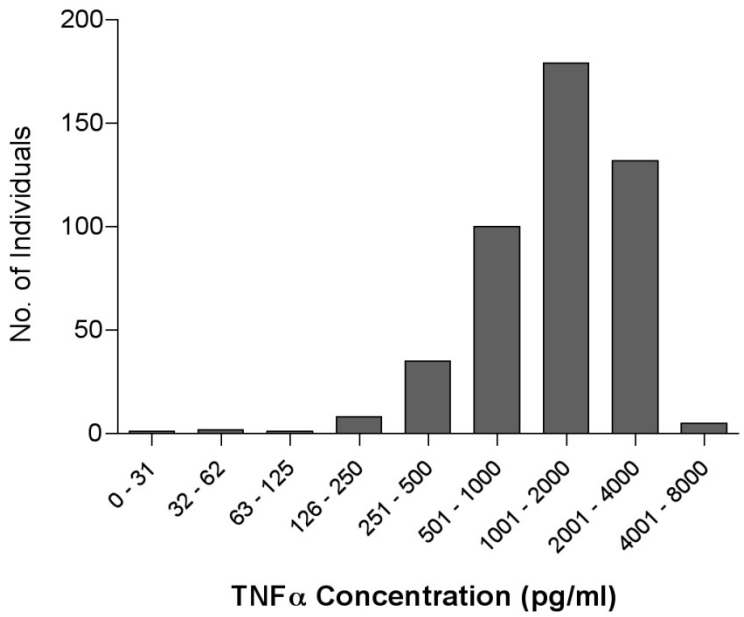
134 nuclear families [2-6 sibs]; 390 children; whole blood assays

TNF production by whole blood after stimulation by:

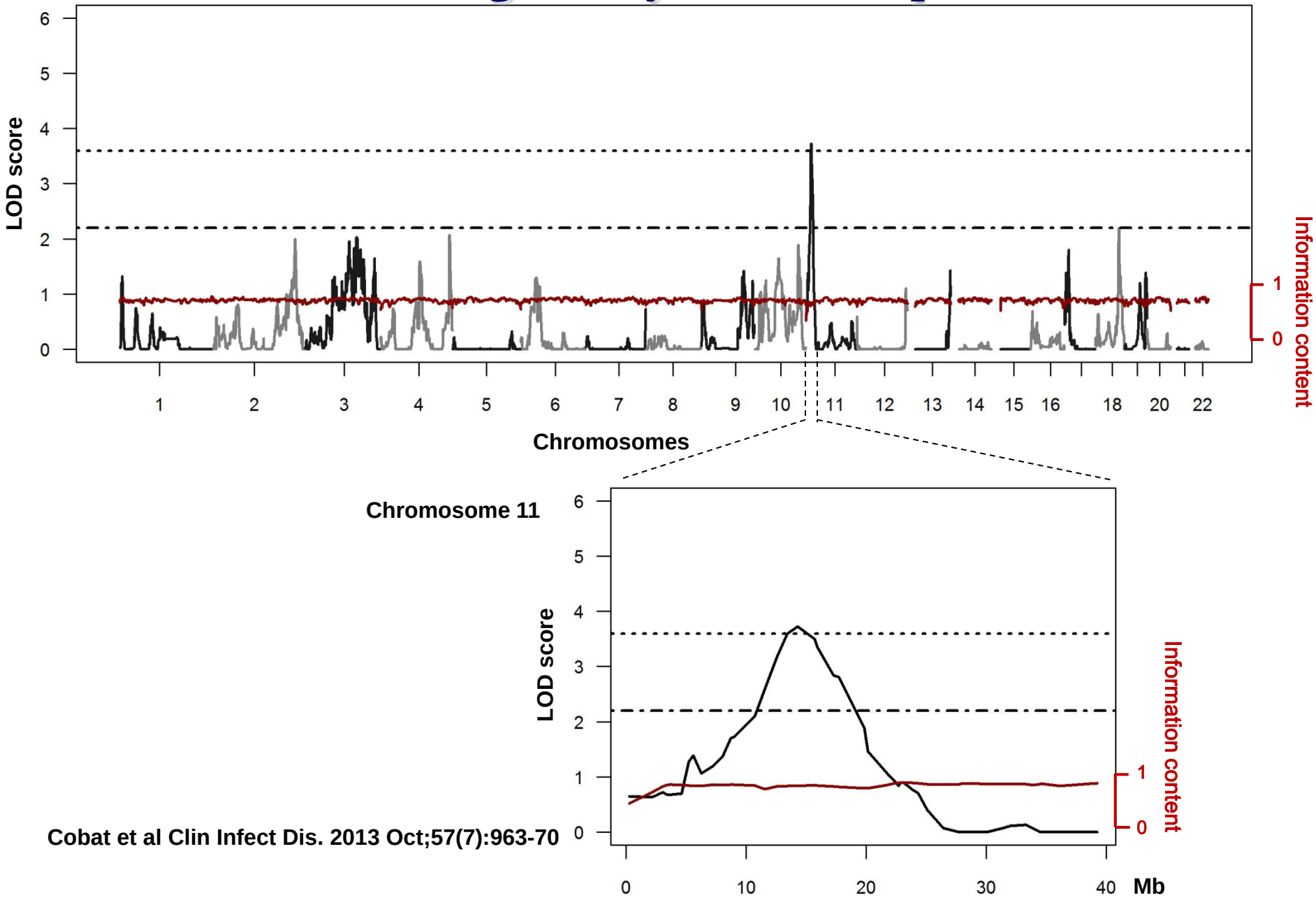
BCG



BCG + IFN-γ

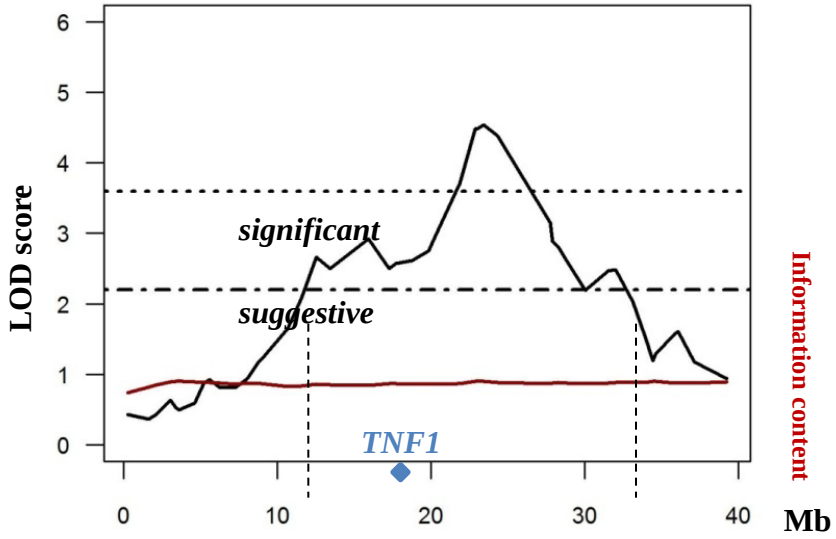


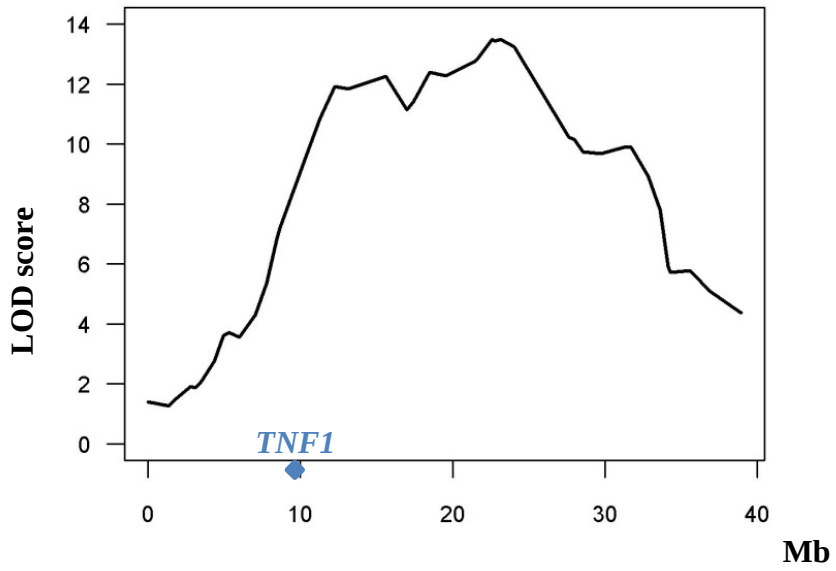
Bivariate linkage analysis of TNF production



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

Take-home message II

Genetics suggests a connection between innate resistance to *Mtb* infection with innate efficiency to produce TNF

CIHR IRSC
Canadian Institutes of Health Research | Instituts de recherche en santé du Canada

Inserm
Institut national de la santé et de la recherche médicale

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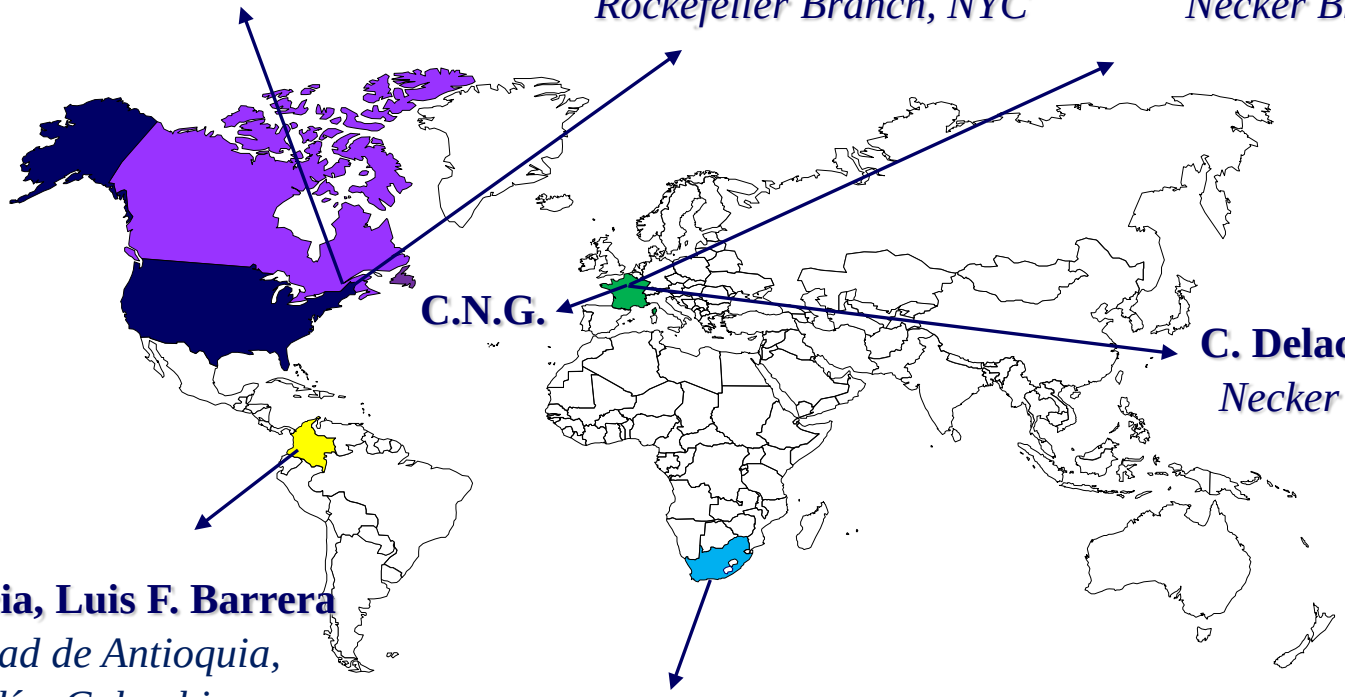

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