JNI – 11 Journées Nationales d'Infectiologie

Du mercredi 9 au vendredi 11 juin 2010 – Le Corum, Montpellier

Vers une théorie génétique des mycobactéries humaines

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Déclaration de conflits d'intérêts:

Jacinta Bustamante MD, PhD

Absence de conflits d'intérêt

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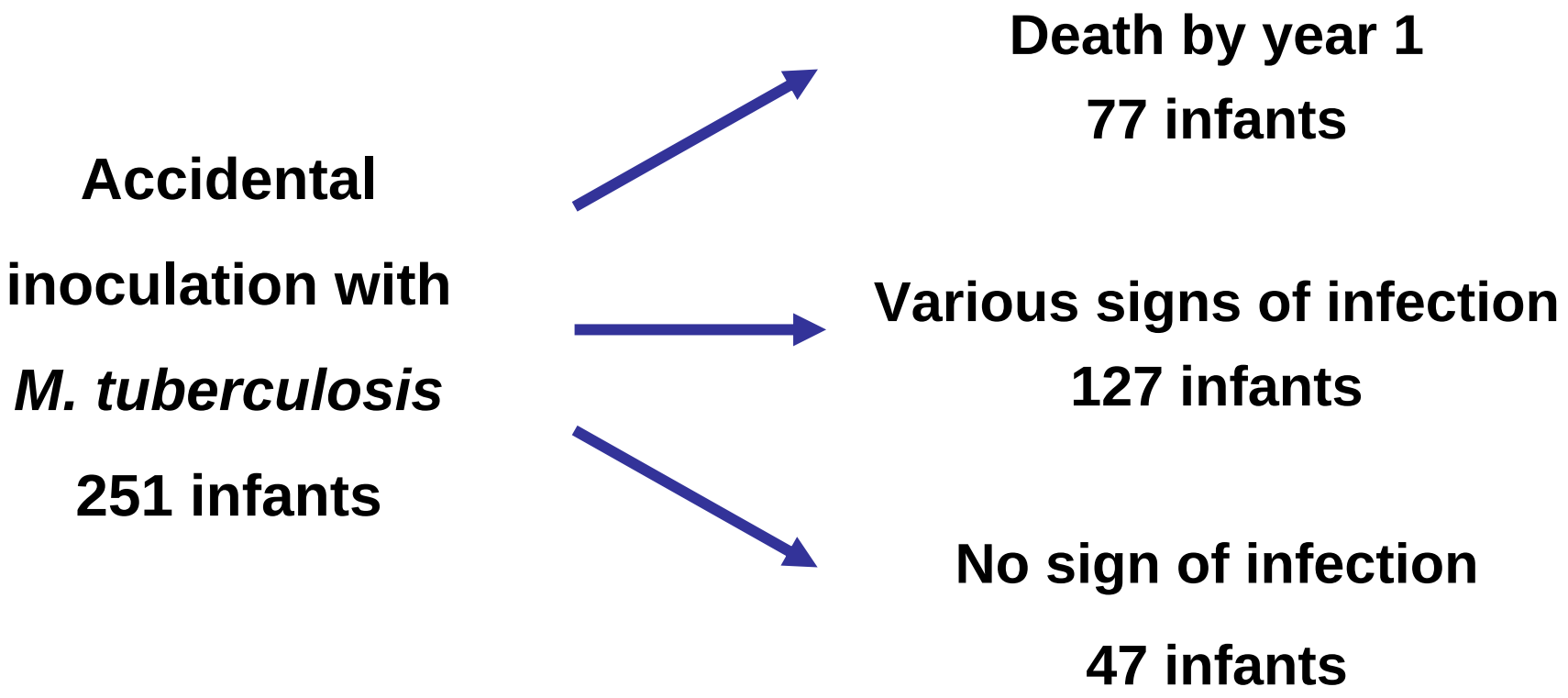
The genus *Mycobacterium*

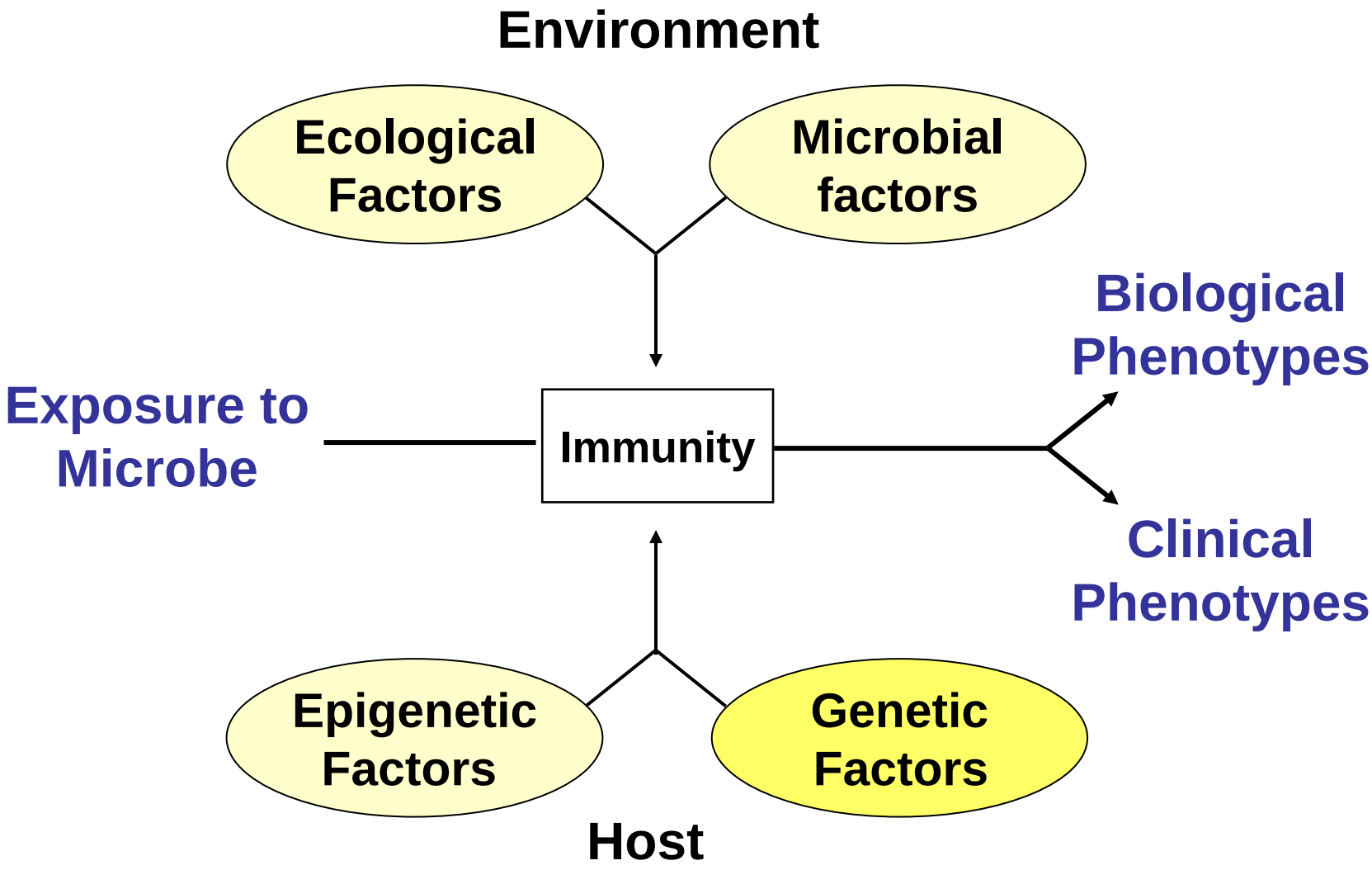
‘Virulent’	**‘Weakly virulent’**
M. tuberculosis complex	> 80 species (e.g. *M. avium*,
M. leprae	*M. marinum, M. fortuitum…)*
Human transmission	Environmental
transmission	
(airborne)	(water, soil, air…)
M. ulcerans (Buruli ulcer)	BCG vaccine
Aquatic bug transmission?	Injection transmission

Inter-individual variability in tuberculosis: the key question

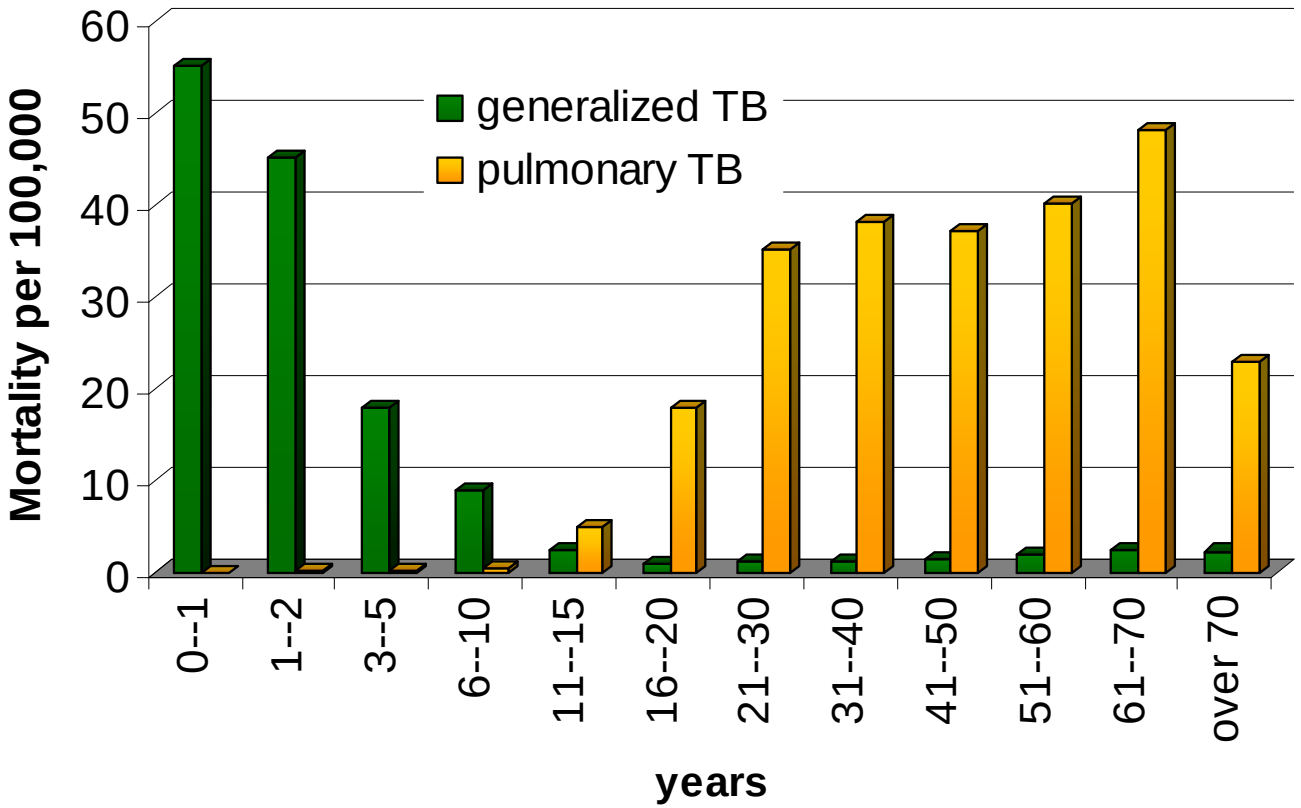
- **The Lübeck disaster before world war II**



Four theories of infectious diseases

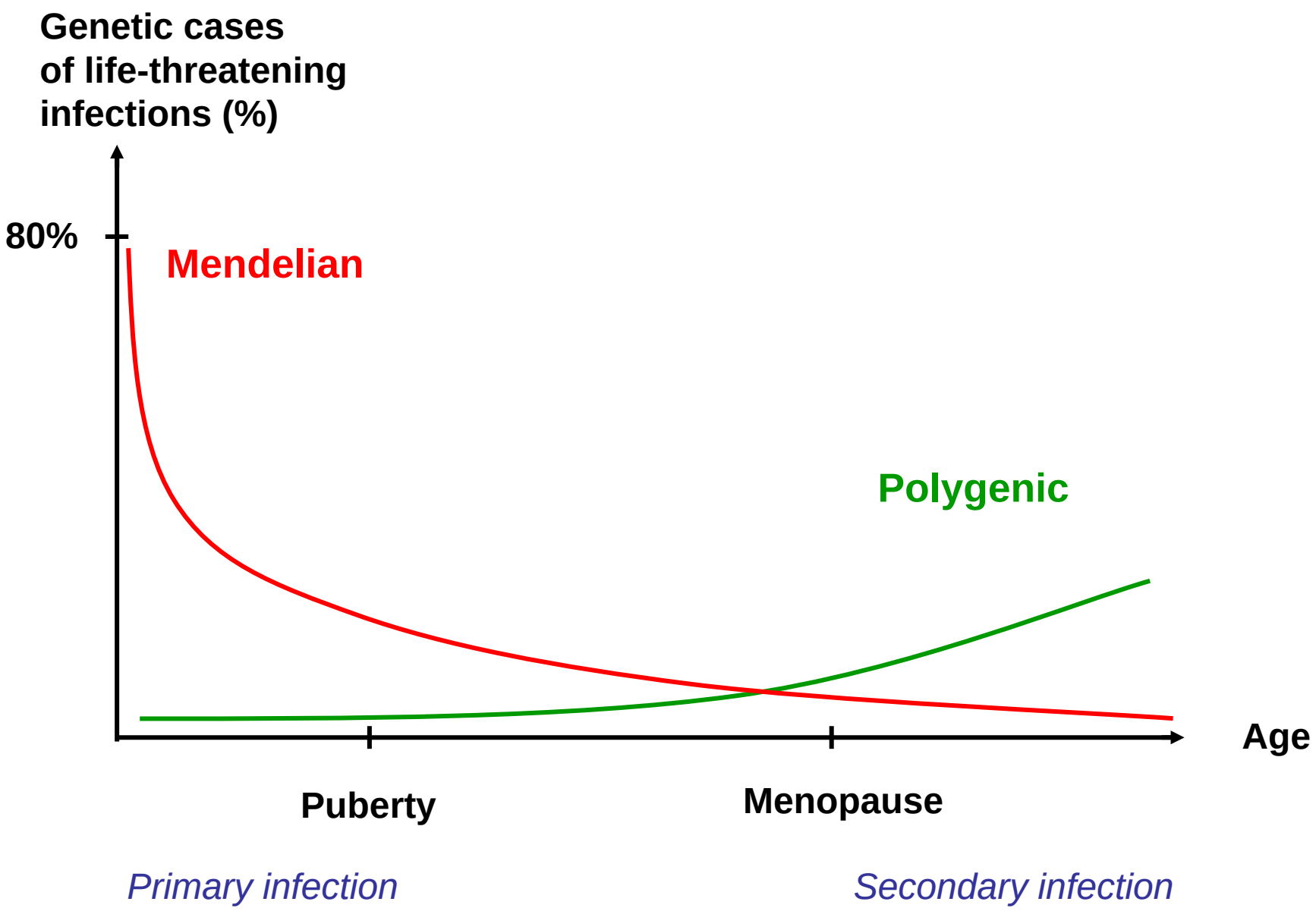


Tuberculosis in children and adults



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

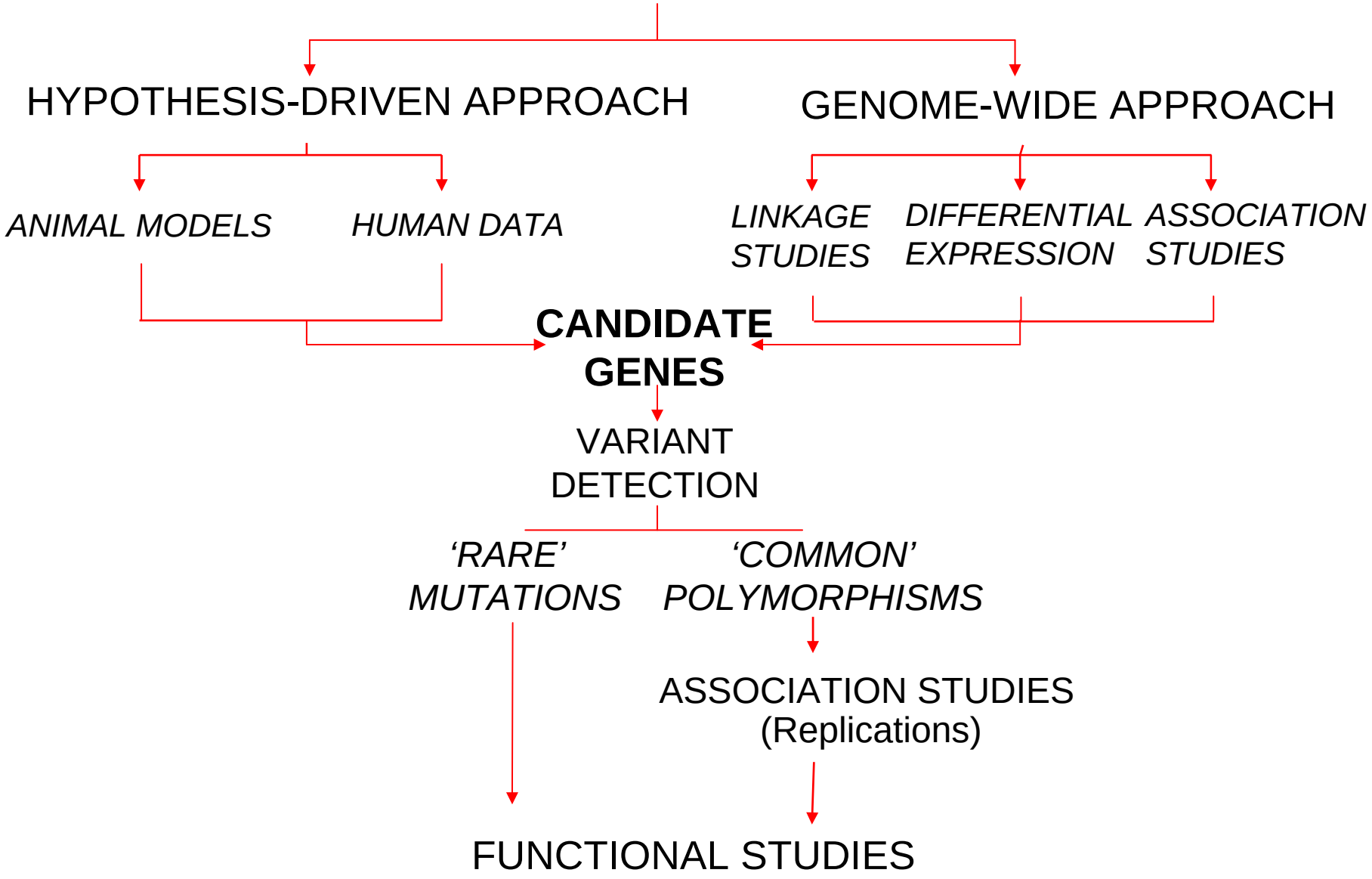
A genetic architecture of tuberculosis



Methods of investigation in humans

Phenotype	Rare (disseminated infection)	Common (TB, leprosy)
Tools	**Mendelian Genetics**	**Genetic Epidemiology**
Sample	Small	Large
	⇩	⇩
	Rare mutation	Common polymorphism

MENDELIAN and COMPLEX INHERITANCE

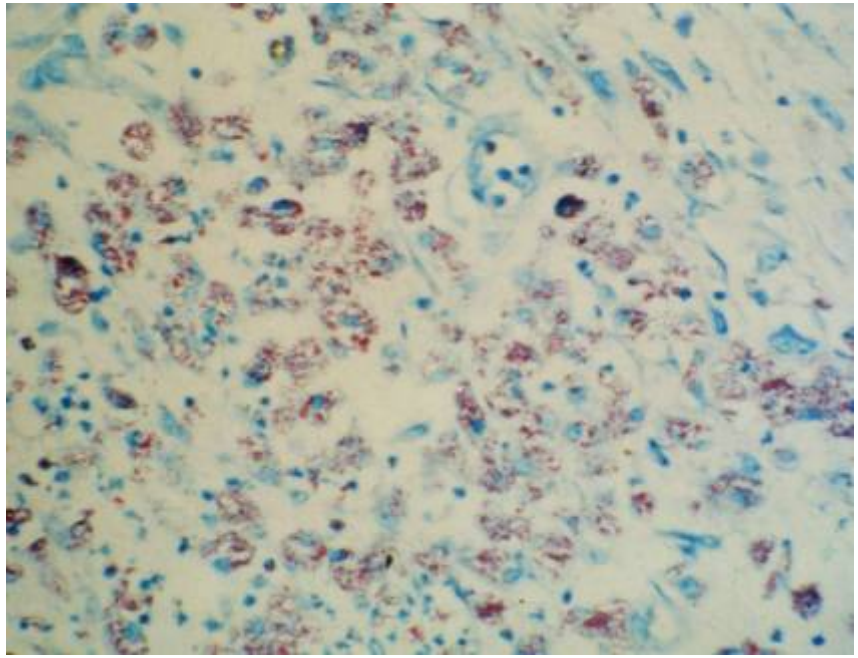


Mendelian susceptibility to mycobacterial diseases (MSMD)

* Infections by BCG and environmental Mycobacteria

* Otherwise healthy individuals

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



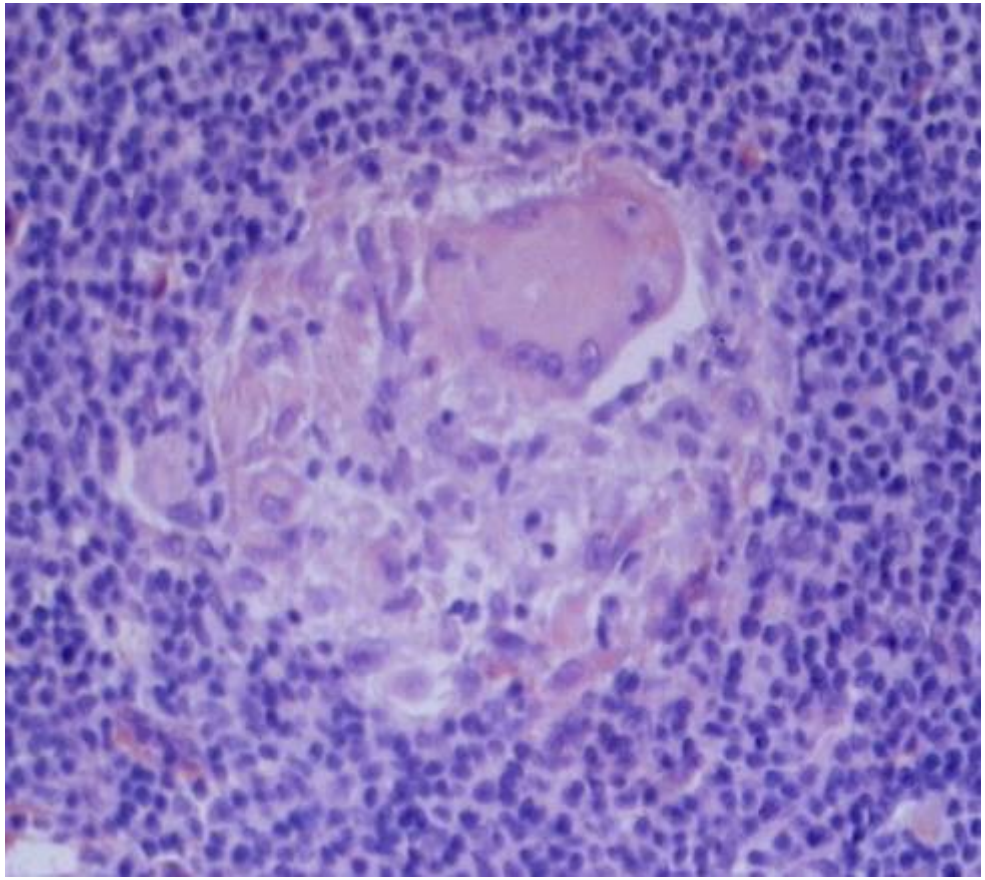
Mendelian Susceptibility to Mycobacterial diasease

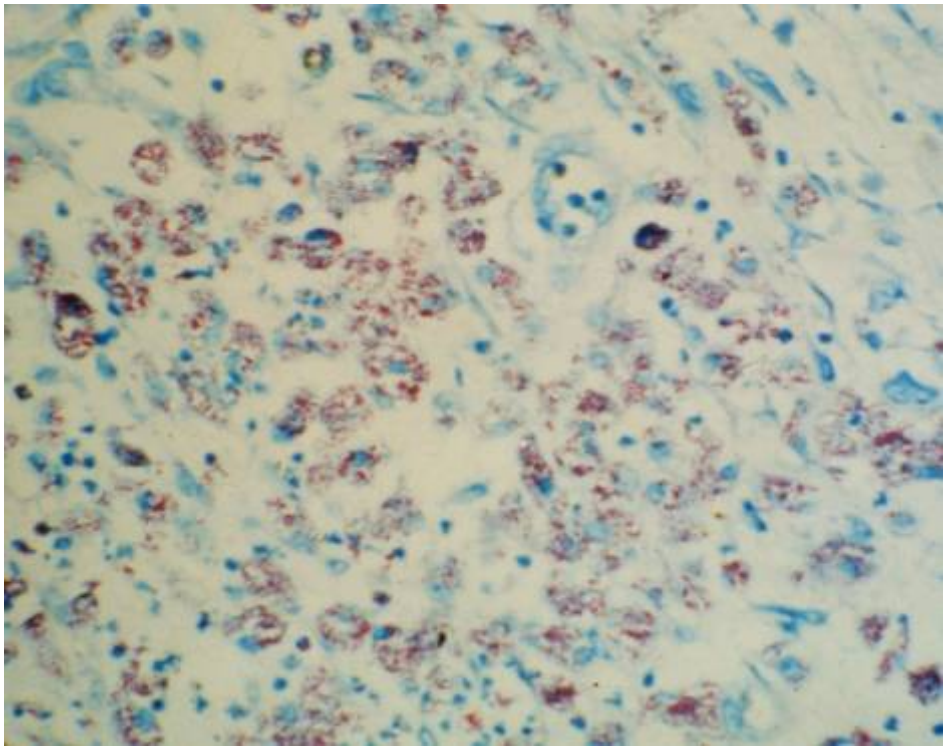
HETEROGENEOUS

Genetic basis: autosomal or X-linked inheritance

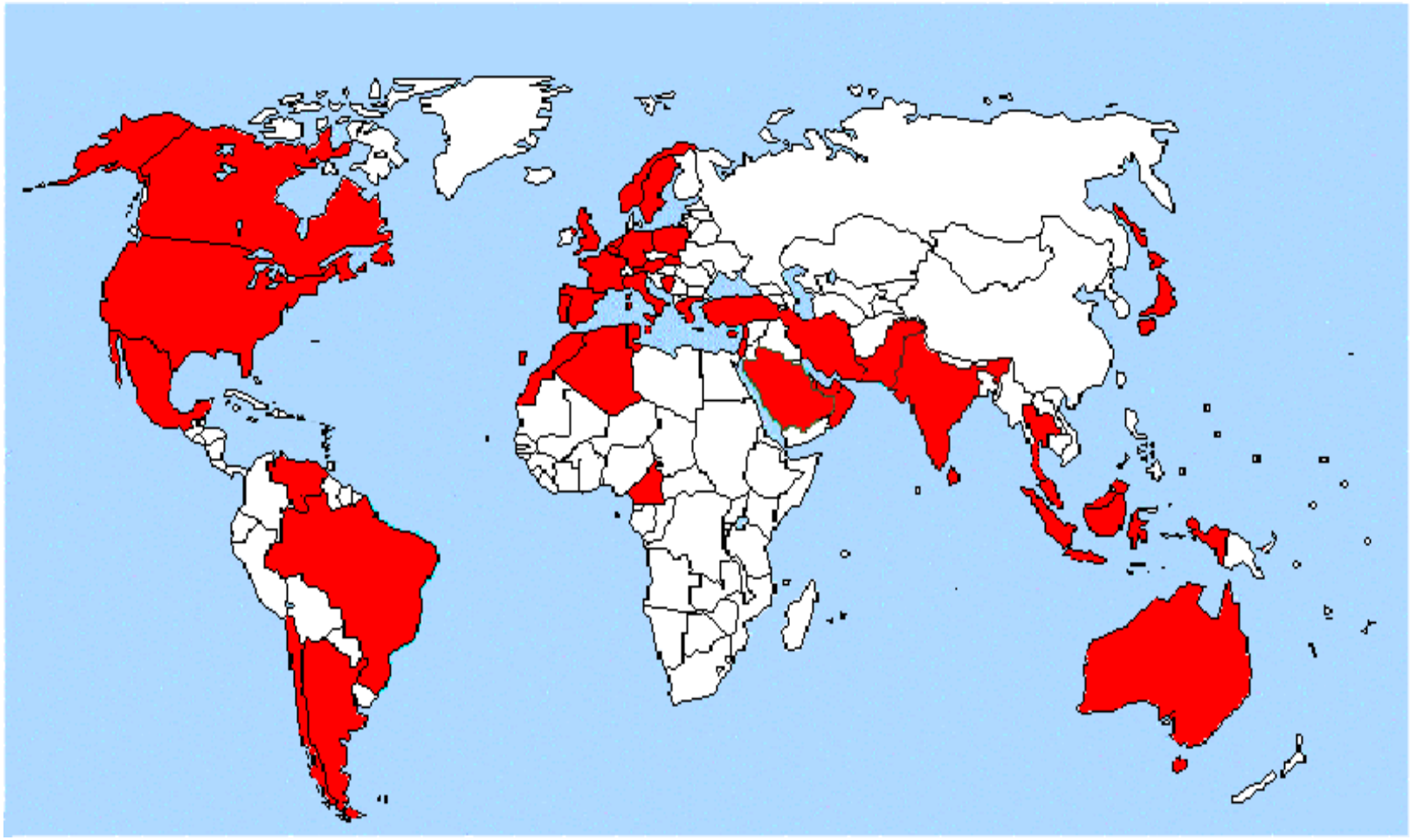
Clinical outcome: reccurent, disseminated or local

Granulomatous lesion: lepromatous type
tuberculoid type

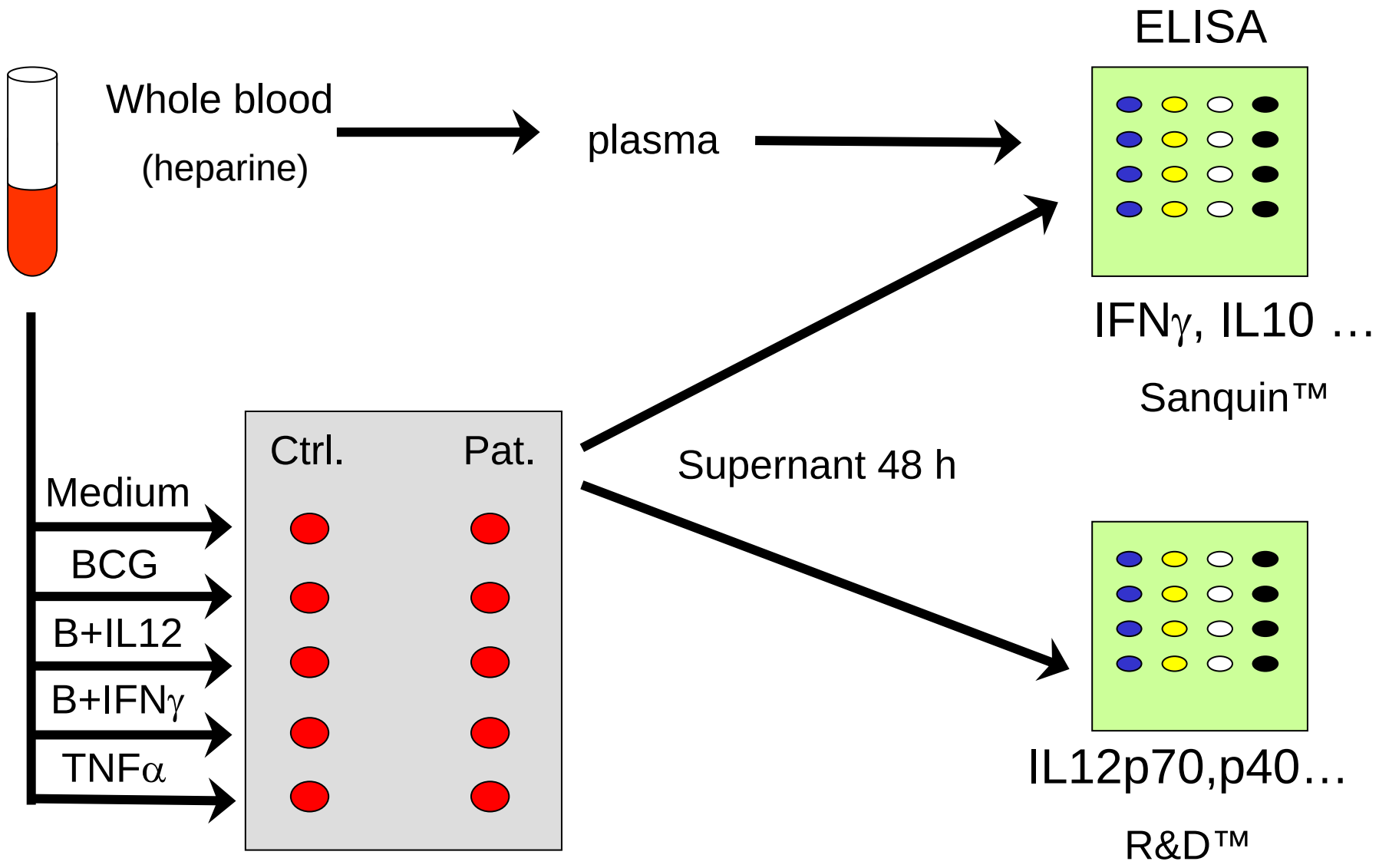




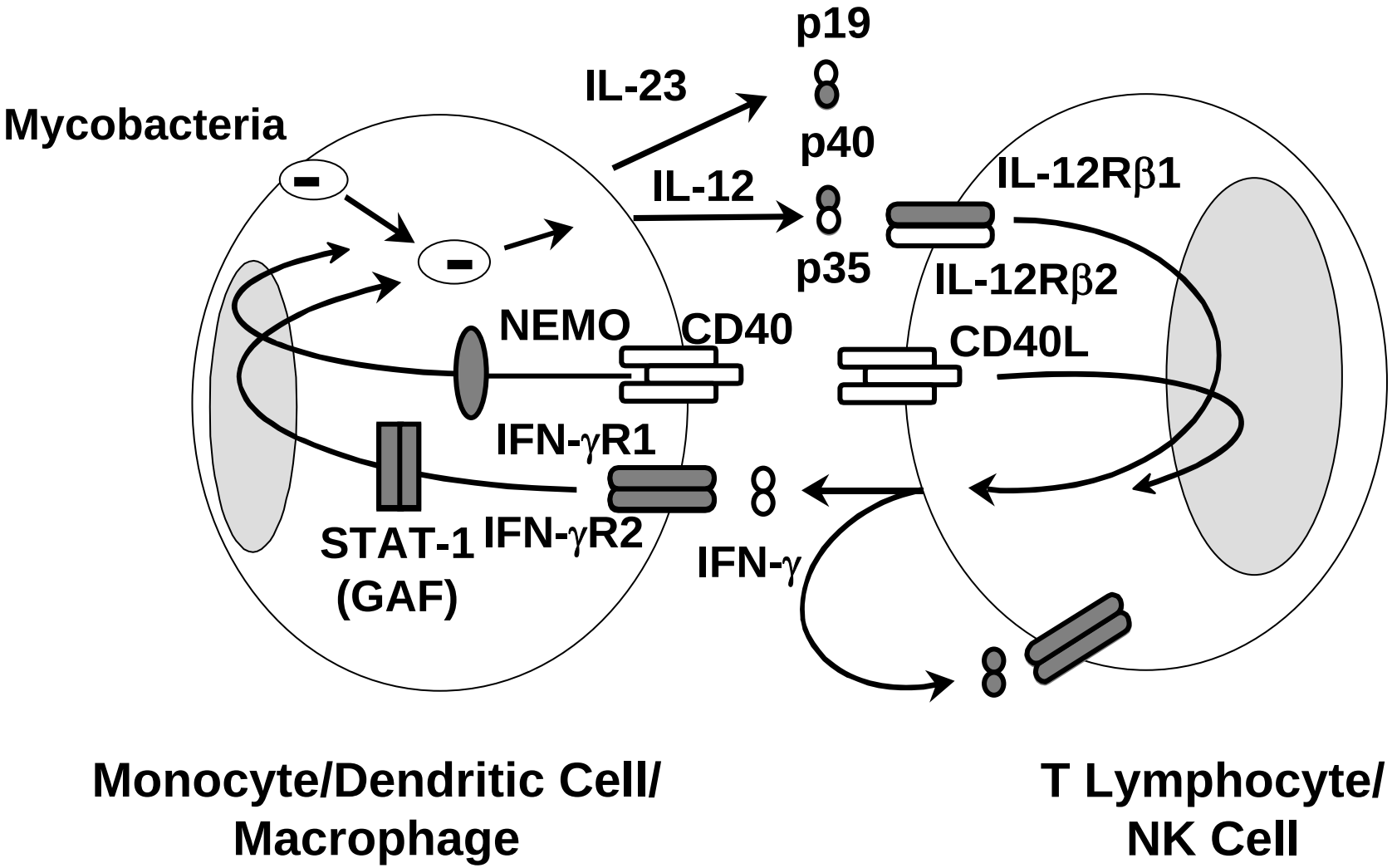
Over 300 patients in 45 countries



Functional screening *in vitro*



MSMD: IFN-γ/IL-12/IL-23 pathway

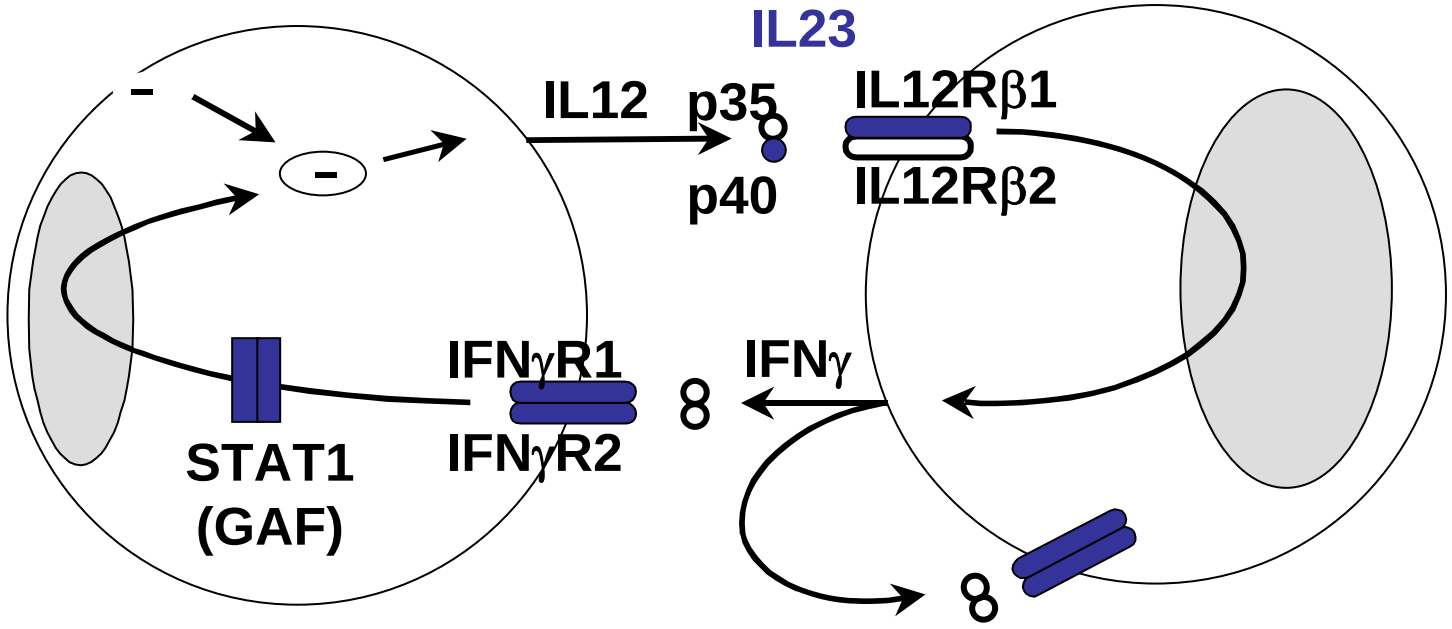


Mendelian Tuberculosis

- Mendelian disorders of the IL12-IFNγ axis are genetic etiologies for severe forms of tuberculosis:

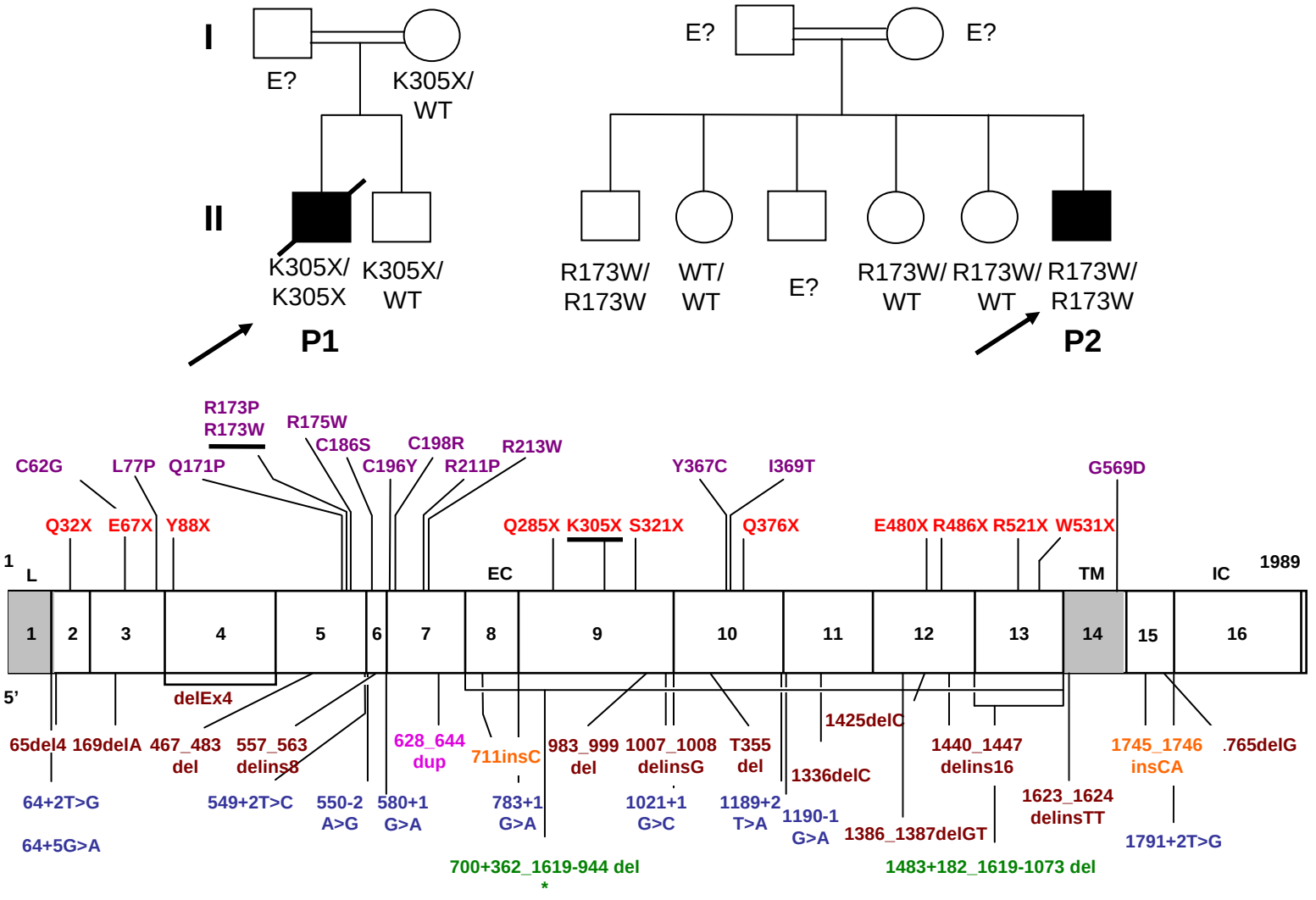
- What is the proportion of ‘Mendelian’ tuberculosis? (in children) …

Proof of principle: Mendelian tuberculosis



IL12-Rβ1 deficiency and tuberculosis

Patients with extra-pulmonary TB from Iran and Morocco



MSMD and severe childhood tuberculosis Strategy

Functional screening of the pathway

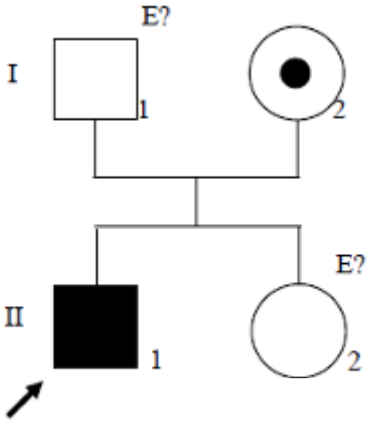
I. IFN-γ Production
II. IFN-γ Response
III. IL-12 Production
IV. Genetic Screening

Positional cloning

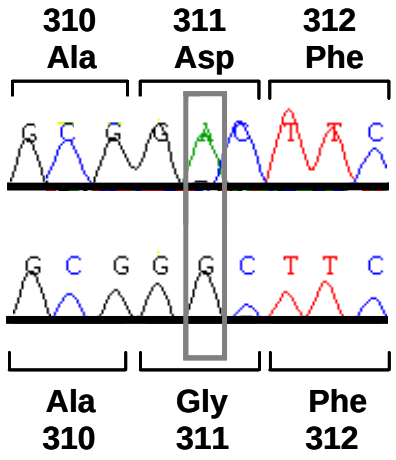
30 consanguineous families
2 X-linked families

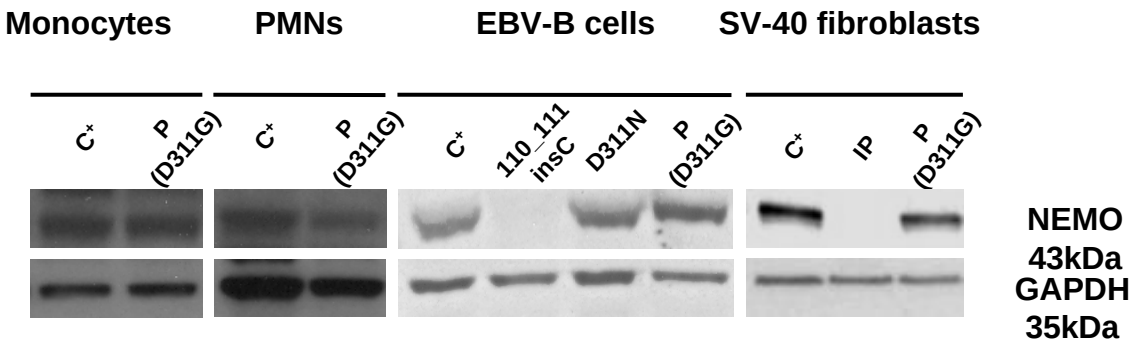
I. Genome Scan
II. Fine Mapping
III. Exome sequencing
IV. Candidate Gene

Novel mutation in *NEMO*

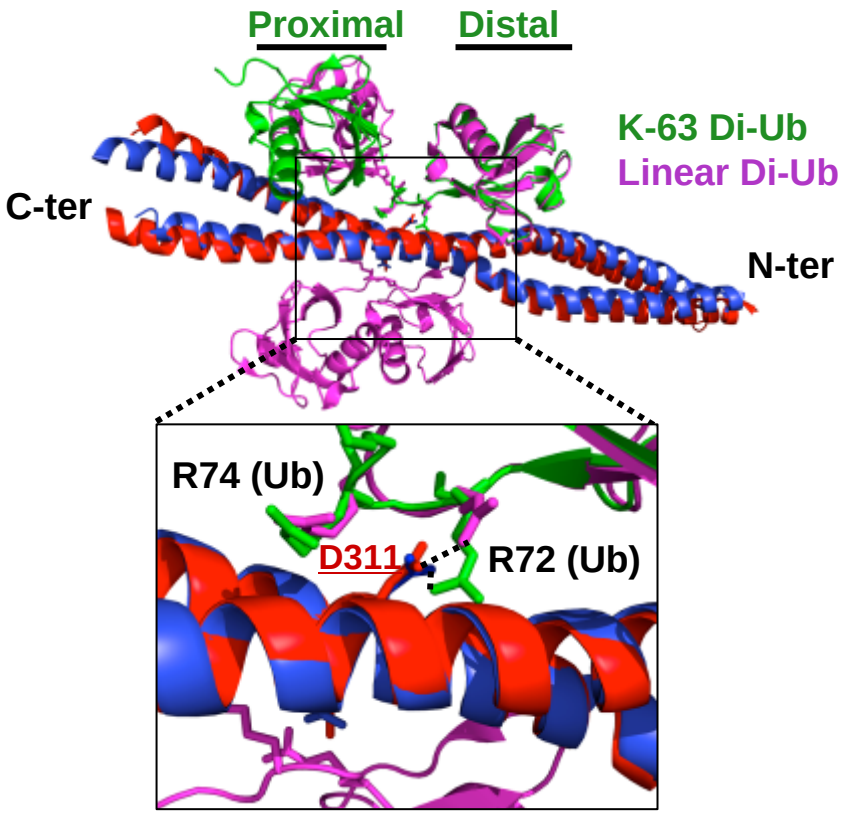


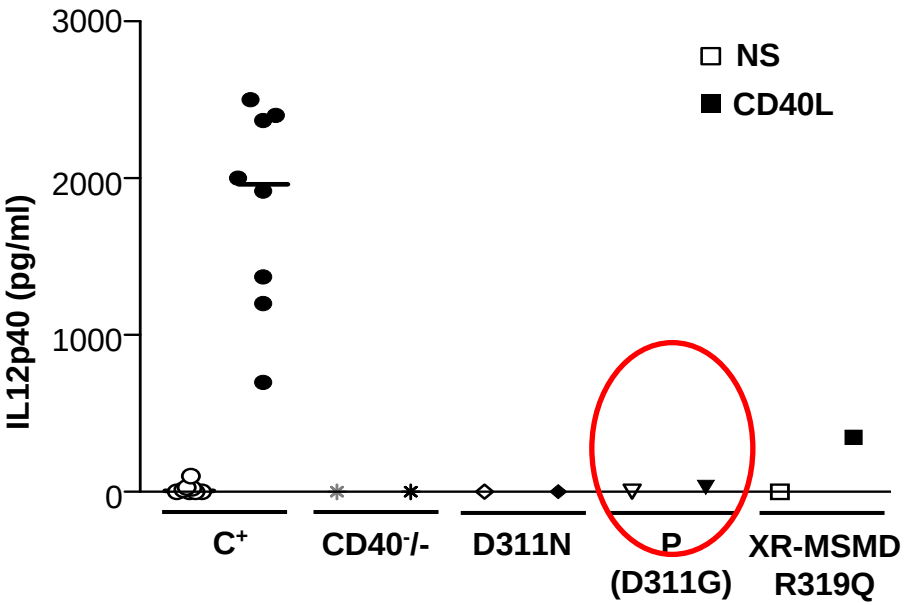
P (D311G)

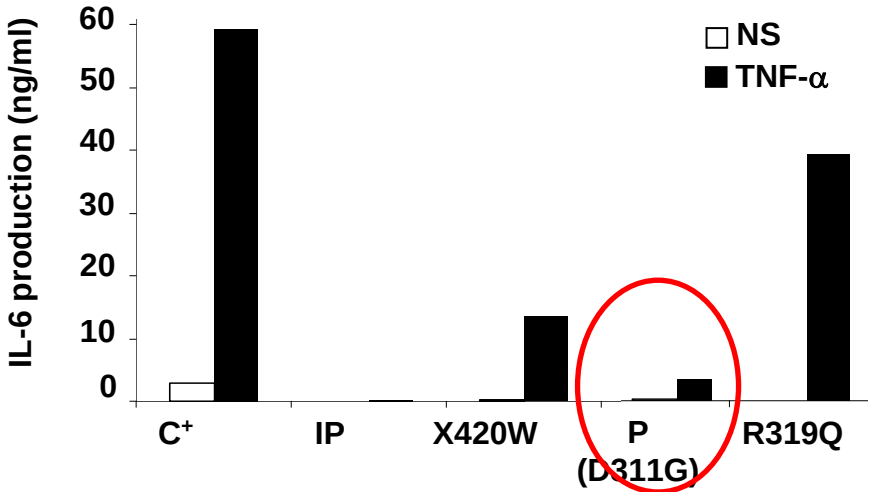




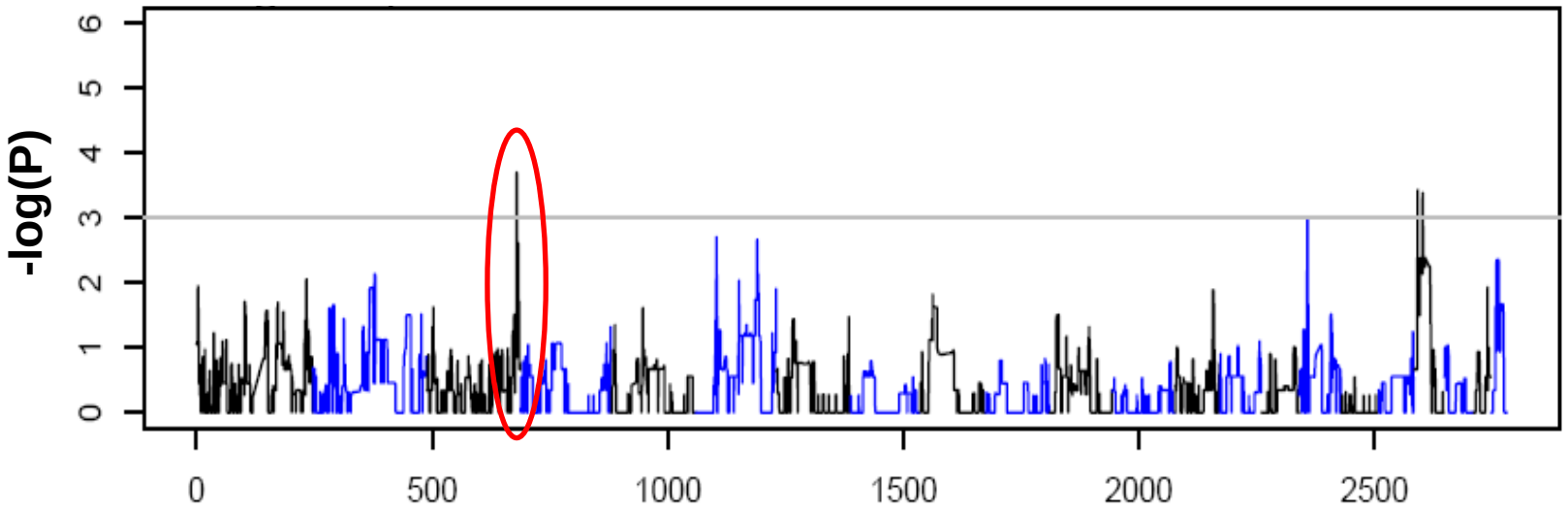
Impairment of ubiquitin binding of NEMO

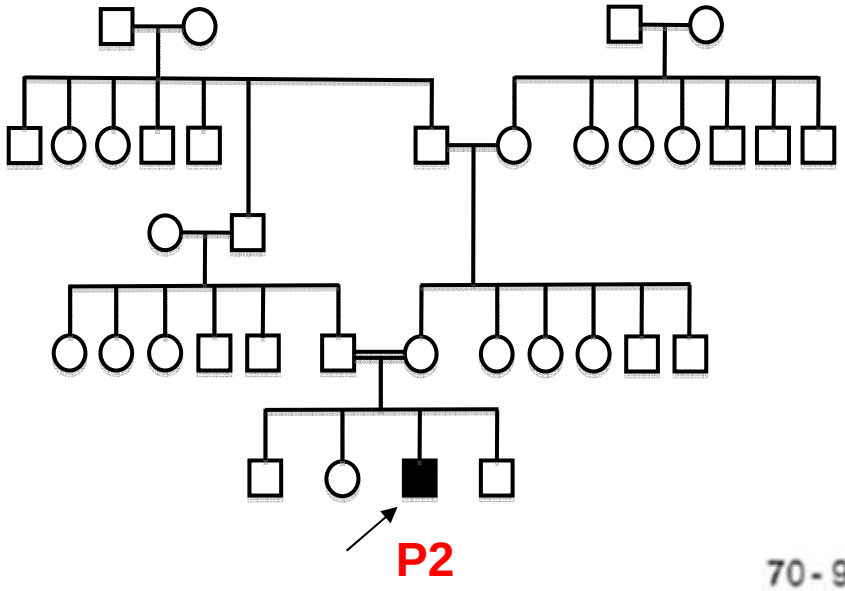


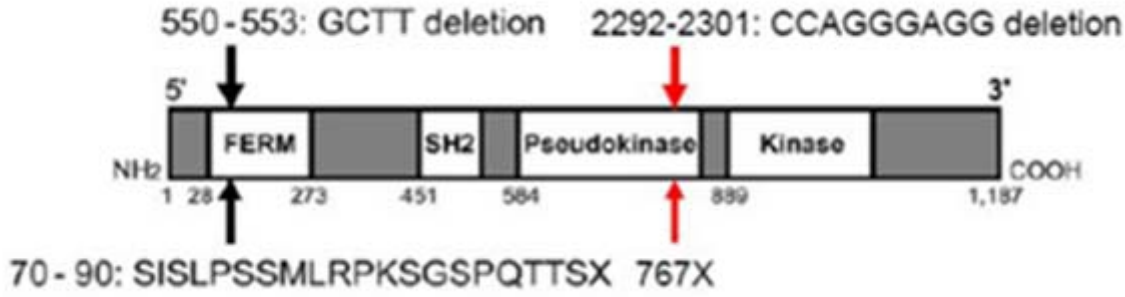




Positional Cloning: autosomal recessive





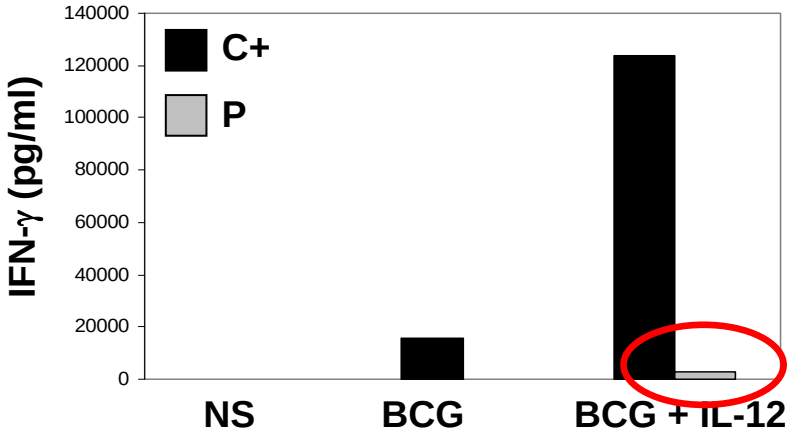


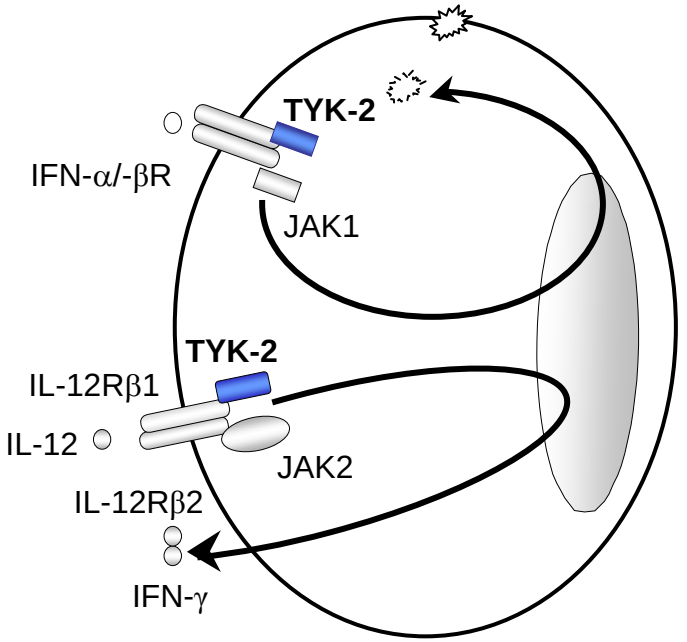
P1 **P2**

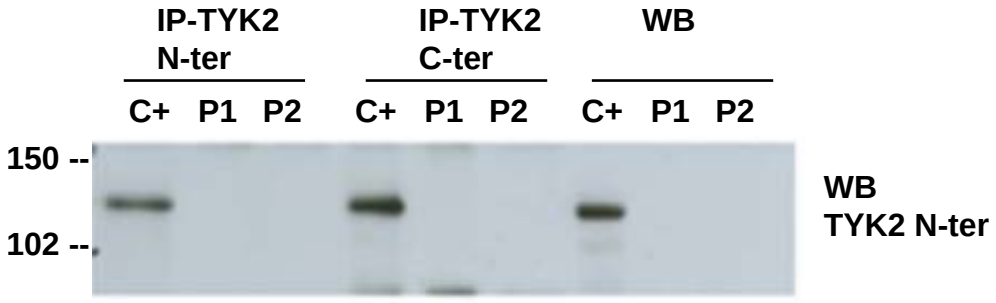
Tyk2 deficiency

Clinical features

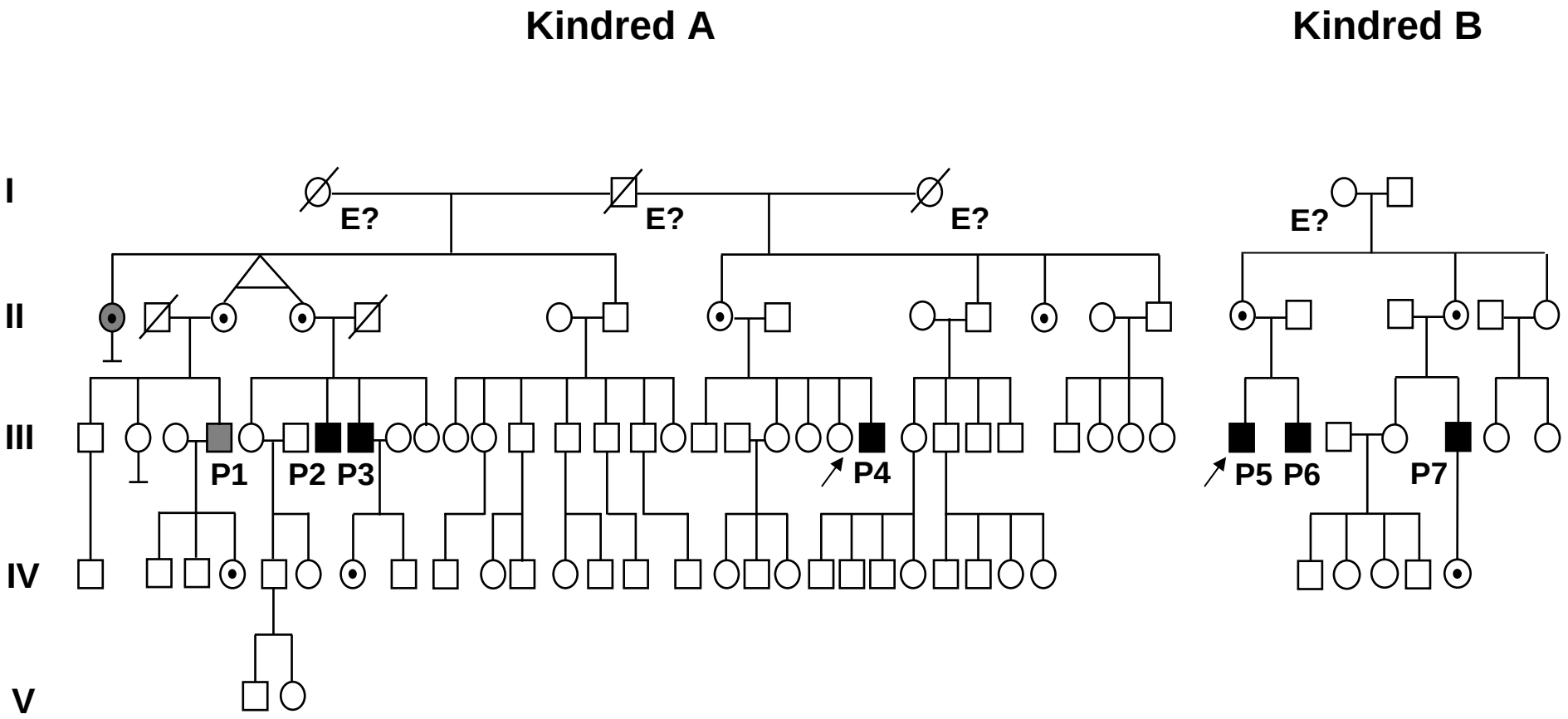
- **BCG-osis**
- **Cutaneous herpes simplex virus infections**







A novel form of XR- MSMD



Case reports – Kindred A

P1
Born 1953
No BCG vaccination
10 yo: pulmonary TB
34 yo: disseminated TB
Now 57 yo and well
off all treatment

P2
Born 1950
BCG inoculation at birth
3 mo: BCG-itis
12 yo: BCG-itis
29 yo: BCG cervical adenitis
Now 60 yo and well
off all treatment

P3
Born 1955
BCG inoculation at birth
3 mo: BCG-itis
Now 55 yo and well
off all treatment

P4
Born 1974 (index case, 1998)
BCG inoculation at 2 years
3 mo: BCG-itis
21 yo: BCG abdominal adenitis
24 yo: BCG cervical adenitis
27 yo: BCG cervical adenitis
Now 36 yo and well
off all treatment

Case reports – Kindred B

P5
Born 1974
BCG inoculation at birth
3 mo: BCG-itis
Now 36 yo and well
off all treatment

P6
Born 1969
BCG inoculation at birth
6 mo: BCG-itis
4 yo: BCG-itis
39 yo: BCG-osis
Now 41 yo well off all treatment

P7
Born 1974
BCG inoculation at birth
3 mo: BCG-itis
11 yo: BCG-osis
17 yo: BCG-itis
Now 36 yo and well
off all treatment

Cellular phenotype

Known MSMD etiologies:

AD and AR: *IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2*, *STAT1*

XR: *NEMO* (gene structure and function)

IL-12 and IFN-γ loops

…

Known XR primary immunodeficiencies:

XR WAS (platelets, skin, Ig)

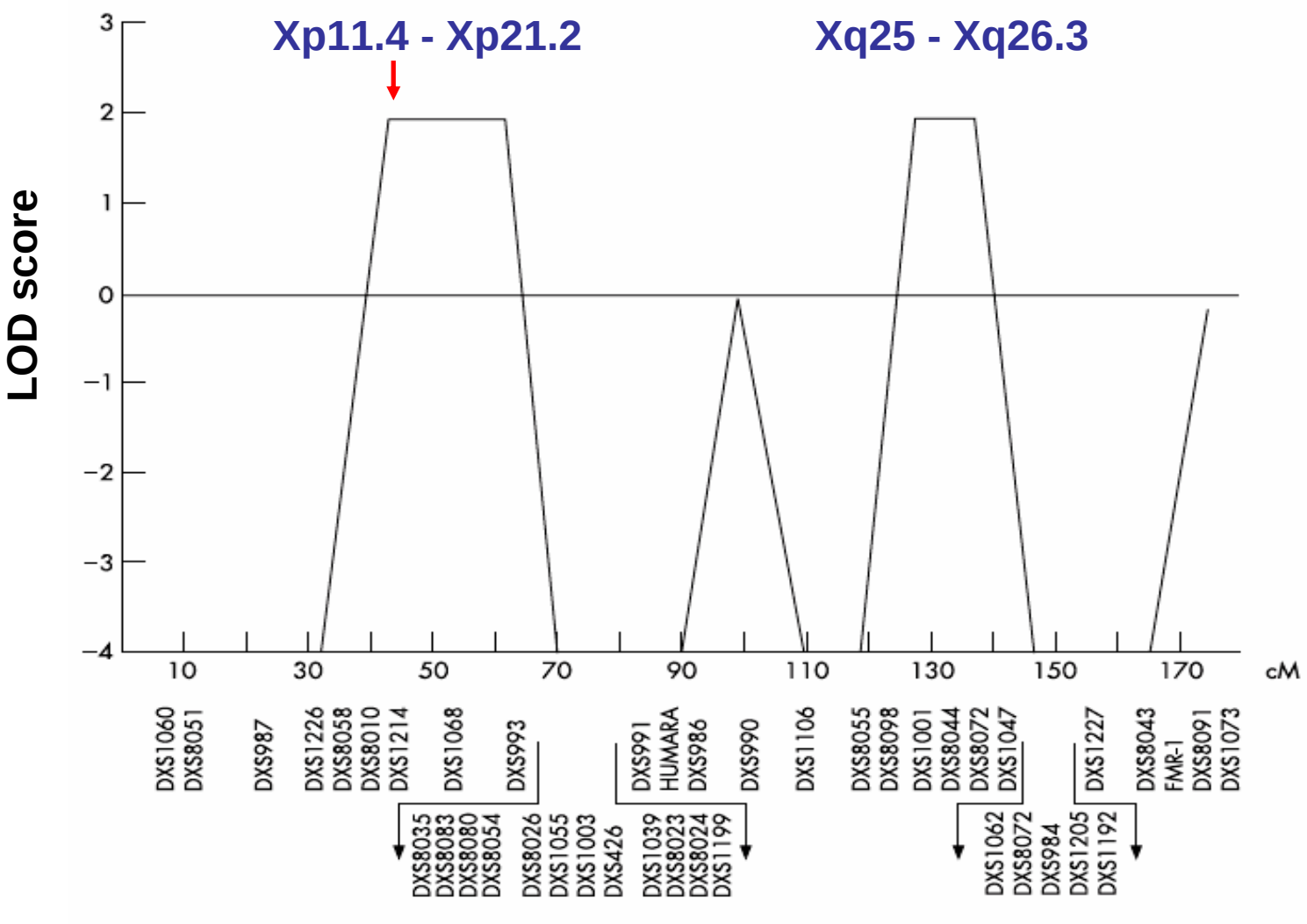
XR (S)CID (T cell number and function)

XR CGD (NBT, chemiluminescence)

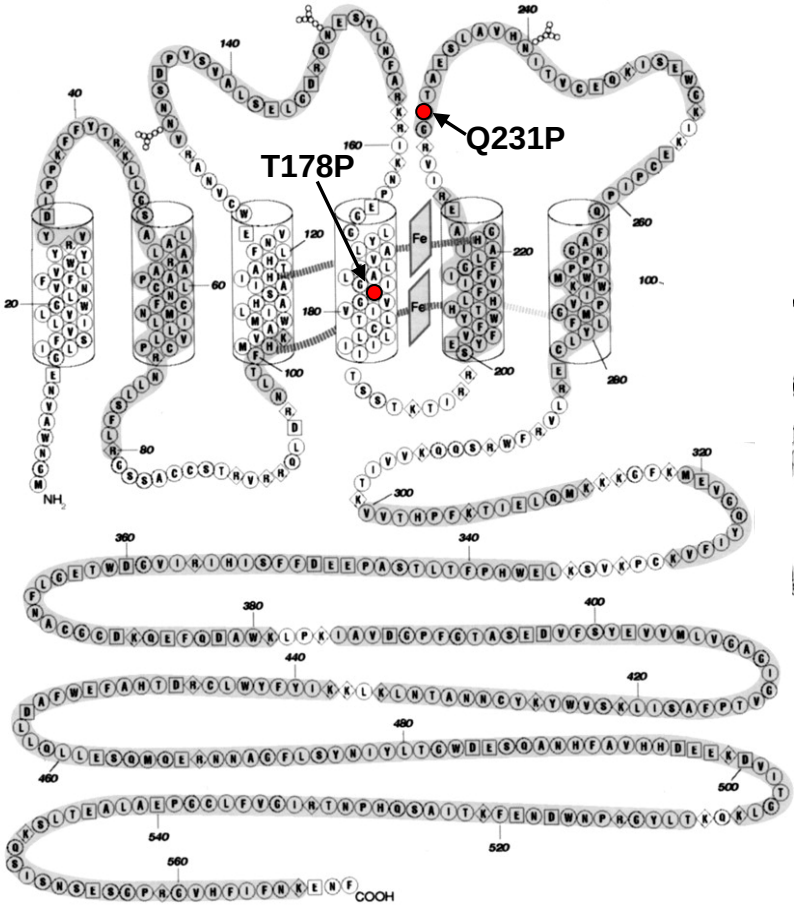
CD40L (expression)

…

Positional cloning: X-linked



Strict co-segregation of the *CYBB* (gp91^{phox}, NOX2) mutation



Homo sapiens	RIVRG**Q**TAESLAVHNITVCEQKISEWG-KIKECPIP
Mus musculus	RIVRG**Q**TAESLEEHNLDICADKIEEWG-KIKECPVP
Rattus norvegicus	RIVRG**Q**TSDSLKEHNLDVCADKIKEWG-KIKECPVP
Bos Taurus	RIVRG**Q**TAESLLKHQPRNCYQNISQWG-KIENCPIP
Sus scrofa	RIVRR**Q**TPKSLLVHDPKACAQNISQWG-KIKDCPIP
Oryctolagus cuniculus	RIVRG**Q**TEESLKKHDPVMCEQHISDWG-KIKDCPVP
Xenopus tropicalis	KIVRG**Q**TDKSLEKHNSTECEDKFTEWG-NITSCPIP
Danio rerio	RIVRG**Q**TDADLQVHDPTICHSKFEKWGQNVTDCPVP
Takifugu_rubripe	---RG**Q**TPASLKSNDPTVCADQFEDWGRNGSNCAVP

CYBB (226-260)

Homo sapiens	EGGLYLAVTLLAGI**T**GVVITLCLILII
Mus musculus	EGGLYVAVTRLAGI**T**GIVITLCLILII
Rattus norvegicus	EGGLYVAVTRLAGI**T**GIVITLCLILII
Bos Taurus	EGGLYVAVTRLAGI**T**GVVITLCLILII
Sus scrofa	EGGLYVAVTRLAGI**T**GVVITLCLILII
Oryctolagus cuniculus	EGGLLVAVTRLAGV**T**GIIITLCLILII
Xenopus tropicalis	IGGINVAFTFLAGL**T**GVVITLALILII
Danio rerio	TNPTIVMFTTVAGL**T**GVVITLALILII
Gallus gallus	VGGLYVAFTYLAGL**T**GVIITLALILII
Canis lupus familiaris	EGGLYVAVTLLAGI**T**GIVITLCLILII

CYBB (164-190)

These missense mutations are not SNPs, with 1,300 X chromosomes sequenced

They are not known CGD mutations

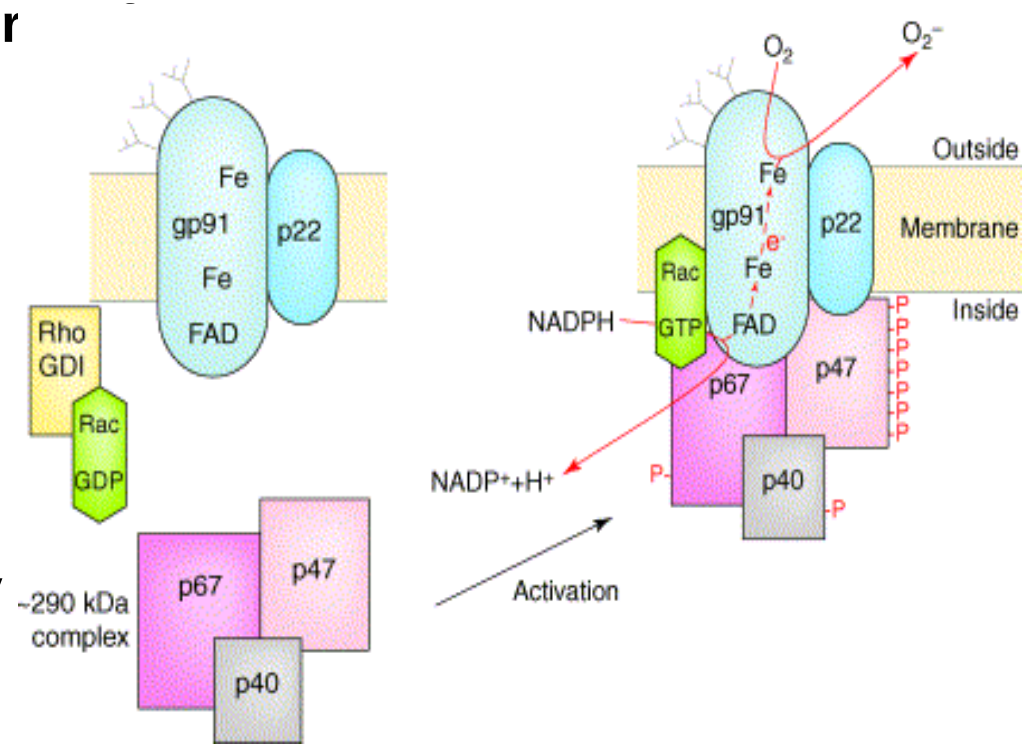
CGD and the phagocyte NADPH oxidase

CYBB/gp91phox **most frequent for**

Early-onset, severe, recurrent,
& multiple bacterial
& fungal infections

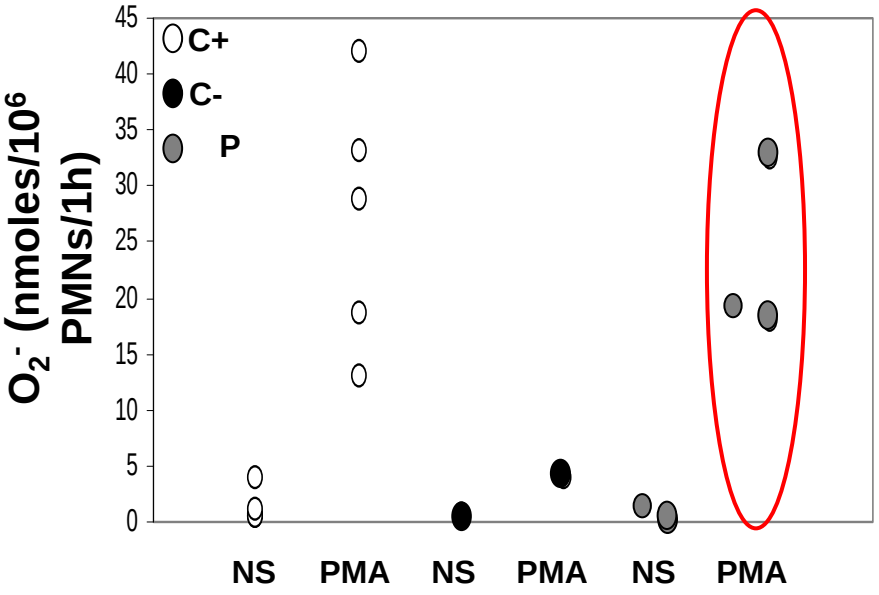
Also vulnerable to BCG & TB
in endemic regions

Defect of respiratory burst activity
in all phagocytic cells
(granulocytes, monocytes &
macrophages)

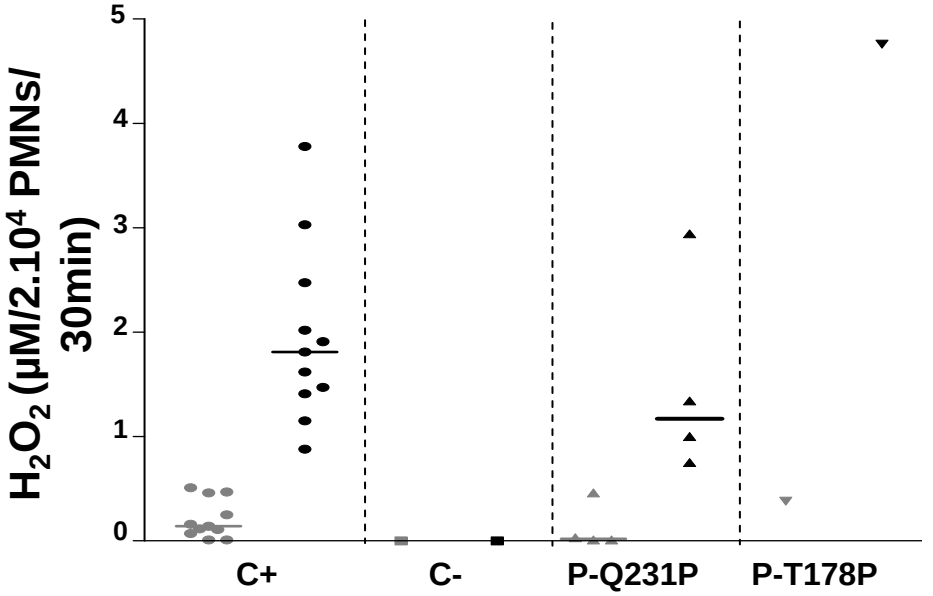


Our patients: seven otherwise healthy adults with a pure MSMD phenotype

The patients' PMNs produce O_2^- and H_2O_2

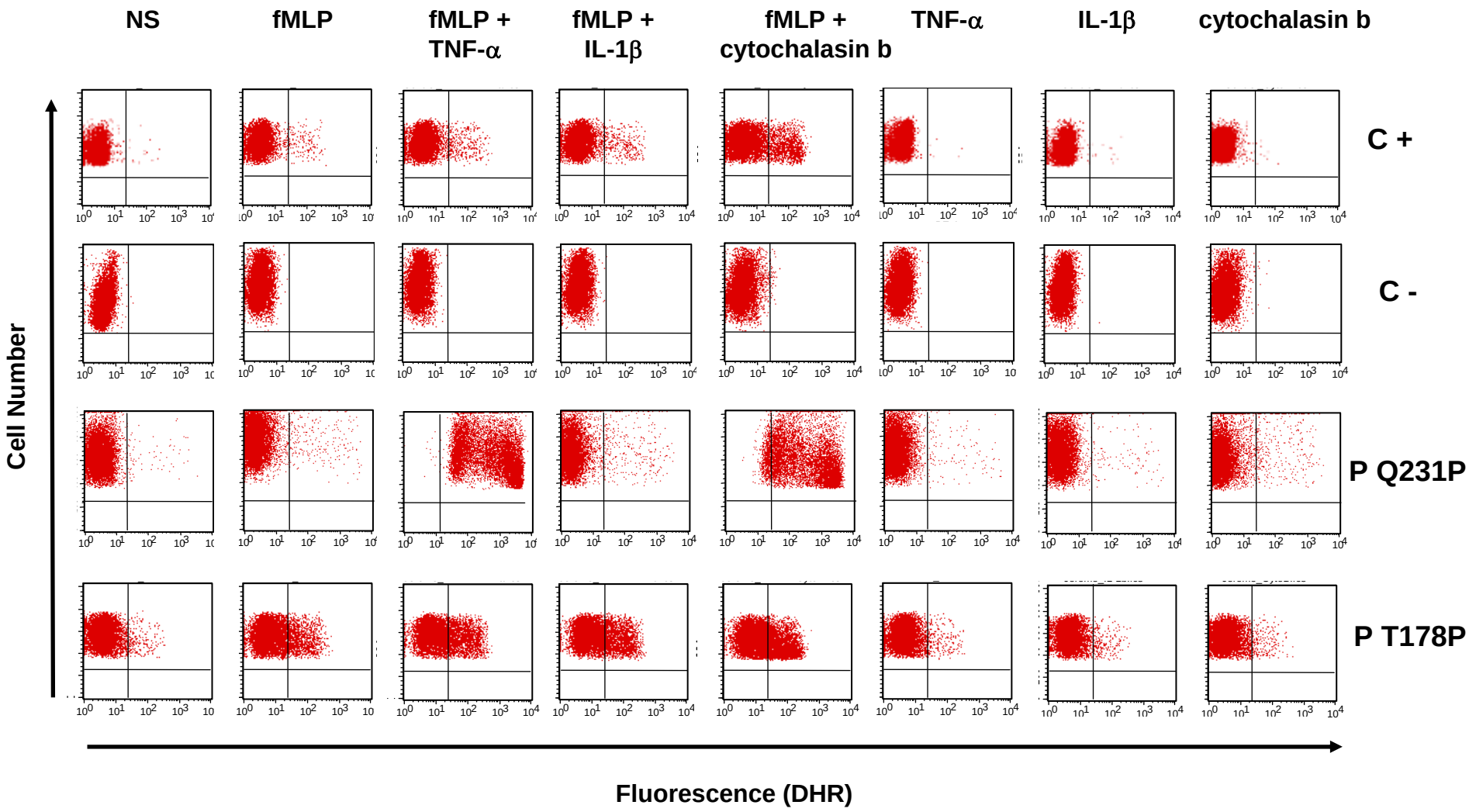


Cytochrome-*c*

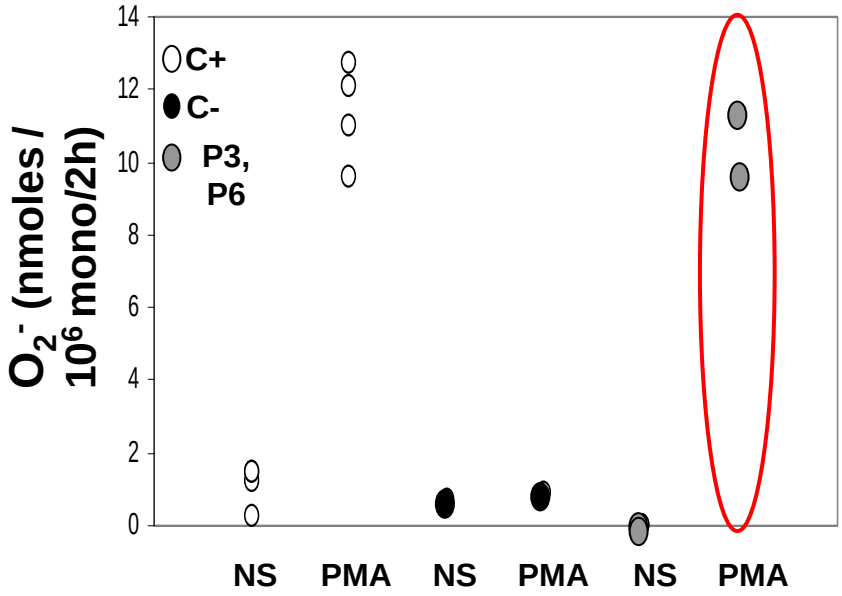


Resorufin

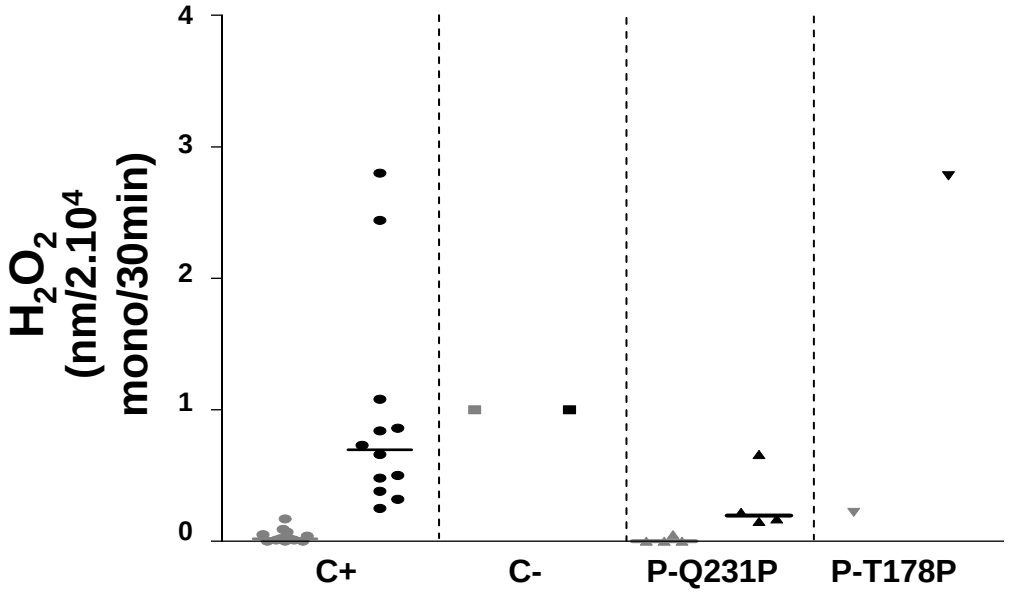
The PMNs respond to physiological stimuli



The patients' monocytes produce O_2^- and H_2O_2

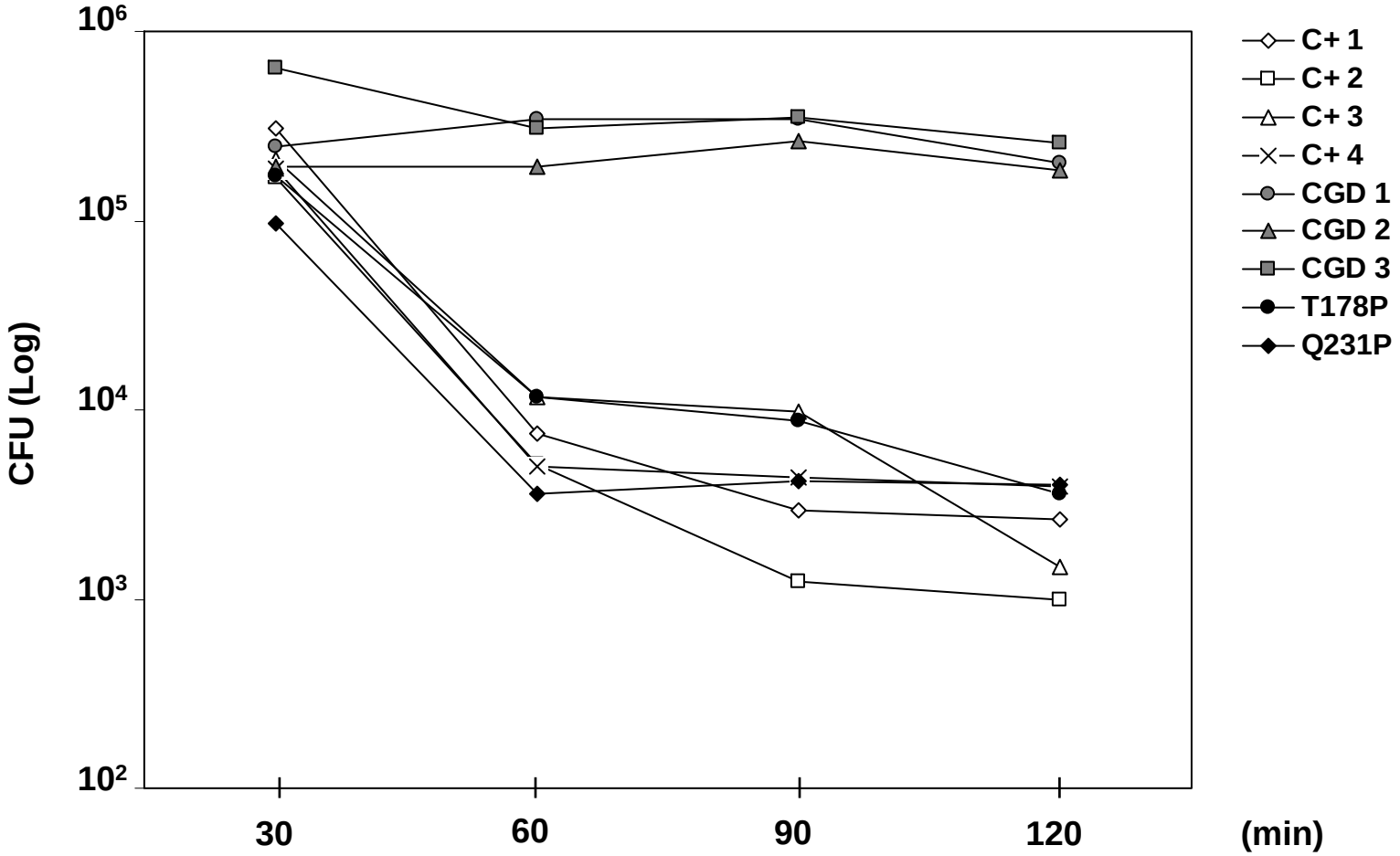


Cytochrome-*c*

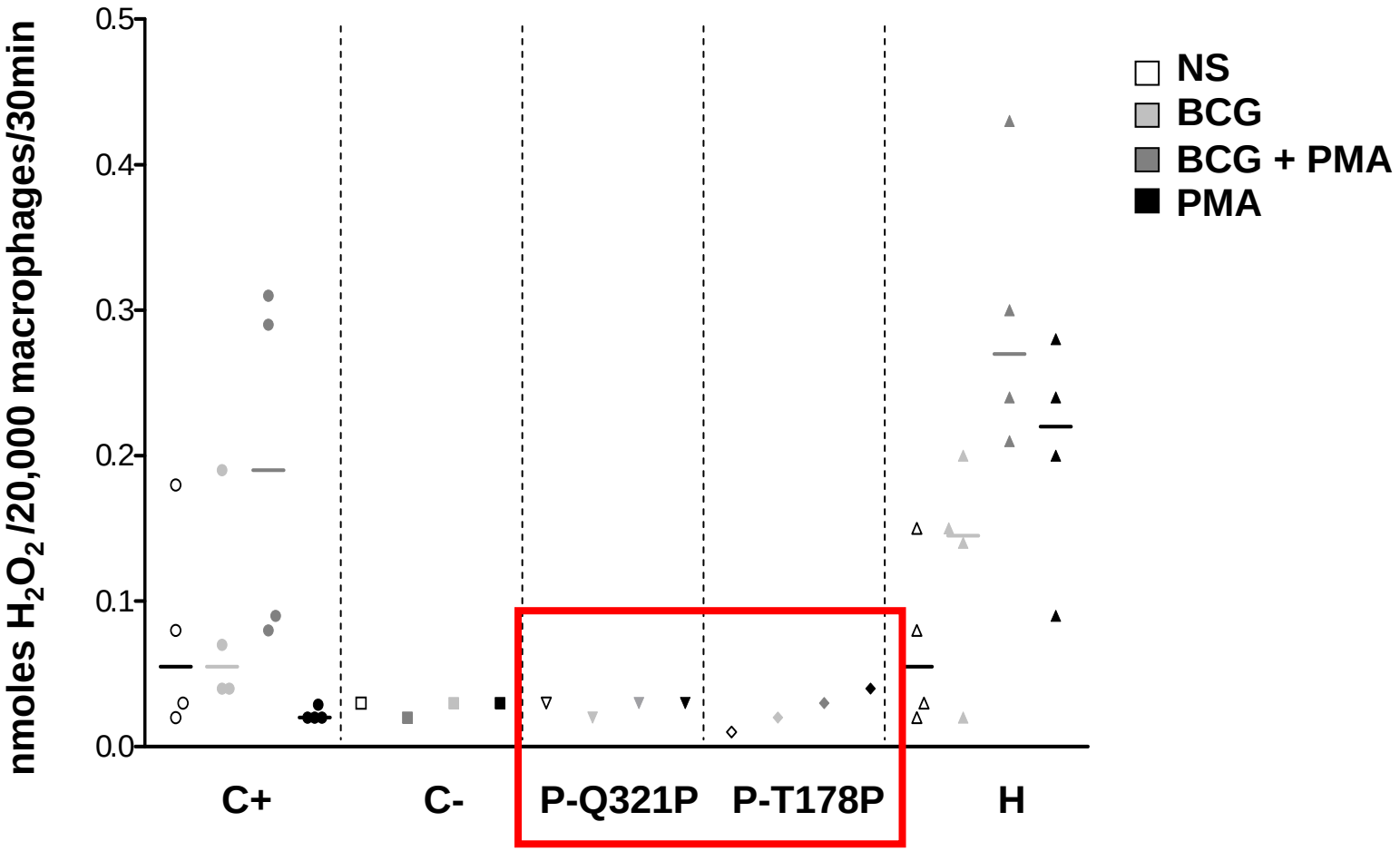


Resorufin

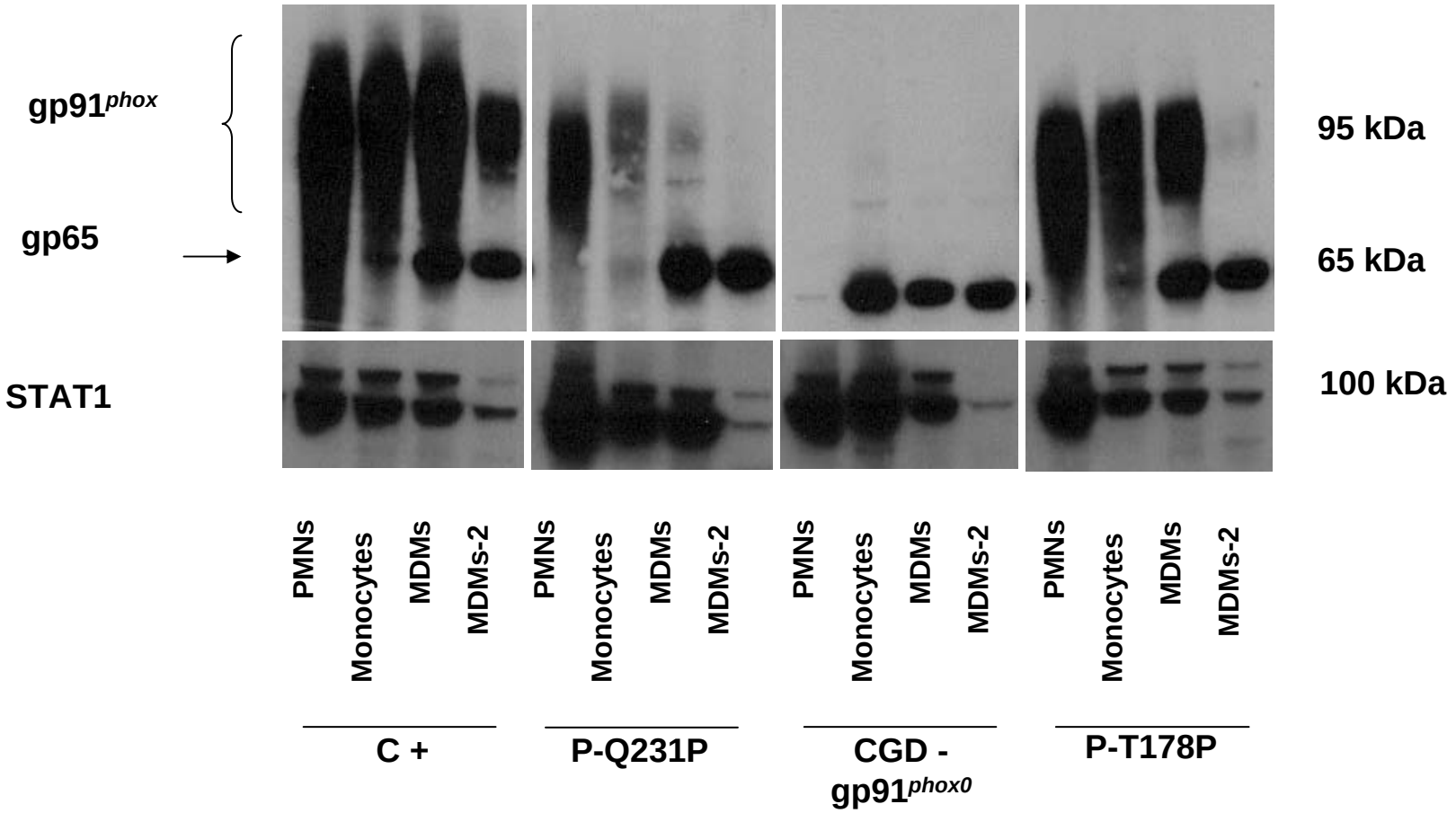
Normal killing of *S. aureus* by neutrophils



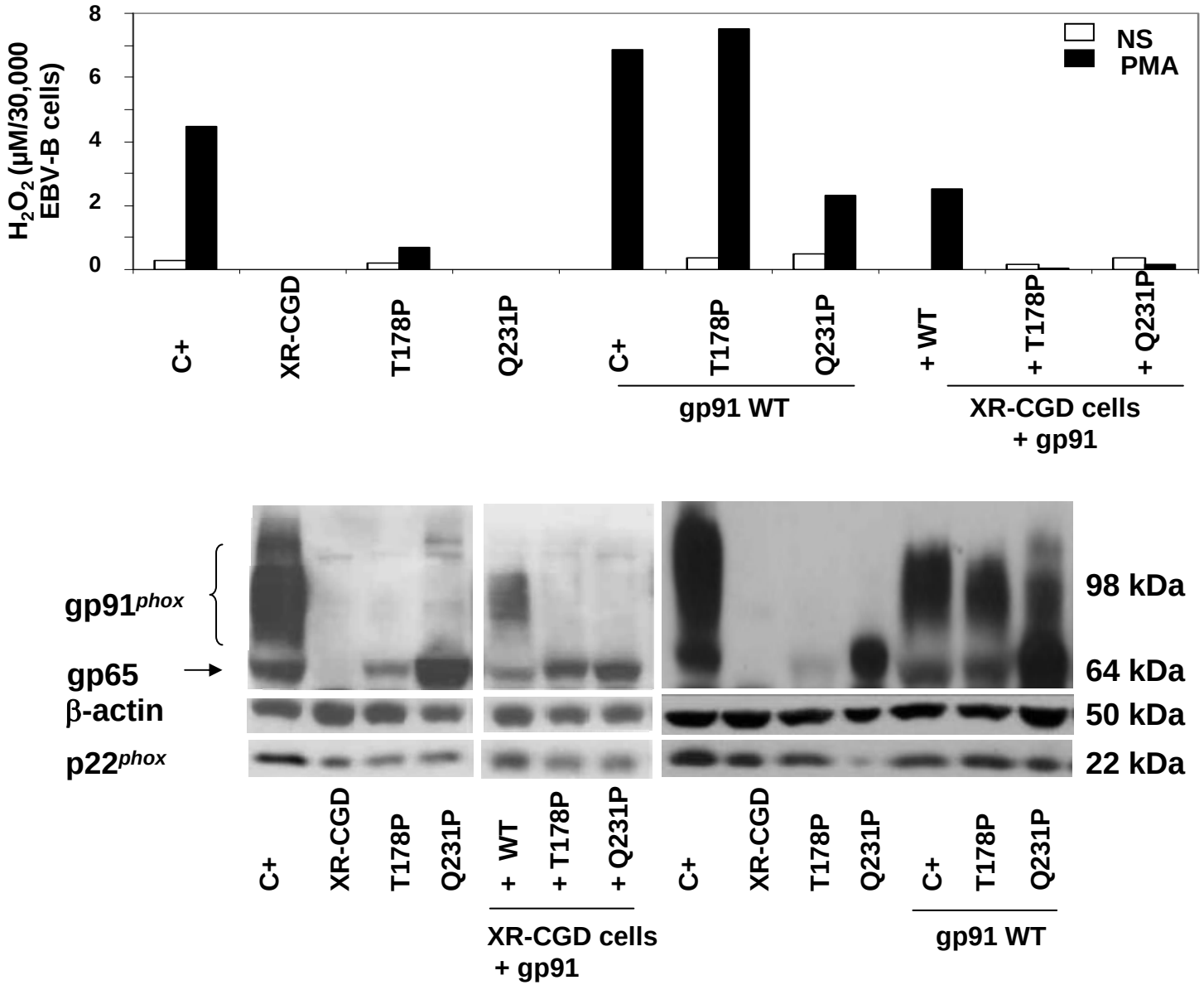
The patients' macrophages do not produce H_2O_2



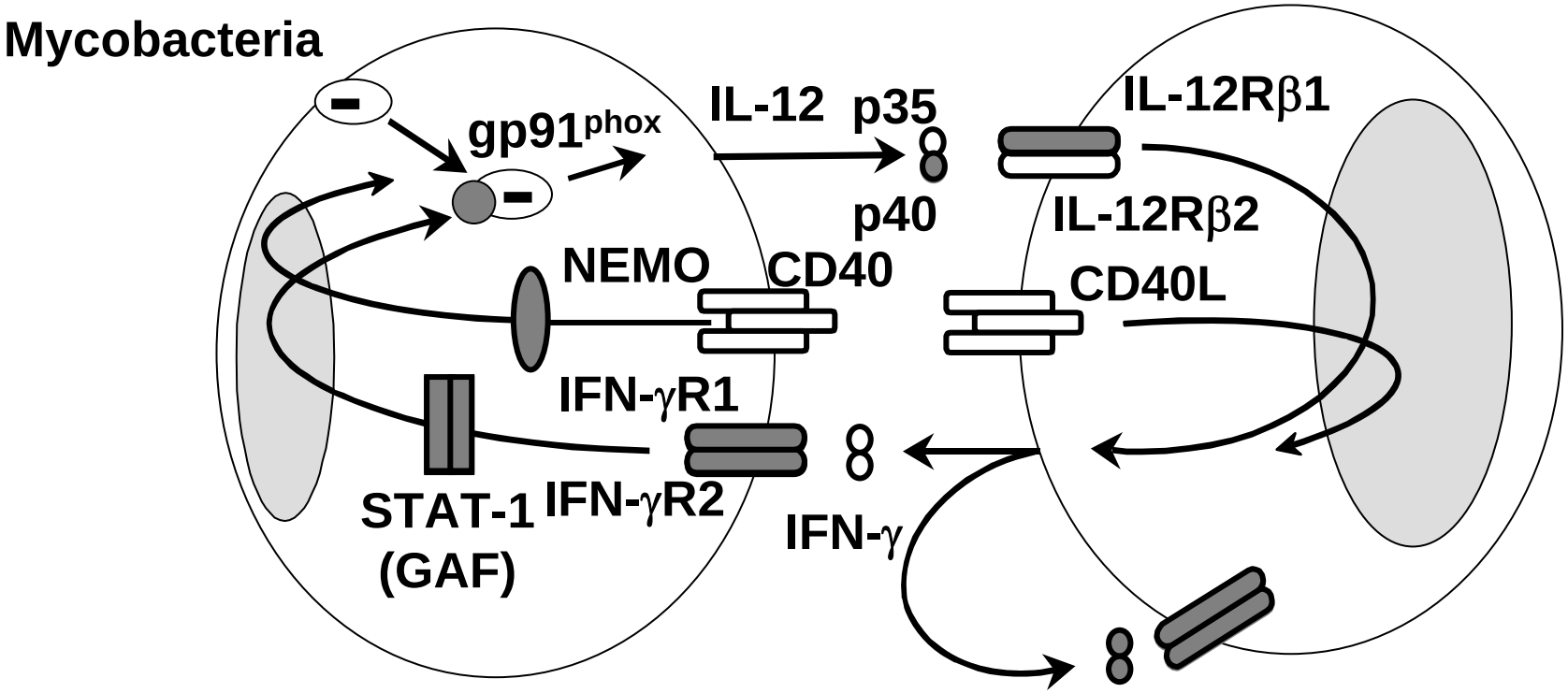
Impaired expression of gp91*phox*



The Q231P and T178P alleles are null in EBV-B cells



Connexion between CYBB and IL-12-IFN-γ ?



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1901

Collaborators, children and their families