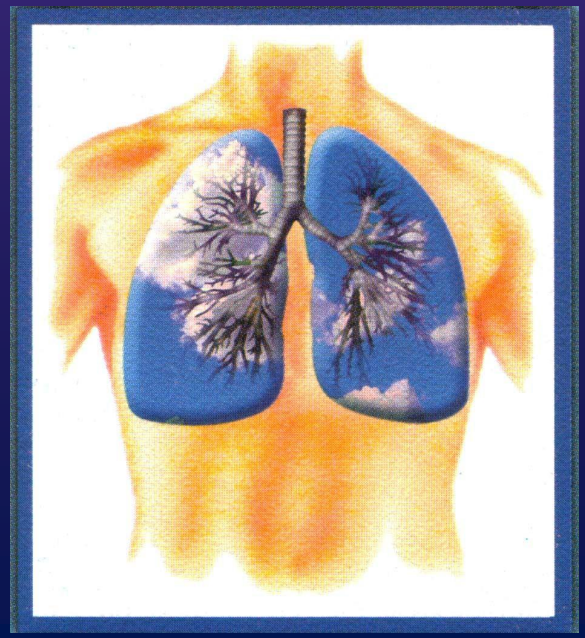
Tomsk Research Institute of Medical Genetics
Siberian Brunch of Russian Academy for Medical Science

# Genetics of tuberculosis susceptibility in Siberian populations

**Rudko Alexey**



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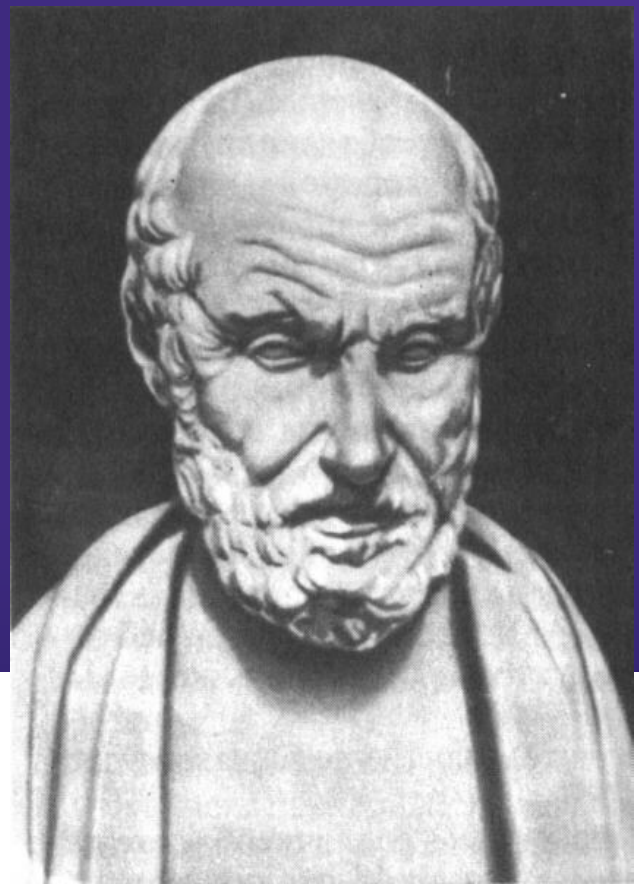
# Tuberculosis is a world-wide health problem

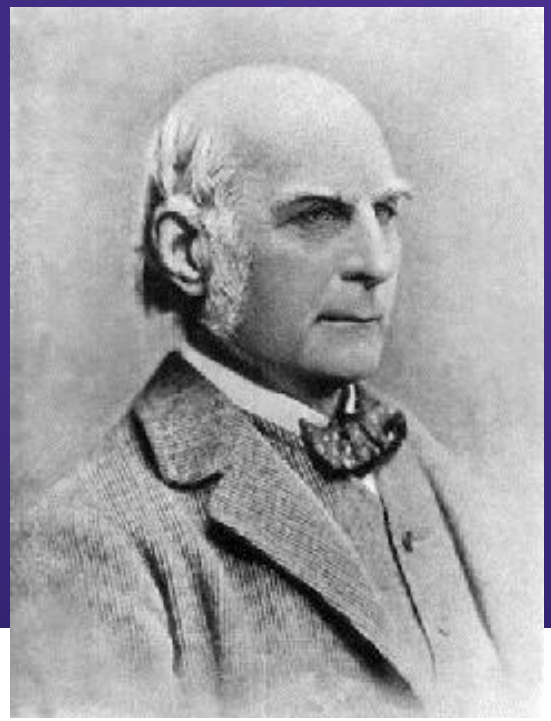
- From 1850 y. to 1950 y. there are 2 billion peoples have died from tuberculosis.
- Now every year is about 2 million deaths are becoming from this infection.
- 10% of TB cases are occured on the base of HIV infection.



## *Hippocratic*

**(460-377** yy.b.c.**)**

«Some peoples are more resistance to diseases, others absolutely cannot withstand to. As children with epilepsy are born from epileptic parents, simply the children predisposing to tuberculosis are born from consumptive».



## *Francis Galton*

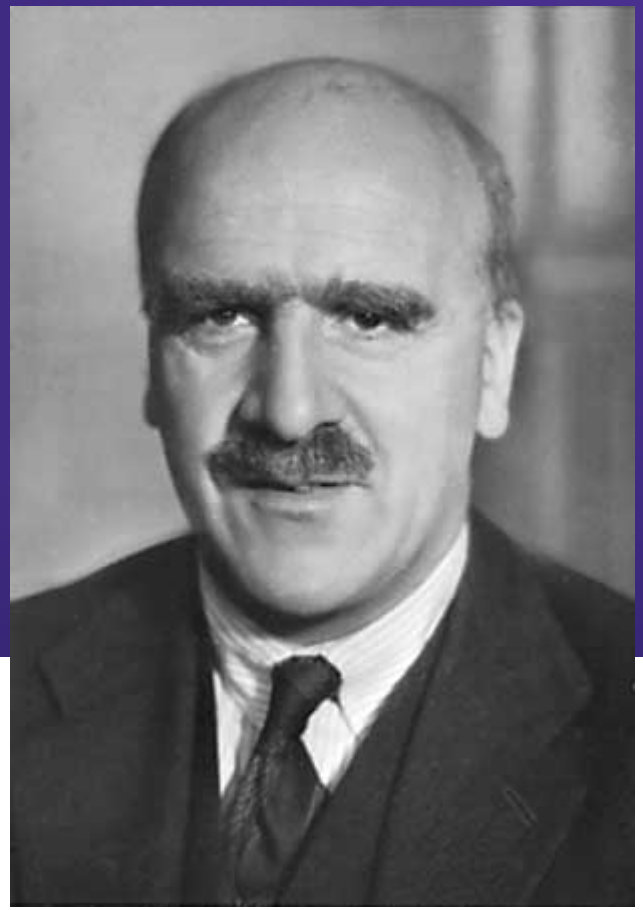
## (1822-1911)

«Not only capacities and talent are inheriting, but also another psychic and biologic properties such as tendency to drunkenness, vagrancy, **tuberculosis**, cardiac diseases and longevity, as well as morale and religion». (1870).

# *Solomon Levit*

(1894–1938)

Levit S. organized spacious medical-genetics research  of different diseases in Soviet Russia used of different approaches, including studying of twines. Studying of inherited factors influence to development of tuberculosis he performed in collaboration with Shtefko V.G. (Central Research Institute of Tuberculosis).



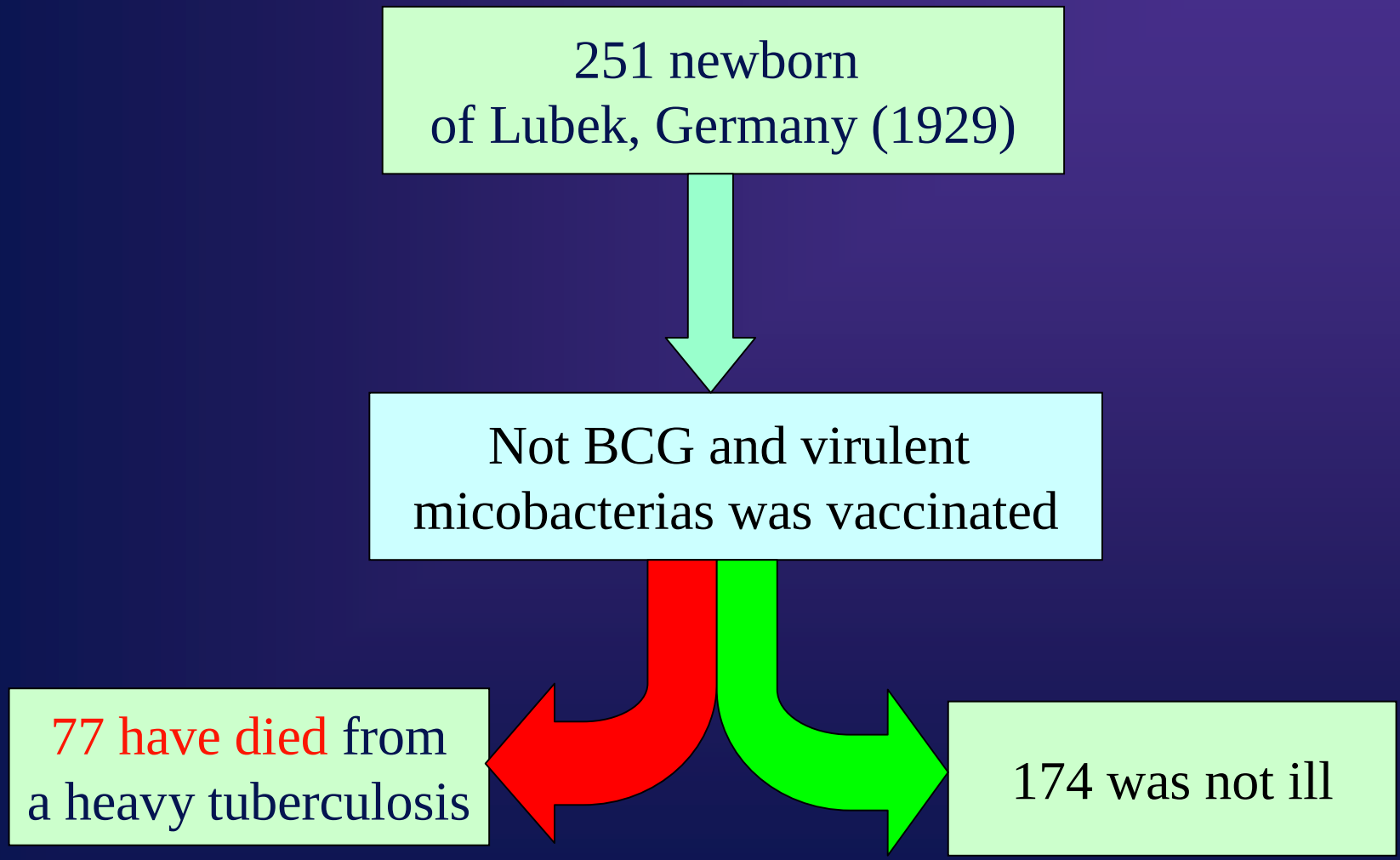
## John .B.S. Haldane
## (1892-1964)

J. Haldane proposed that the majority of the genetic diversity found within human populations has been selected for and maintained by infectious diseases. It has been claimed that *Mycobacterium tuberculosis* was responsible for one-fifth of all deaths occurring in western Europe after the industrial revolution. Therefore it is likely that *M. tuberculosis* has had a major effect on the evolution of the human genome (1949).

# Tuberculosis is a multifactorial disease

- **Social and environmental factors: poverty, bed nutrition, over-population, migrations.**
- **Micobacterium: potogenity and drug resistance.**
- **Host condition: adequate immune defense dependent on human genome.**
- **HIV-infection: is about 8-10% of tuberculosis is associated with AIDS.**

# Proof of genetic components influence



# Family-based study of tuberculosis

- Accumulation of TB at separetad relatives
- Studying of twines

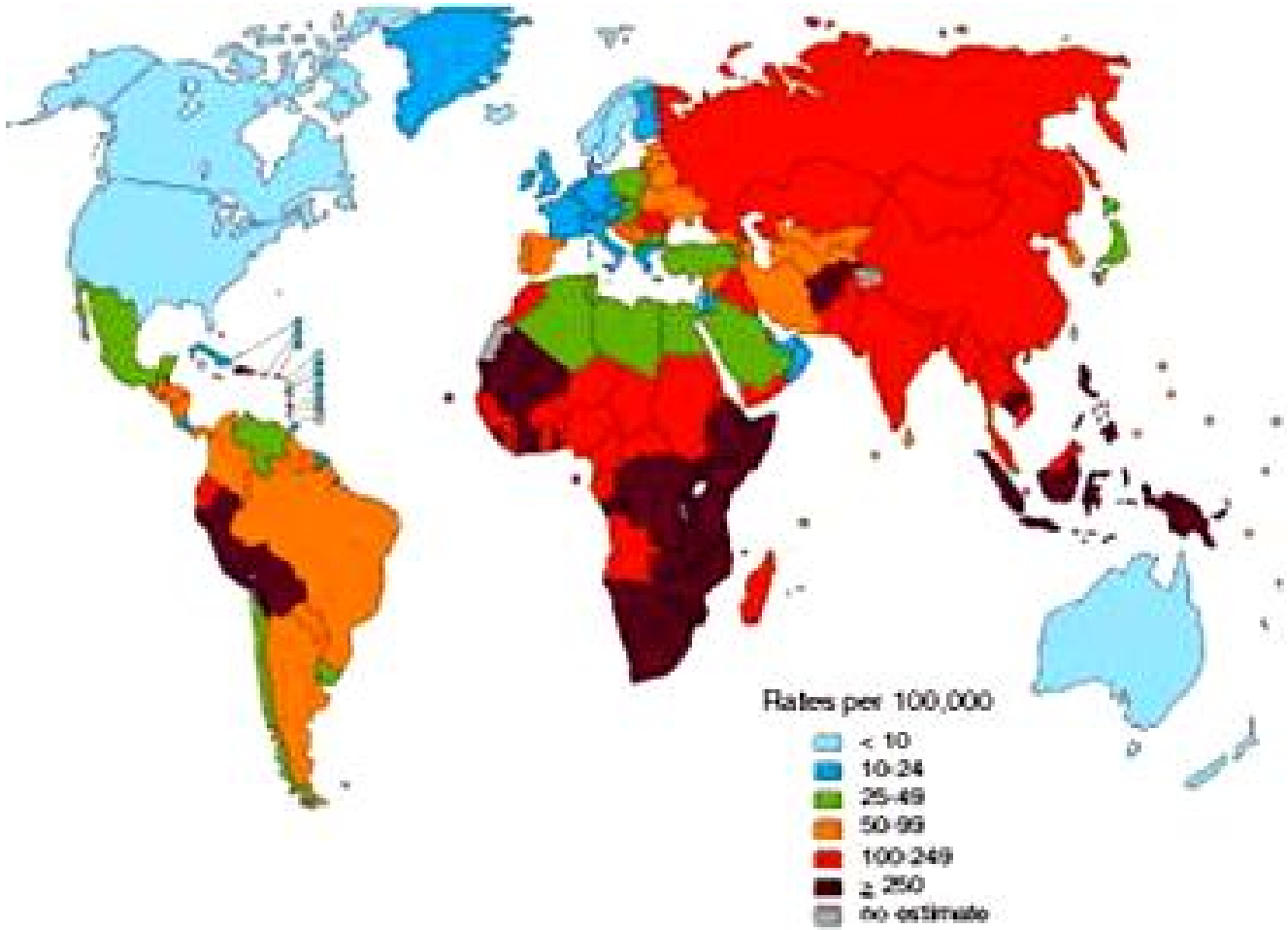
| Country | Concordance | |
|---|---|---|
| | MZ (%) | DZ(%) |
| Germany | 65 | 25 |
| USA | 62 | 18 |
| Grate Britain | 32 | 14 |

Bellamy R., 2001

# Evidence of involvement of inherited factors to TB development at man

## Clinicoepidemiologyc studying

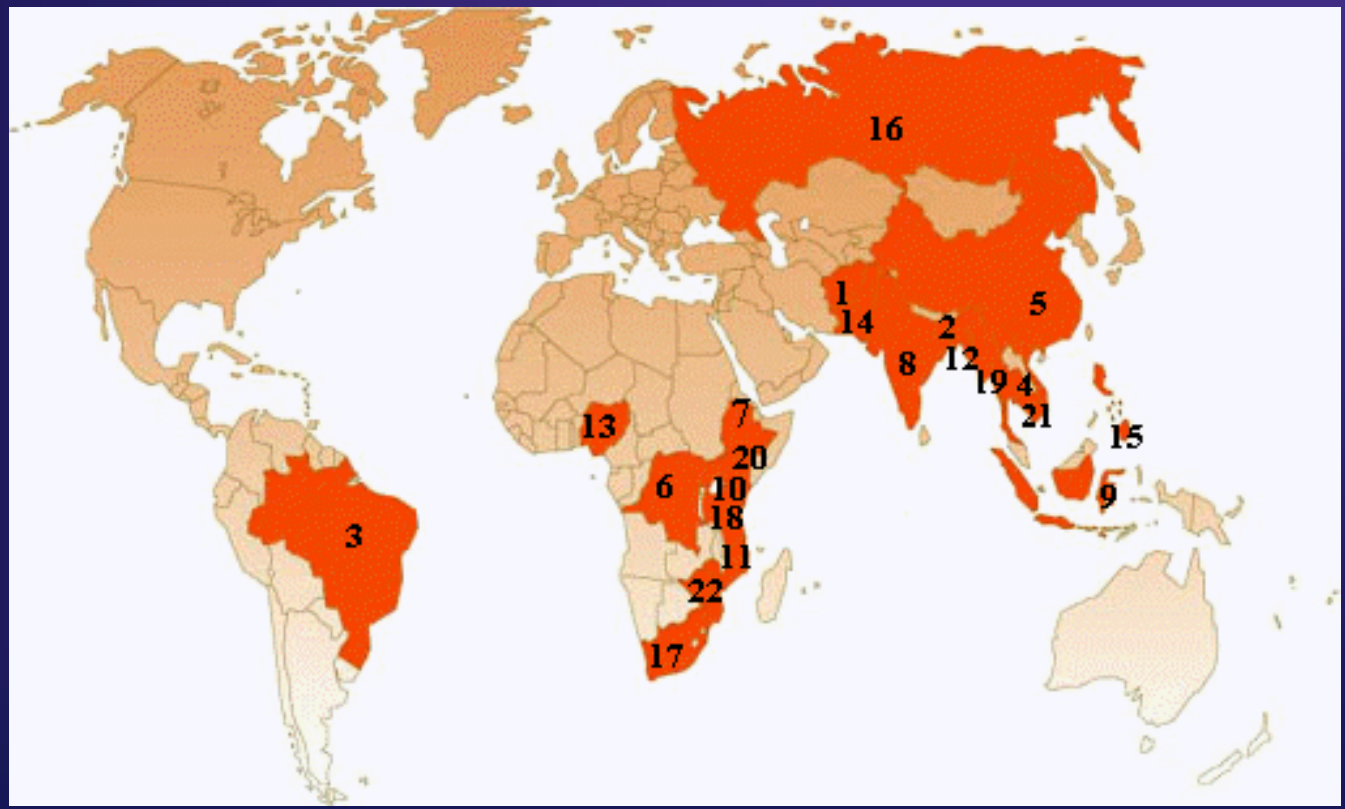
► ***Different prevalence of TB in different races and populations***



# 22 countries - 80% of all tuberculosis patients in the world

**Burden ranking**

1. India
2. China
3. Indonesia
4. Nigeria
5. South Africa
6. Bangladesh
7. Pakistan
8. Ethiopia
9. Philippines
10. Kenya
11. Congo
12. Russian Federation
13. Viet Nam
14. Tanzania
15. Uganda
16. Brazil
17. Afghanistan
18. Thailand
19. Mozambique
20. Zimbabwe
21. Myanmar
22. Cambodia



# TB-morbidity in Russia

| region | Rate of new TB cases | |
|---|---|---|
| | 2000 y. | 2001 y. |
| **Russion Fedration** | **90,7** | **88,2** |
| **Siberian Federal County** | **126,4** | **127,8** |
| Altai Republic | 92,1 | 96,2 |
| Altai region | 137,8 | 140,1 |
| Buryat Republic | 140,9 | 132,1 |
| **Tiva Republic** | **322,9** | **348,3** |
| Khakas Republic | 116,7 | 123,1 |
| Krasnoyarsk region | 100,2 | 102,7 |
| Irkutsk region | 131,8 | 143,0 |
| Kemerovo region | 149,0 | 130,8 |
| Novosibirsk region | 138,6 | 137,4 |
| Omsk region | 108,9 | 110,6 |

# Different approaches for identifying genes of predisposition to tuberculosis

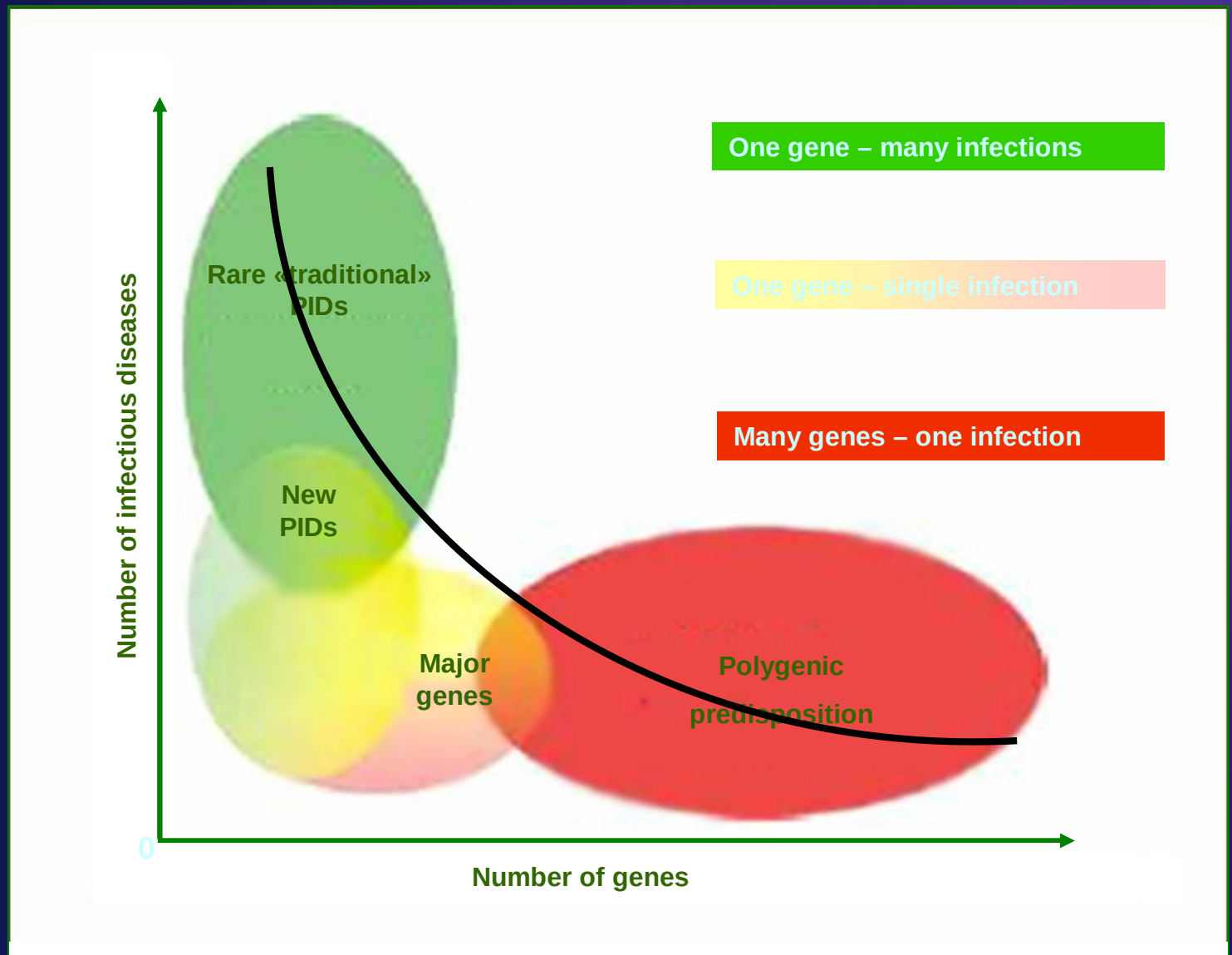
- Using of animal models.
- Studying of individuals with high susceptibility to nonpathogenic mycobacteries.
- Case-control study. Search of associations between polymorphic variants of candidate genes and tuberculosis.
- Genome-wide linkage studies.

*Bellamy R., 2006*

# Genetic loci involved in susceptibility to tuberculosis at man

| Локус | Описание |
|---|---|
| *HLA-DRB1* | HLA, класс II |
| *SLC11A1 (NRAMP1)* | Транспорт двухвалентных катионов |
| *INFG* | Цитокин интерферон-гамма |
| *VDR* | Рецептор витамина D |
| *SP110* | Фактор транскрипции |
| *IL8* | Цитокин интерлейкин-8 |
| *UBE3A* | Убиквитин лигаза |
| *MAL/TIRAP* | Адаптор TLR сигнального пути |
| *P2RX7* | Рецептор ATP |
| *IL10* | Цитокин интерлейкин-10 |
| *DC-SIGN* | Рецептор С-типа лектина дендритных клеток |
| *SP-A* | Белок сурфактанта |
| *CCL2* | Хемокин белок-1 хемоаттрактант моноцитов |
| *IL12RB1* | Рецептор цепи цитокина интерлейкина-12 |
| *INFGR1* | Рецептор цепи цитокина интерферона-гамма |
| *CR1* | Рецептор комплемента, CD35 |
| *TLR2* | Toll-подобный рецептор-2 |
| *MBL2* | Маннозо-связывающий лектин |

*(Hill A.V.S., 2006)*




# Spectrum of genes of susceptibility to infectious diseases

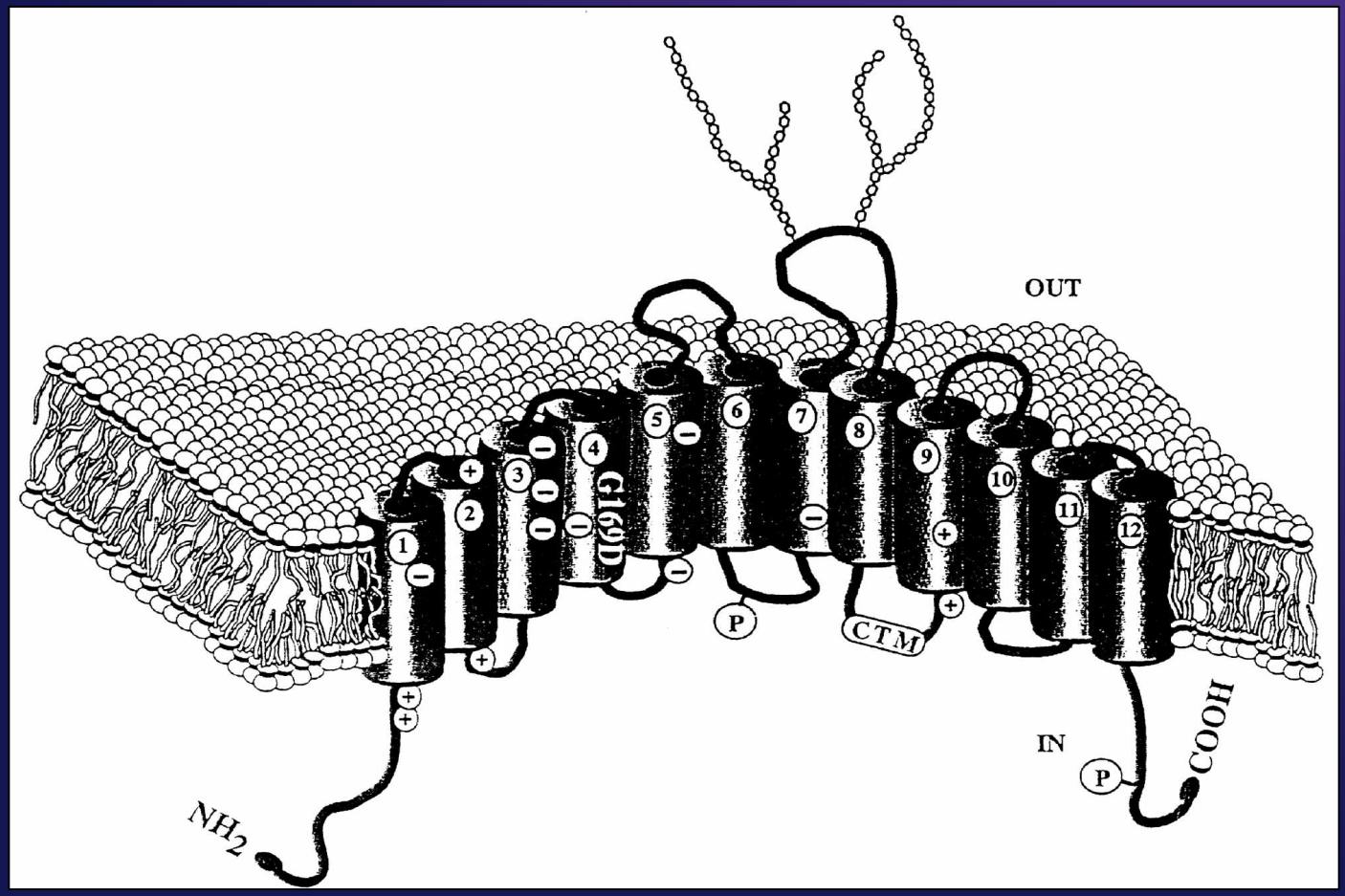
***(Casanova u Abel, 2007)***

***PIDs – Primery immunodeficites**

# *NRAMP1 (SLC11A1)* gene.

- 1974 y. – variable growth of *Leishmania donovani* in inbred strains of mice, terminated by *Lsh* locus.
- 1982 y. – susceptibility of mice to *Leishmania donovani*, *Salmonella typhimurium*, *Mycobacterium bovis* controlled by 1 locus (***Bcg/Lsh/Ity*** ), on the 1-st chromosome.
- 1992 y. – the *Nramp1* gene (Natural resistance associated macrophage protein 1) was mapped in mice by positioning cloning in locus ***Bcg/Lsh/Ity.***
- 1994 y. – human homologous gene of *Nramp1* was mapped on 2q35. It was named *NRAMP1 (SLC11A1).*
- *1996 y.* – the mutation at mice *Nramp1* gene was detected (G169D), which cause the susceptibility of mice to *Leishmania donovani*, *Salmonella typhimurium*, *Mycobacterium bovis* .

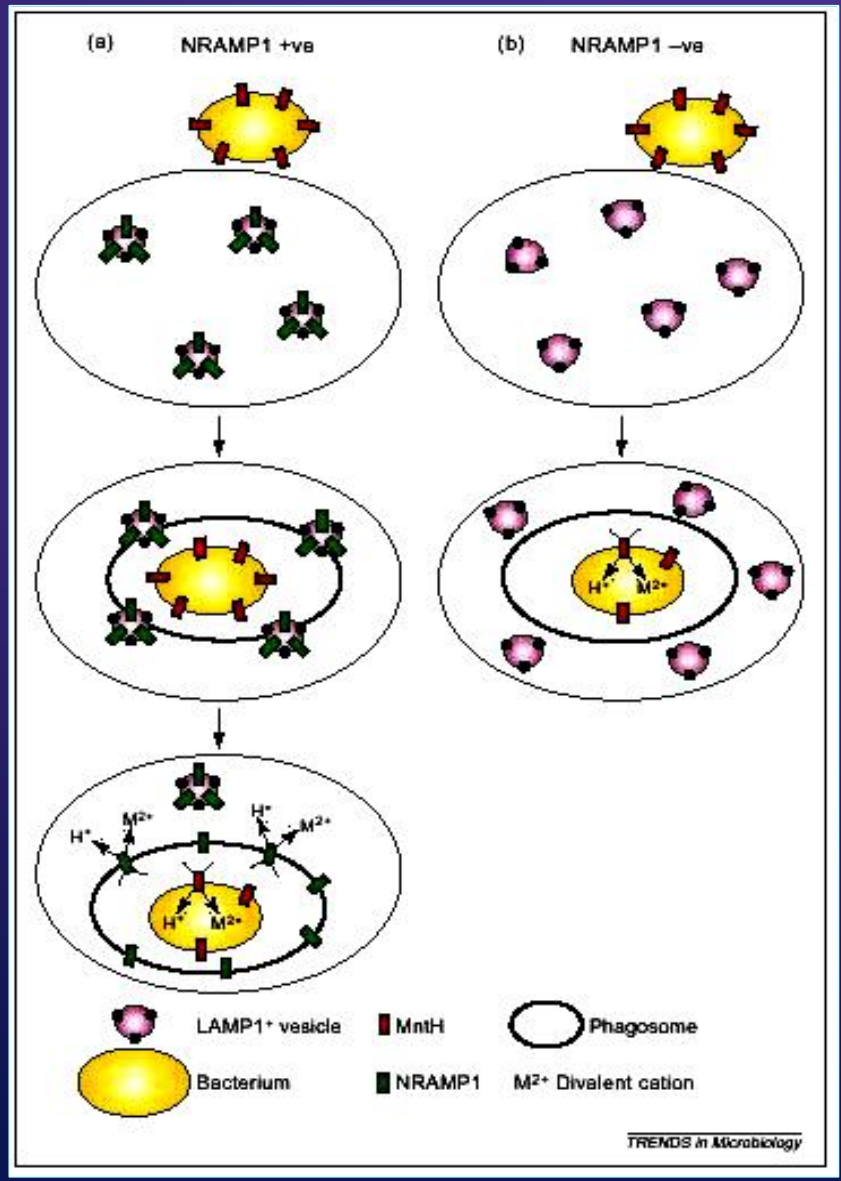
# NRAMP1 protein of mice



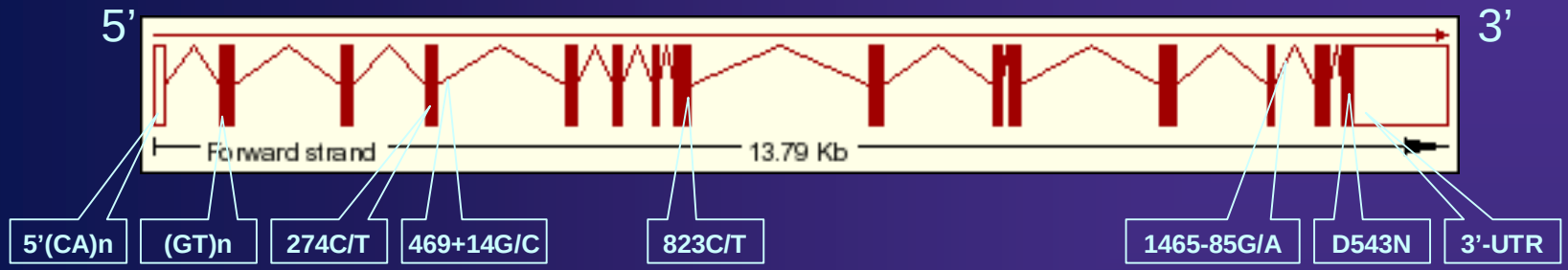

Localization at man - 2q35;  MIM - 600266

# Mechanism of Anti-tuberculosis activity of NRAMP1 protein

The NRAMP1 protein is believed to be a transmembrane iron transporter, which is localized to the late endosomal compartment of resting macrophages and is recruited to the phagosome on phagocytosis.



# *NRAMP1* gene polymorphism




- *NRAMP1* – is one of the most actively studied gene at human.
- There are 58 knowing SNPs of human *NRAMP1* in databases.
- Is about 10 SNPs of *NRAMP1* are most studied.
- There are more than 15 associations with tuberculosis were detected in different world populations.
- Discrepancies of results: from mayor gene effect (Canadian aborigines) to absence of associations (Dania, Brazil, Morocco).

# ***Toll*-like receptors genes**

- Stimulation of TLRs lead to activation of the nuclear factor of transcription NF-kB including a transcription of genes cytokines (*TNFa*, *IL1B*, *IFN* $\gamma$ etc.), hemocines and molecules of adhesion which are important at an inflammation.

- *TLR2* – (4q32), MIM – 603028

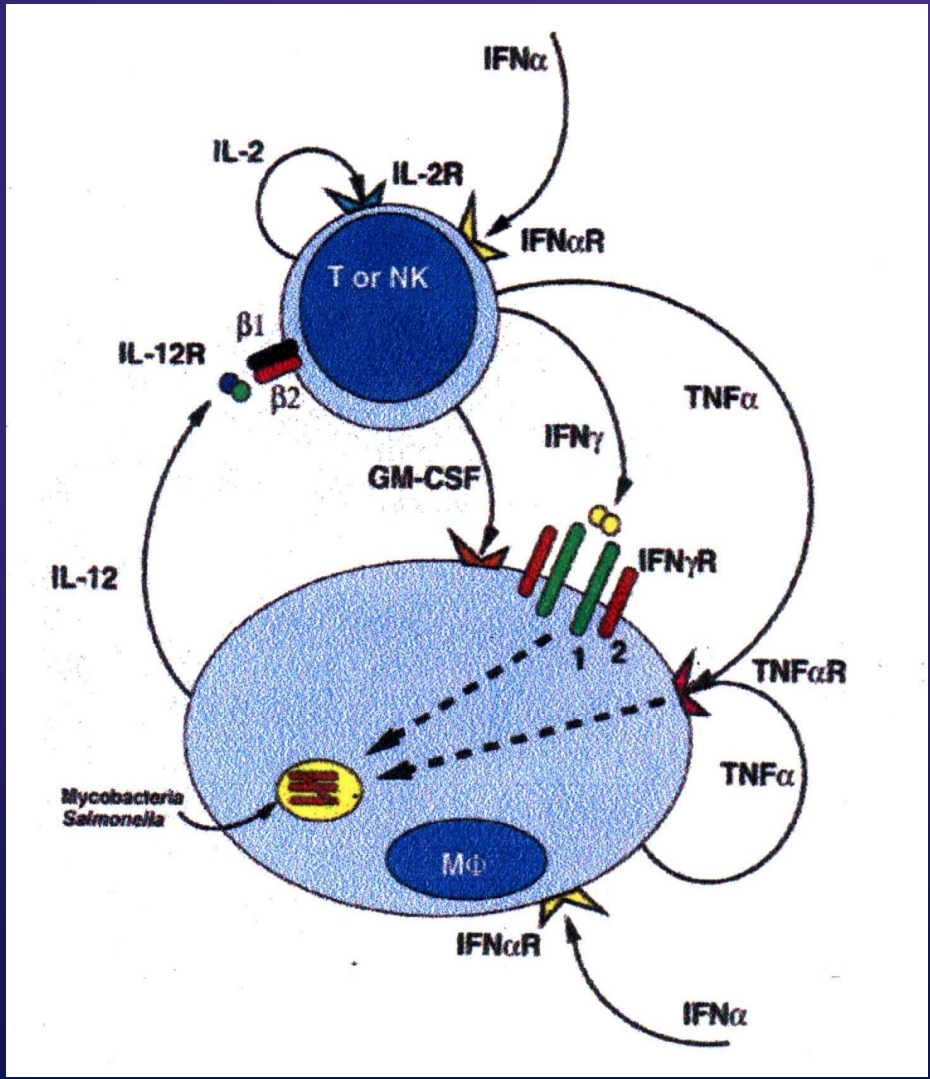
- *TLR4* – (9q32-q33), MIM – 603030

- Polymorthic variants of *TLR2* gene (P681H, Arg753Glu, (GT)n et al.) are associated with decriase of immunity, *M. tuberculosis* and other intracellular infections at man.

# Monogenic forms of nonpathogenic micobacterial infections

**Recently, some individuals with severe infections due to otherwise weakly pathogenic mycobacteria (non-tuberculous mycobacteria or *Mycobacterium BCG*) or *Salmonella* species have been shown to be unable to produce or respond to interferon-γ. This inability results from deleterious mutations in any one of five different genes involved in the type 1 cytokine cascade: *IL12B, IL12RB1, IFNGR1, IFNGR2* or *STAT1.***

**First description of such infections was in 1951 y.**



# Mutations in genes associated with type 1 cytokines and receptors and their phenotypes

| Gene | Extent of defect | Pattern of inheritance | N of cases reported | Number of deaths <11 | Main inefctions diagnosed (n) |
|---|---|---|---|---|---|
| *IL12B* | Complete | AR | 16 | 7 (44%) | *M. bovis BCG* (13)<br>*Salmonella* (5) |
| *IL12RB1* | Complete | AR | 21 | 4 (19%) | *M. bovis BCG* (11)<br>*M avium* (6)<br>*Salmonella* (8) |
| | Partial | | 1 | | - |
| *IFNGR1* | Complete | AR | 22 | 10 (45%) | *M avium* (8)<br>*M. bovis BCG* (11) |
| | Partial | AR | 2 | - | *M. bovis BCG* (1)<br>*S. enteritidis* (1)<br>*Clinical tub.* (1) |
| | Partial | AD | 32 | 2 (7%) | *M avium* (8)<br>*M. bovis BCG* (11) |
| *IFNGR2* | Complete | AR | 2 | - | *M. avium* (1)<br>*M. fortuitum* (1)<br>*M. abscessus* (1)<br>*M. bovis BCG?* (1) |
| | Partial | AR | 1 | - | *M. bovis BCG* |
| *STAT1* | Partial | AD | 3 | - | *M. bovis BCG* (1)<br>*M avium* (1) |

(Ottenhoff T. et al., 2002).

- **The rate of disseminate BCG infections in France is 0,59 per 1 million of births.**

**(Altare et al., 1998).**

- **Clinical tuberculosis (*M. tuberculosis*) has been diagnosed in at least four individuals with deficiencies in the type 1 cytokine cascade.**

**Why not more?**

**- This may be a result of lack of exposure, as many of the individuals studied come from countries where *M. tuberculosis* is no longer endemic.**

**- Individuals with such defects that are affected with tuberculosis die before a genetic diagnosis is made, or, alternatively, that such genetic defects are not considered once individuals present with tuberculosis.**

**- It is possible that resistance to *M. tuberculosis* in humans is less dependent on IL-12 and IFN-γ immunity than is resistance to *M. bovis* BCG or non-tuberculous mycobacteria.**

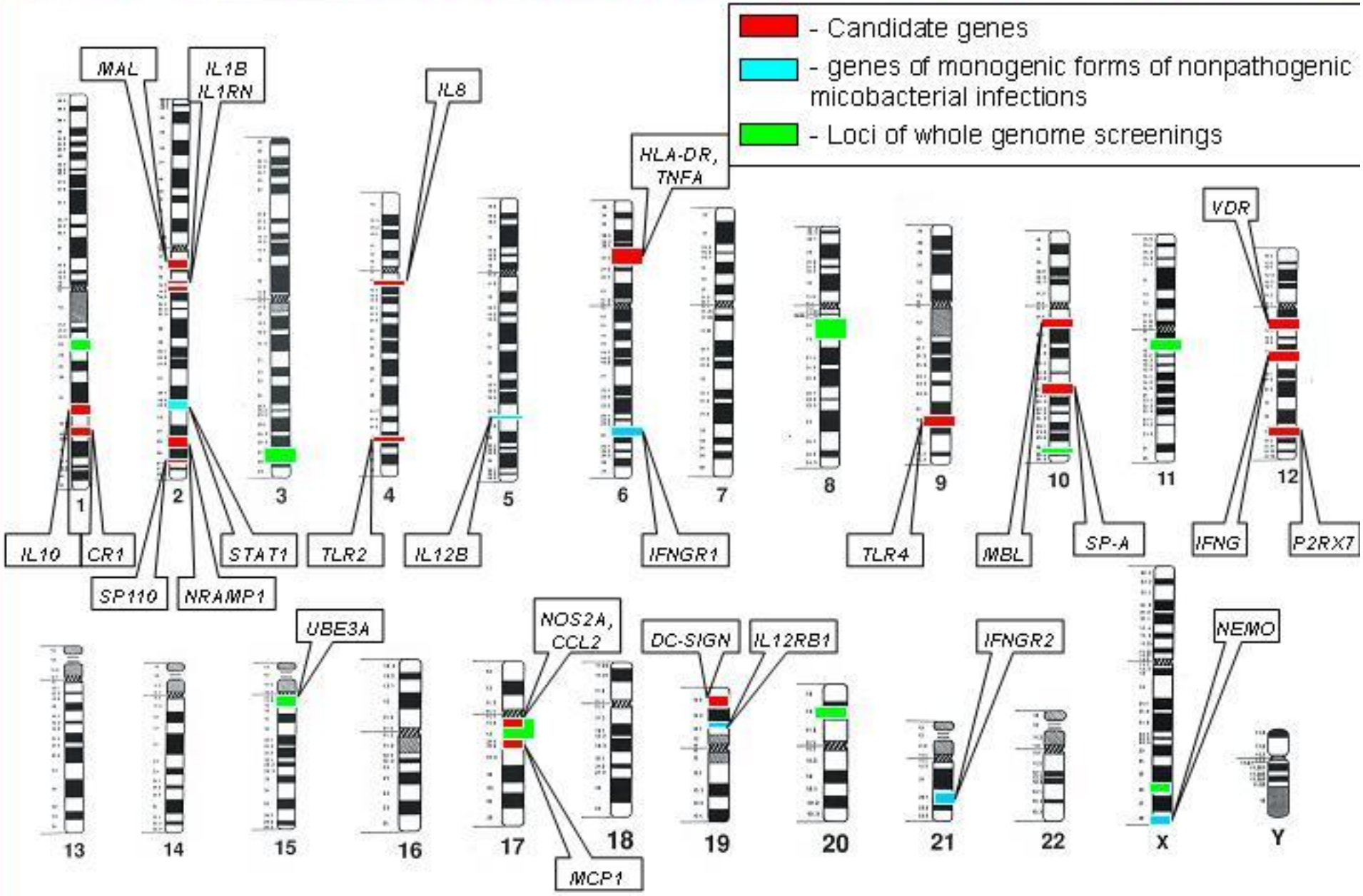
**(Ottenhoff T. et al., 2002).**

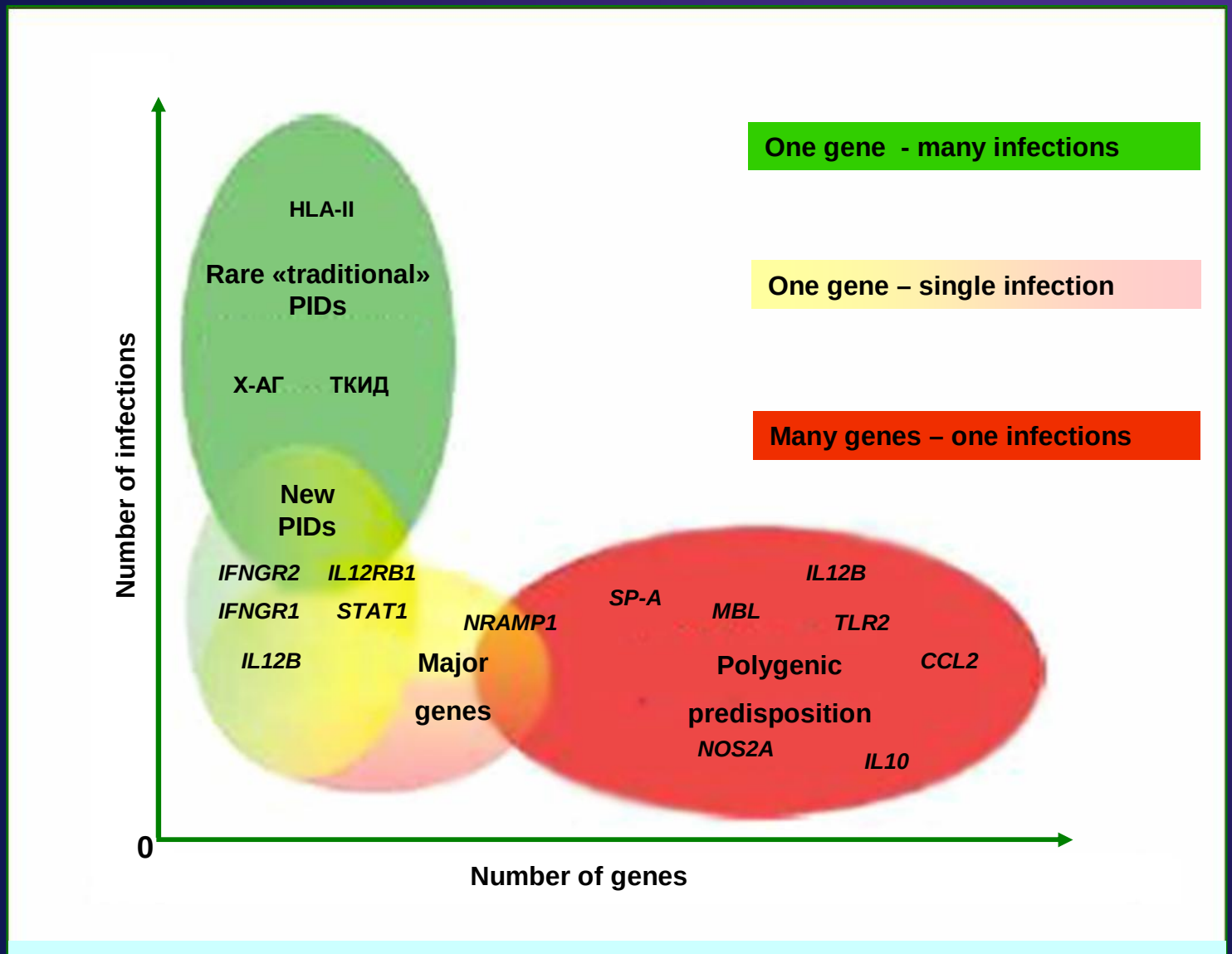
ü The primery disseminate form of TB at children can be result of the mutations of genes of the type 1 cytokine cascade (*IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2* or *STAT1).*

# Results of genome-wide screens at tuberculosis

| population | material | Linked loci | LOD | articles |
|---|---|---|---|---|
| Gambia | 67 families – 73 sibs pairs with TB, 16 families with TB - 19 sibs pairs (Kuazulu-Natal) | Xq26<br>15q11-13 | 1,84<br>2,00 | Bellamy R. et al, 2000 |
| Brasilia | 16 families with TB – 178 individuals | 10q26.13<br>11q12.3<br>20p12.1 | 1,31<br>1,85<br>1,78 | Miller E.N. et al, 2004 |
| Morocco | 96 families - 227 siblings | 1q22<br>3q27-q28<br>8q12-q13 | 2,00<br>1,93<br>3,38 | El Baghdadi J. et al, 2006 |

# Candidate loci and genes of susceptibility to tuberculosis






**Spectrum of genes of susceptibility to infectious diseases**

***(Casanova и Abel, 2007)***

***PIDs – Primery immunodeficites**

# Results of studying of genetic predisposition to tuberculosis in Siberian populations

# Description of studied groups

| | Tuvinians (Tuva Republic) | Russian (Tomsk) |
|---|---|---|
| TB patients | N = 238<br>M / W = 121 / 117<br>Age = 33,4±12,9 | N = 304<br>M / W = 205 / 99<br>Age = 30,6±15,4 |
| Health control | N = 263<br>M / W = 201 / 62<br>Age = 33,1±8,5 | N = 140<br>M / W = 60 / 80<br>Age = 64,3±18,0 |
| TB family | 129 families<br>370 individuals<br>215 TB patients | 42 families<br>109 individuals<br>64 TB patients |

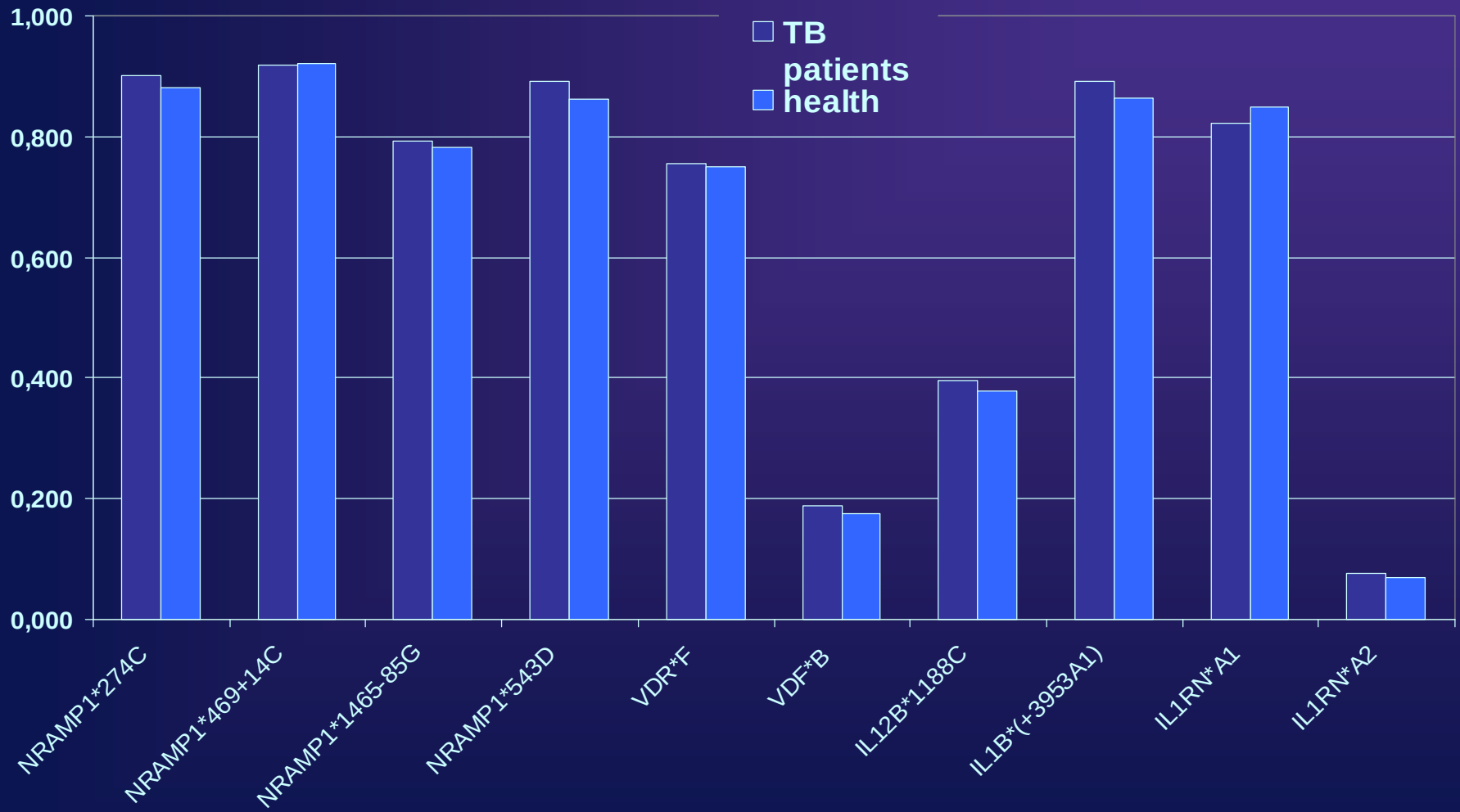
**Summary: it was studied more than 1500 individuals**

# Studied genes

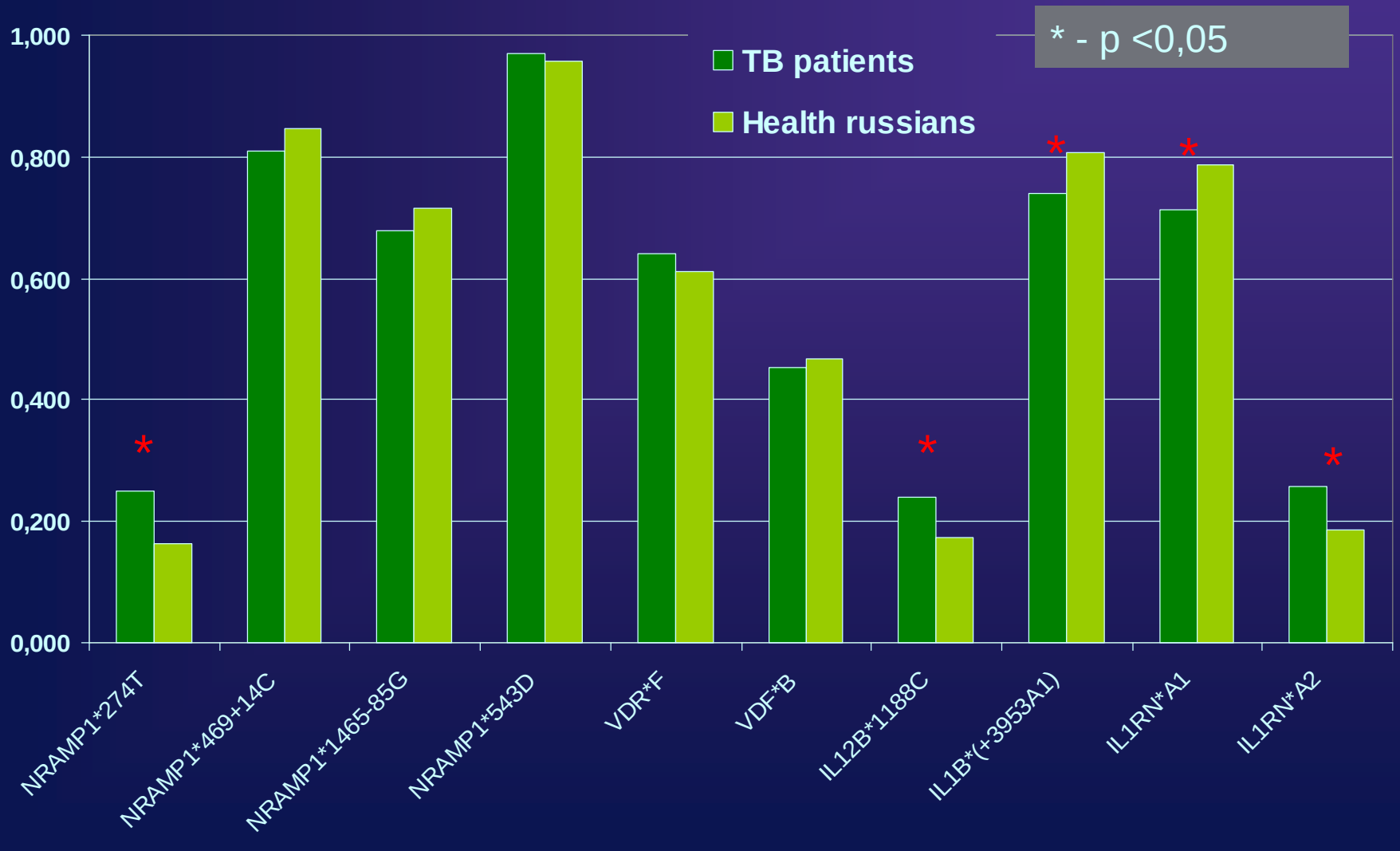
| gene | Polymorphic vaiant | Localization in gene | Enzyme |
|---|---|---|---|
| ***NRAMP1*** | 274C/T | exon 3 | *Mnl* I |
| | 469+14G/C | intron 4 | Apa I |
| | 1465-85G/A | inton 13 | *Bse1* I |
| | D543N | exon 15 | *Bme18* I |
| ***VDR*** | F/f | exon 2 | *Fok* I |
| | B/b | intron 8 | *Bsm* I |
| ***IL12B*** | 1188A/C | 3'-UTR | *Taq* I |
| ***IL1B*** | (+3953)A1/A2 | exon 5 | *Taq* I |
| ***IL1RN*** | VNTR | intron 2 | - |

**All polymorphisms are SNPs,**
with the exception of VNTR of ***IL1RN***

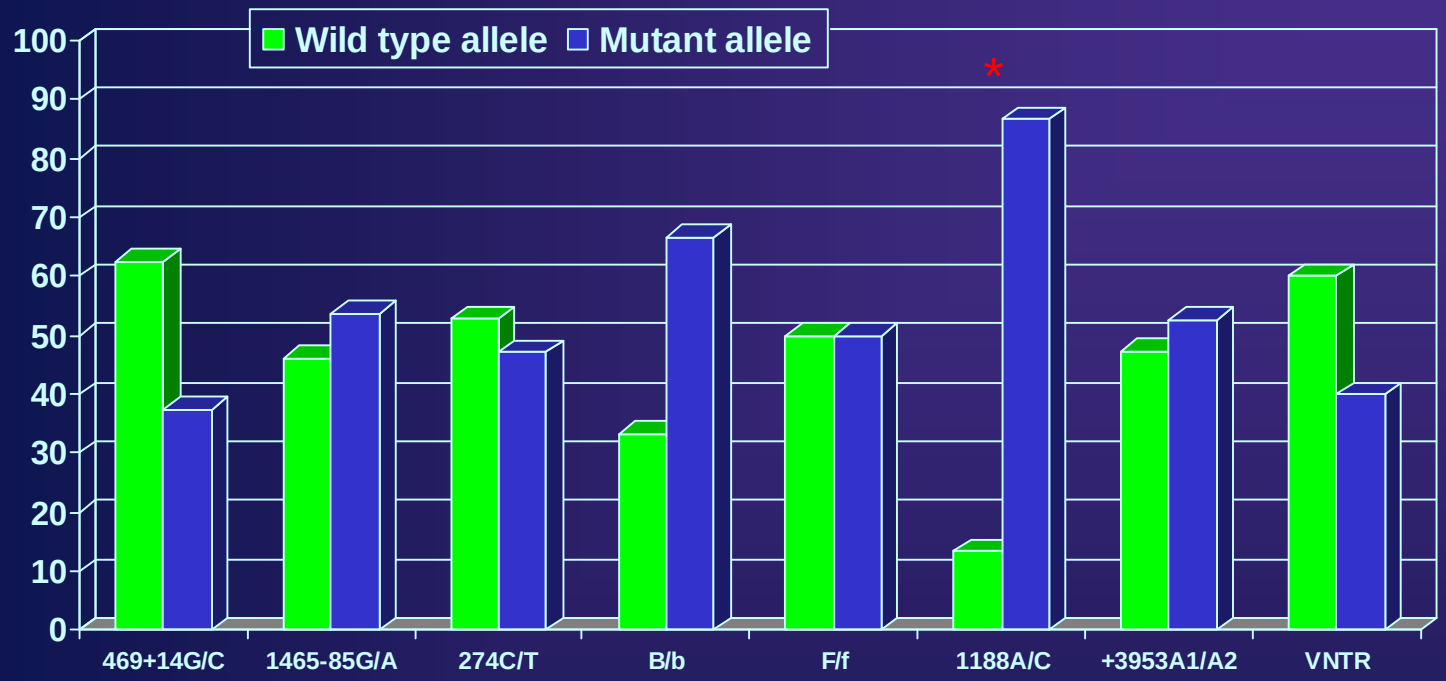
# Allelic frequencies of studied polymorphisms at TB patients and health tuvinians



# Allelic frequencies of studied polymorphisms at TB patients and health Russians



# Results of TDT in Russian families with TB




| | NRAMP1 469+14G/C | NRAMP1 1465-85G/A | NRAMP1 274 C/T | IL12B 1188A/C | VDR B/b | VDR F/f | IL1B +3953A1/A2 | IL1RN A1/not A1 |
|---|---|---|---|---|---|---|---|---|
| TDT | 1,000 | 0,150 | 0,060 | 8,070 | 2,000 | 0,000 | 0,050 | 0.600 |
| P | 0.317 | 0,700 | 0.808 | 0,005 | 0.151 | 1,000 | 0.818 | 0.439 |

# Associations of studied polymorphisms with quantitative and qualitative traits

| **Polymorphisms** | Tuvinians | Russians |
|---|---|---|
| **NRAMP1*274C/T** | level of haemoglobin and leucocytes | Destruction of lung, size of TB lesions |
| **NRAMP1*469+14G/C** | Level of eosinophiles | |
| **NRAMP1*1465-85G/A** | | |
| **NRAMP1*D543N** | Level of neutrophils and monocytes | |
| **IL1B*3953A1/A2** | | |
| **IL1RN*VNTR** | Erythrocytes sedimentation rate, level of neutrophils and lymphocytes; Destruction of lung, size of infiltration | |
| **IL12B*1188A/C** | | |
| **VDR*F/f** | | Erythrocytes sedimentation rate, Level of immature leucocytes |
| **VDR*B/b** | Level of immature leucocytes Destruction of lung, size of cavities | Level of neutrophils; size of TB lesions |

# Conclusions

- The structure of genetic predisposition to tuberculosis in Tuvinians and Russians is not the same. Possibly because they have different models of disease inheriting:

- The model of polygenic inheritance (including *NRAMP1*, *IL12B*, *IL1B*, *IL1RN* genes*)* is more representative for Russians, because of the long *M. tuberculosis* exposition history.

- For Tuvinians the major-gene model is more characteristic, then disease development is caused by one or more genes with relatively strong effect. This effect is reinforced by *NRAMP1*, *VDR*, *IL12B*, *IL1B*, *IL1RN* polymorphic variants, which frequency are more higher in Tuvinians then in other populations.

- Studied genes are important factors in forming of clinical polymorphism of TB in studied populations.

# Our research plans

- Collection and studying of new groups ot TB-patients from other Siberian populations (Jakuts, Burjats et al.).
- Expansion of a spectrum of studied genetic markers in these populations.
- Skrining and studying of mutations of genes involved in Th-1 type of immunity (monogenic forms of TB) at children with active TB.
- Experimental research work - studying of genes expression in monocites at different antigenic stimulation
- Development of the russian and international collaboration.

# Our research collaborations

- Ministry of Health of Tiva Republic
- Siberian State Medical University
- Yakutsk Research Center
- Koch-Mechnikov Forum (Germany-Russia)
- Wellcome Trust Center of Human Genetics (Oxford, UK)
- Novosibirsk Research Institute of Tuberculosis

# Application of new knowledge

- Understanding of infectious diseases pathogenesis and host defense from infectious agents.
- Prediction of disease development risk.
- Design of new and more effective vaccines.
- Design of new drugs.
- Genomic medicine and gene therapy.

Thank you for your attention!