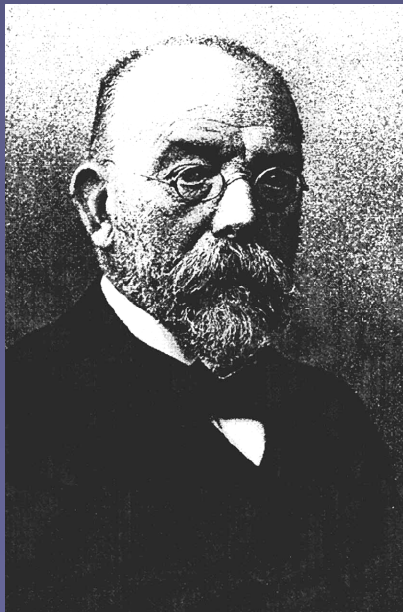
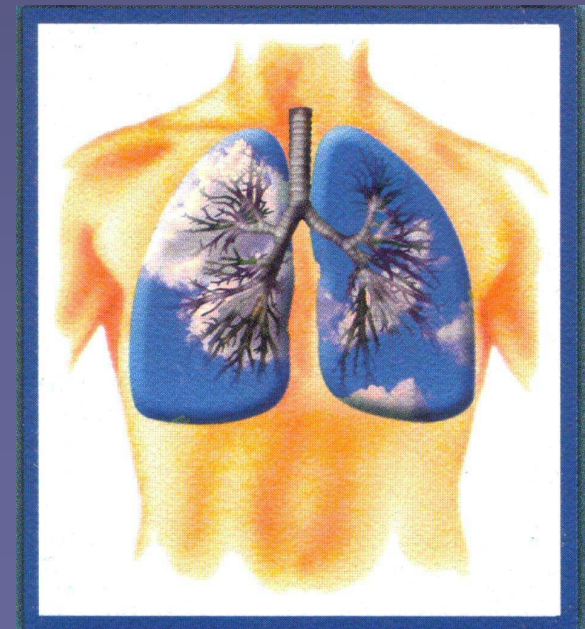


Tomsk Research Institute of Medical Genetics
Siberian Branch of Russian Academy for Medical Science

The study of genetic predisposition to tuberculosis in Siberia



Rudko Alexey



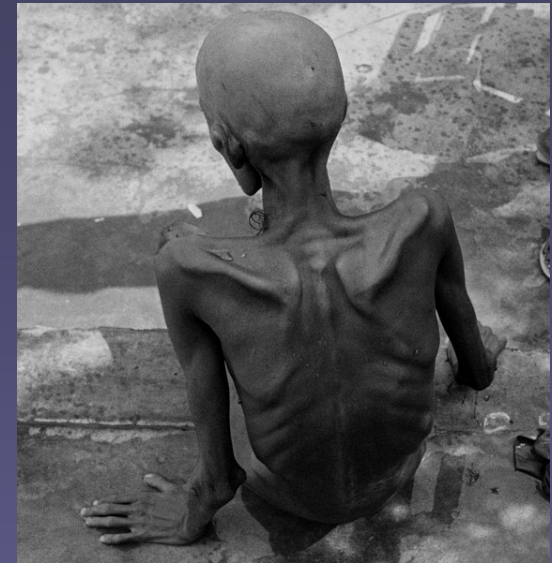
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Tuberculosis is a world-wide health problem

- 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.
- Annually **~500 000 MDR TB** cases are registered.

Tuberculosis is a multifactorial disease

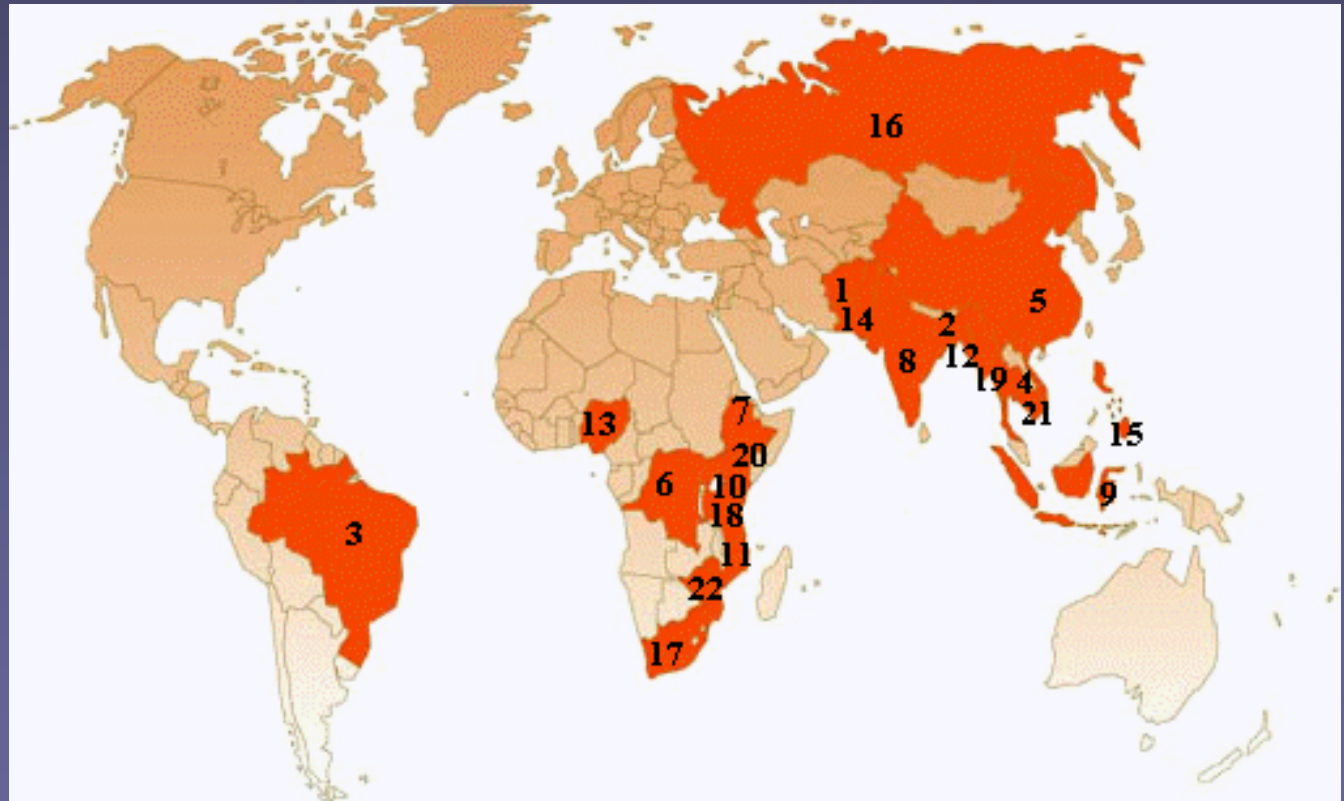
- **Social and environmental factors:** poverty, bed nutrition, over-population, migrations.
- **Mycobacterium:** pathogenicity and drug resistance.
- **Host condition:** adequate immune defense dependent from human genome.
- **HIV-infection:** is about 8-10% of tuberculosis is associated with AIDS.



22 countries - 80% of all TB 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. Vietnam
14. Tanzania
15. Uganda
16. Brazil
17. Afghanistan
18. Thailand
19. Mozambique
20. Zimbabwe
21. Myanmar
22. Cambodia



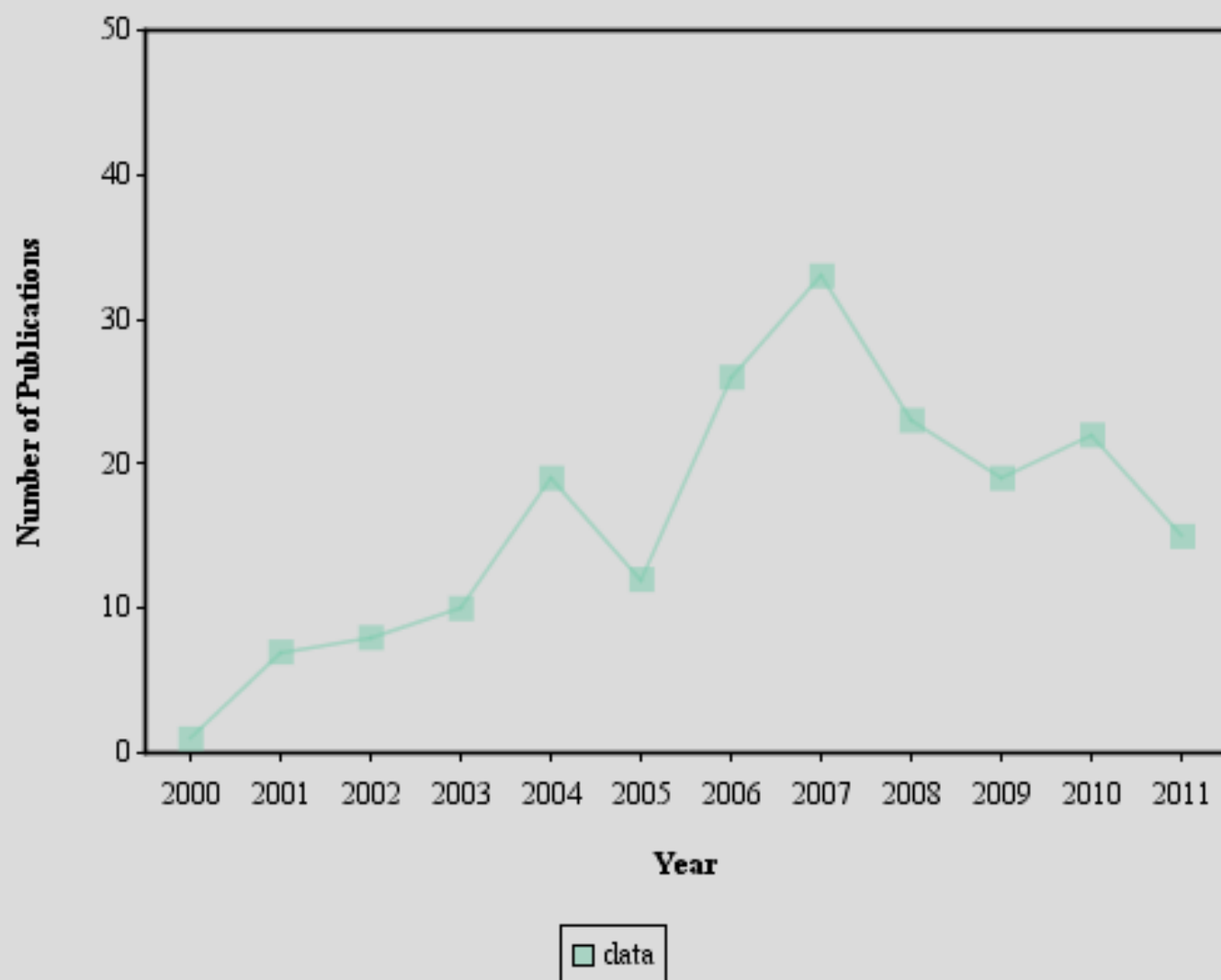
Different approaches for identifying genes of predisposition to TB

- 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.



HuGE Navigator (version
2.0)

HuGE Publications in Studies of Tuberculosis, Pulmonary (All) (Temporal Trend and Spatial Pattern)



195 publications,
104 genes,
5 Meta-analyses

Results of genome-wide association study at TB

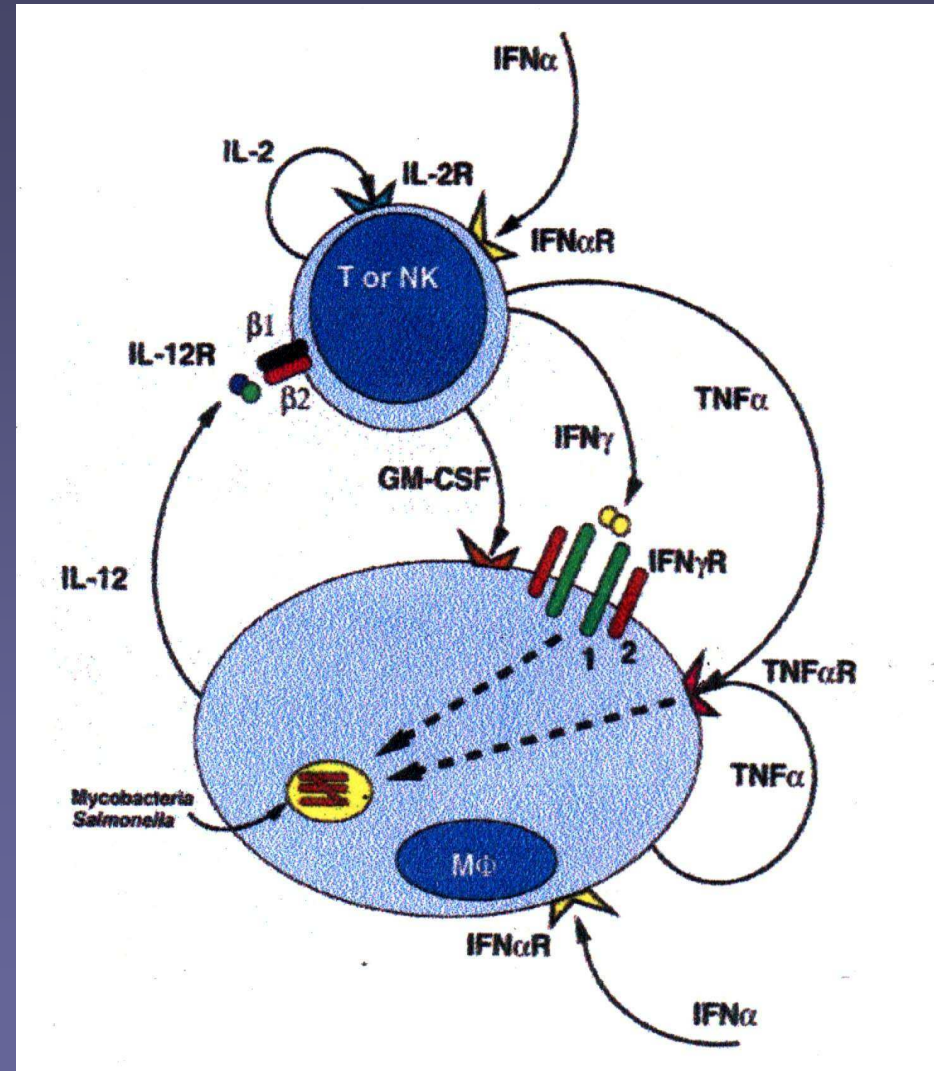
population	material	Linked loci	LOD or p-level	articles
Gambia	67 families – 73 sibs pairs with TB	Xq26 15q11-13	1,84 2,00	Bellamy R. et al, 2000
Brasilia	16 families with TB – 178 individuals	10q26.13 11q12.3 20p12.1	1,31 1,85 1,78	Miller E.N. et al, 2004
Morocco	96 families - 227 siblings	1q22 3q27-q28 8q12-q13	2,00 1,93 3,38	El Baghdadi J. et al, 2006
South Africa	81 families (131 sibs pairs); 24 families (24 sibs pairs)	6p21-q23 20q13.31-33	1,90 2,00	Cooke G.S. et al, 2008
Uganda	193 families (803 individuals)	2q21-q24 5p13-q22 7p22-p21 20q13	p<10-3 p<10-3 p<10-3 p=0.002	Stein C.M. et al, 2008
Thailand	95 families (199 individuals)	5q23.2-31.3 17p13.3-13.1 20p13-12.3	2.29 2.57 3.33	Mahasirimongkol S. et al., 2009
Ghana and Gambia	11425 individuals	18q11.2	p=6.8 × 10 ⁻⁹	Thye T. et al., 2010

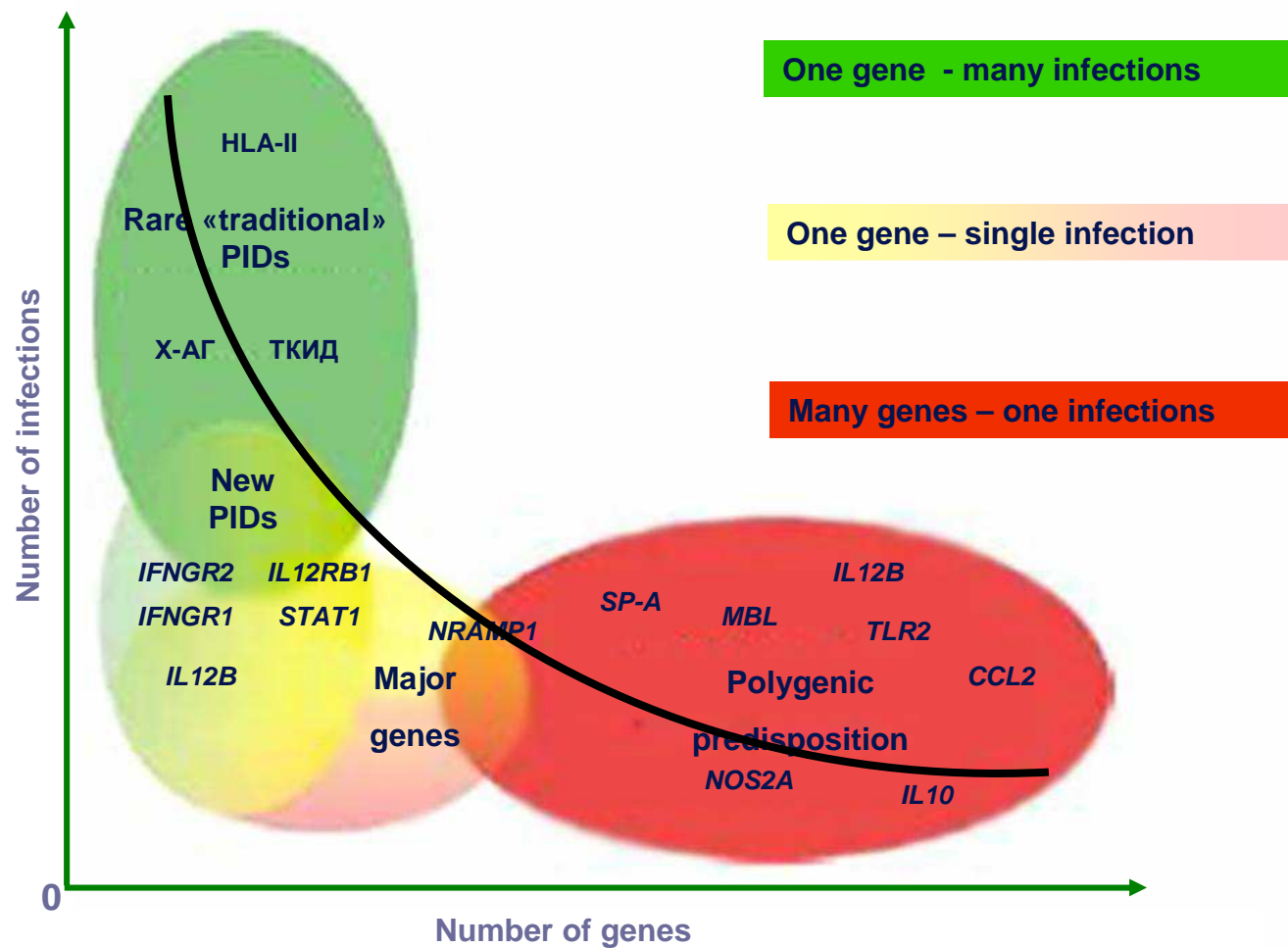
Atypical Familial Mycobacteriosis

MIM 209950

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*, *STAT1* and *NEMO*.

First description of such infections was in 1951 y.

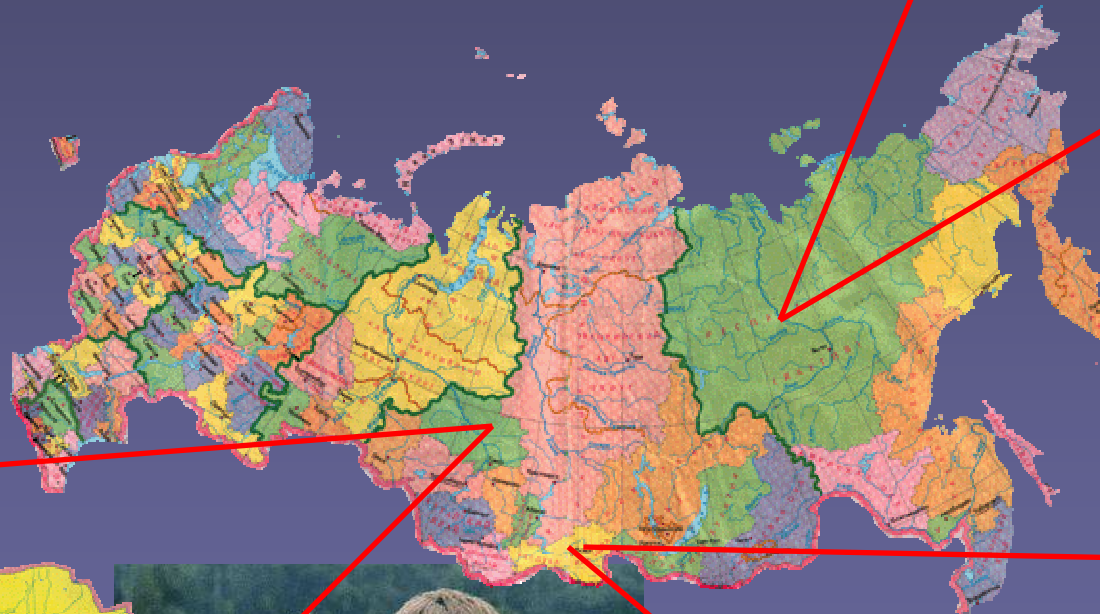




Spectrum of genes of susceptibility to infectious diseases (Casanova u Abel, 2007)

*PIDs – Primery immunodeficitcs

Results of studying of genetic predisposition to tuberculosis in Siberian populations



Studied groups

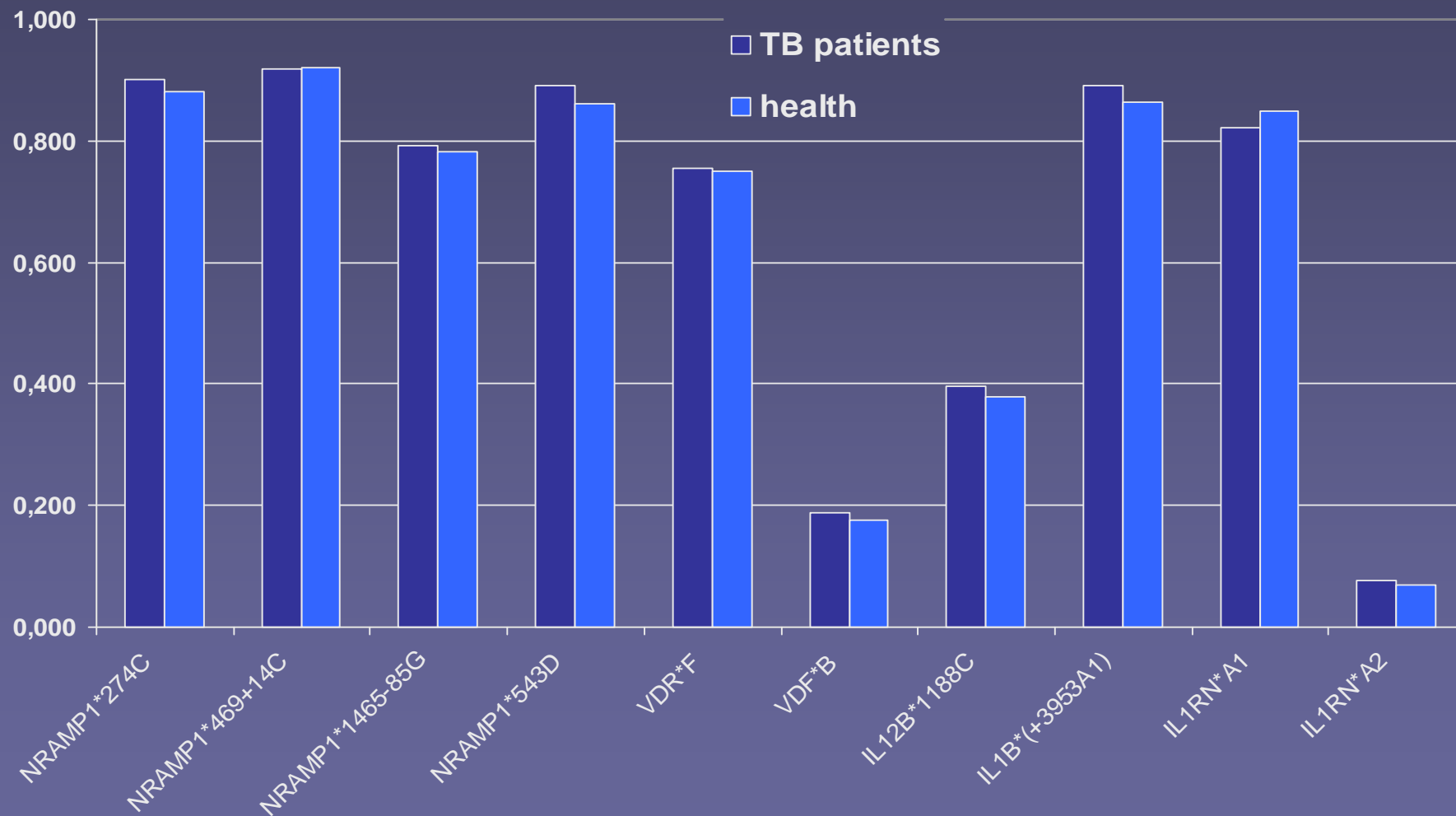
	TB patients	Health control
Tuvinians (Tuva Republic)	N = 238 M / W = 121 / 117 Age = 33,4±12,9	N = 263 M / W = 201 / 62 Age = 33,1±8,5
Russian (Tomsk)	N = 304 M / W = 205 / 99 Age = 30,6±15,4	N = 140 M / W = 60 / 80 Age = 64,3±18,0
Yakuts	N = 150 M / W = 82 / 68 Age = 36,98±10,5	N = 135 M / W = 57 / 78 Age = 41,1±5,76

Studied genes in 2001-2005

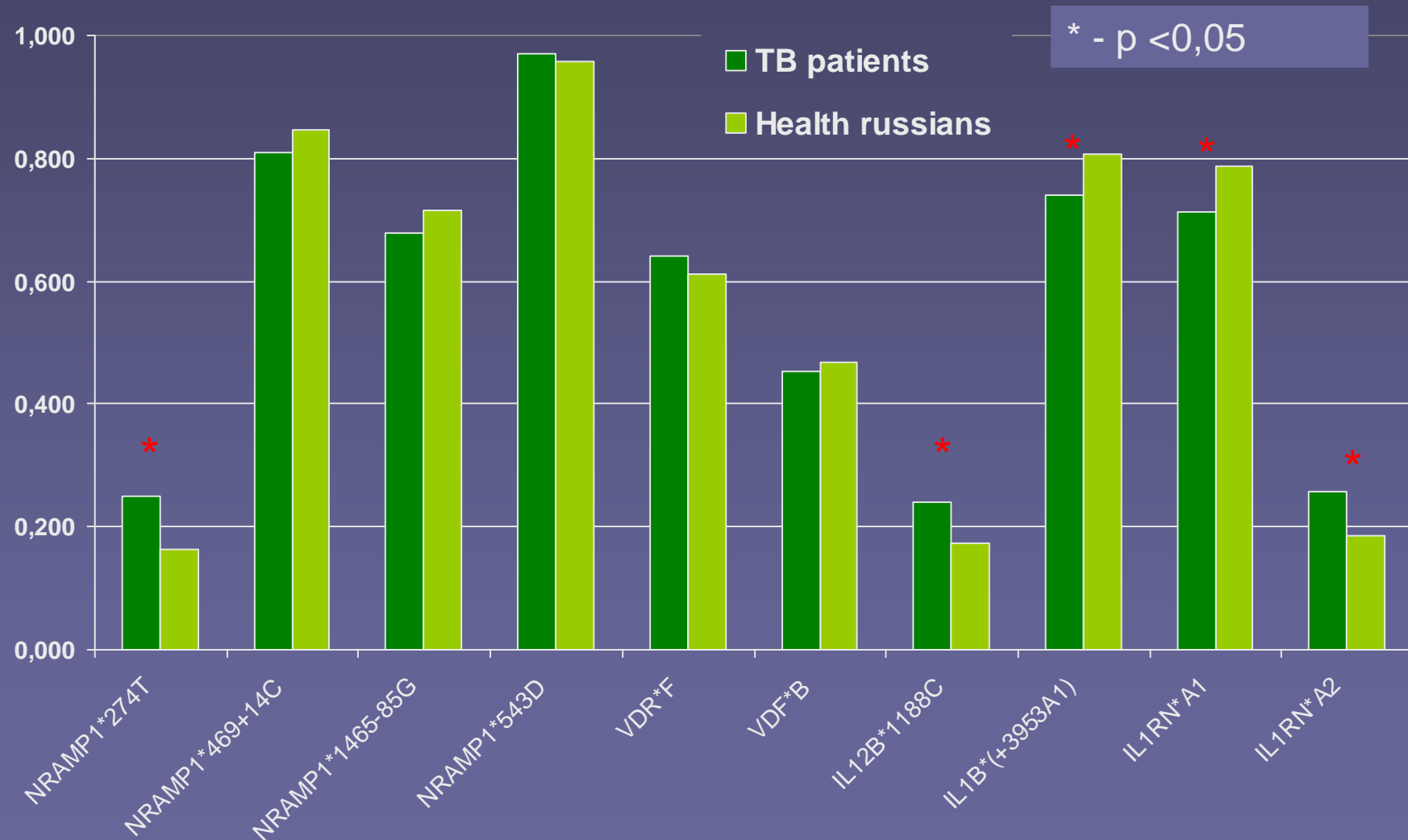
gene	Polymorphic vaiant	Localization in gene	Enzyme
<i>NRAMP1</i>	274C/T	exon 3	<i>Mnl I</i>
	469+14G/C	intron 4	<i>Apa I</i>
	1465-85G/A	inton 13	<i>Bse1 I</i>
	D543N	exon 15	<i>Bme18 I</i>
<i>VDR</i>	F/f	exon 2	<i>Fok I</i>
	B/b	intron 8	<i>Bsm I</i>
<i>IL12B</i>	1188A/C	3'-UTR	<i>Taq I</i>
<i>IL1B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



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.

I. Second stage of case-control study of genetic predisposition to Tb in Siberia

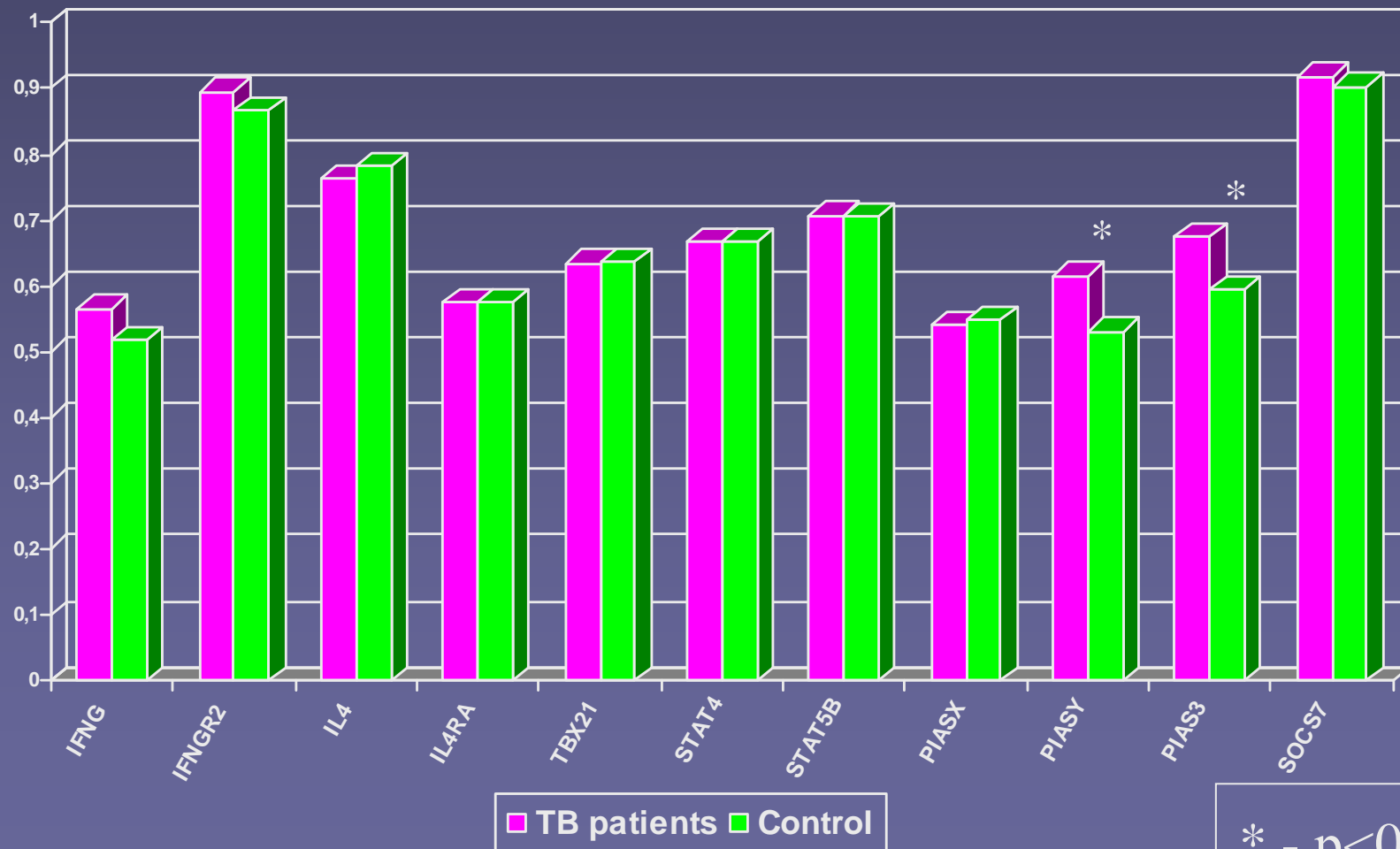
Studied polymorphisms

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graph TD; A([Studied polymorphisms]) --> B[IFNG, IFNGR2, IL4RA, IL4, TBX21, STAT4, STAT5B, PIASX, PIASY, PIAS3, SOCS7, MCP1, MBL, SP110, CD209]; A --> C[IL23R, ITLN1, 1q32.1, ATG16L1, BSN, PTGER4, IRGM, IL12B, CCR6, 7p12.2, 8q24.13, TNFSF15, ZNF365, NKX2-3, C11ORF30, CCDC122, 13q14.11, NOD2, ORMDL3, STAT3, PTRF, NAGLU, PTPN2, 21q21.1]
```

*IFNG, IFNGR2,
IL4RA, IL4,
TBX21,
STAT4, STAT5B,
PIASX, PIASY, PIAS3,
SOCS7,
MCP1,
MBL,
SP110,
CD209*

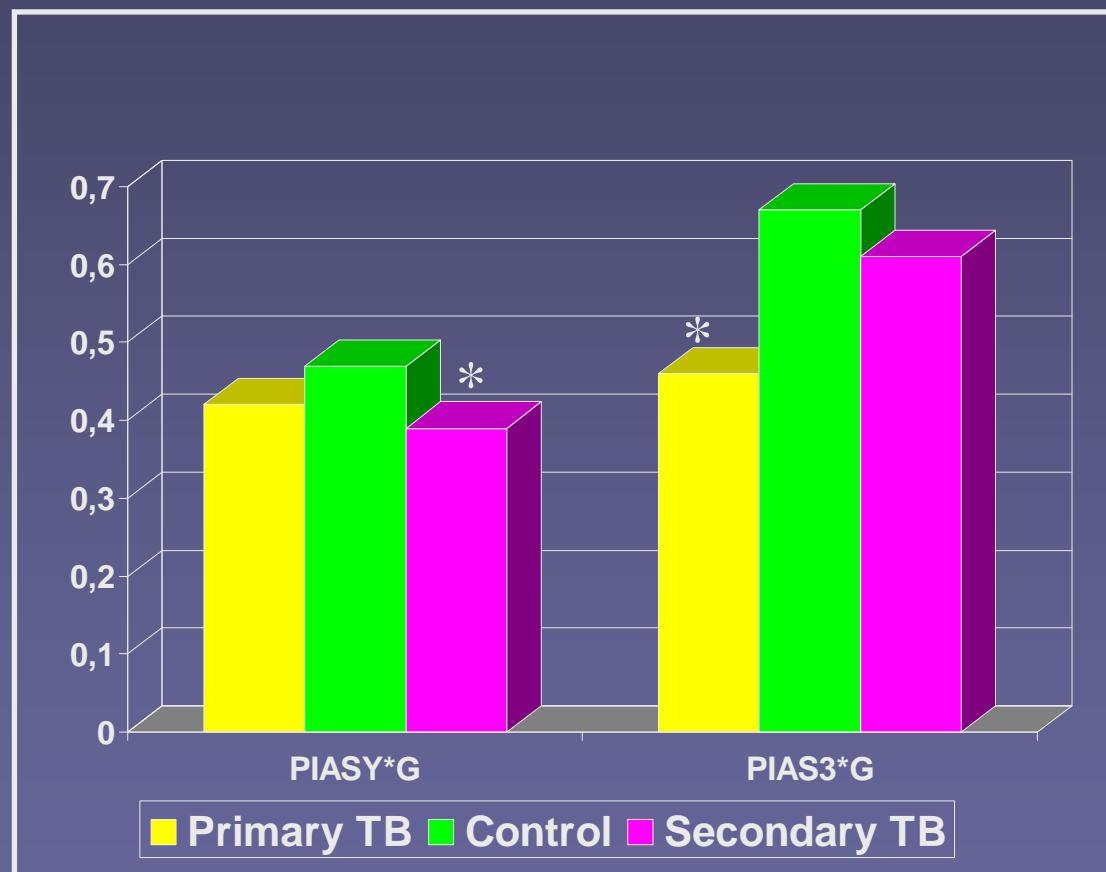
*IL23R, ITLN1, 1q32.1, ATG16L1,
BSN, PTGER4, IRGM, IL12B,
CCR6, 7p12.2, 8q24.13,
TNFSF15, ZNF365,
NKX2-3, C11ORF30, CCDC122,
13q14.11, NOD2, ORMDL3,
STAT3, PTRF,
NAGLU, PTPN2, 21q21.1*

Allelic frequencies of studied polymorphisms at TB patients and health Russians



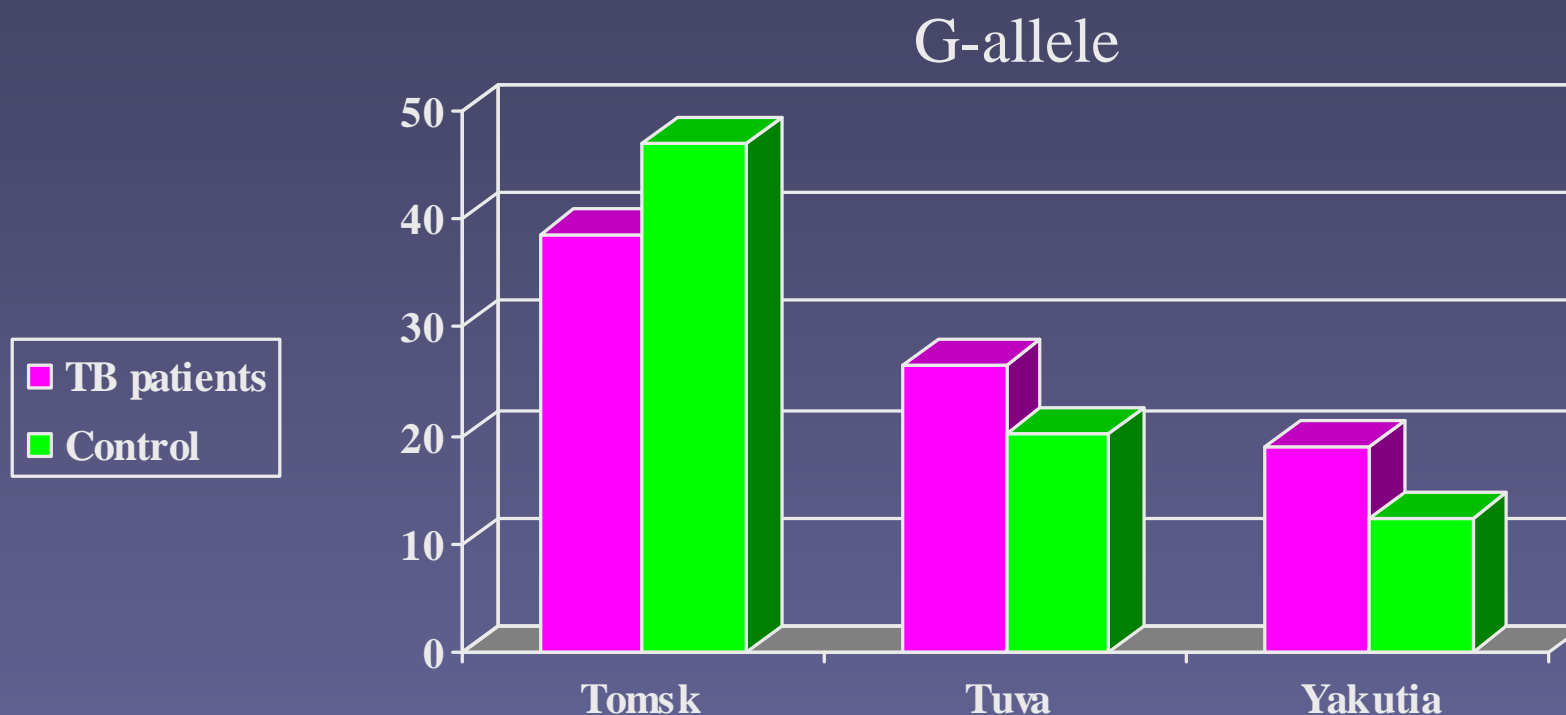
* - $p < 0.05$

Association of studied genes *PIASY* (rs3760903) and *PIAS3* (rs12756687) with clinical forms of TB



* - $p < 0.05$

Association of rs3760903 PIASY gene with TB in Siberian populations



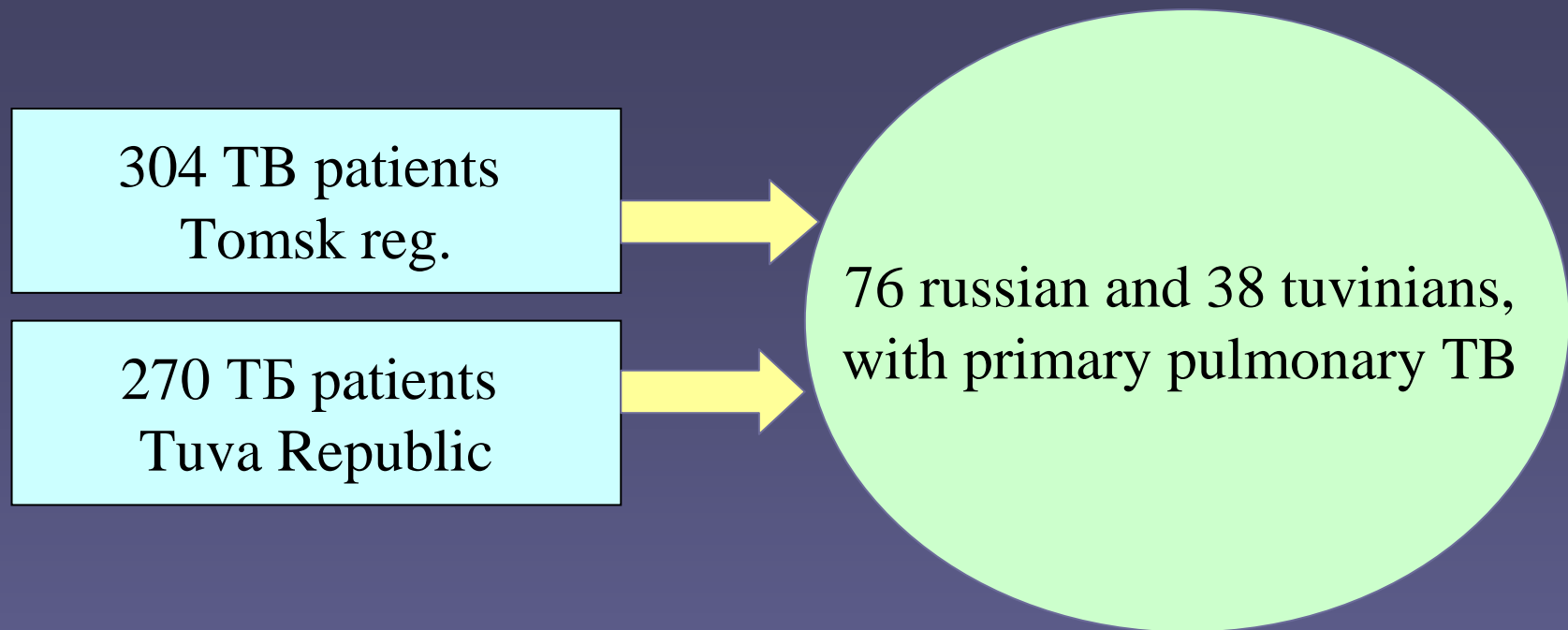
	Confidence level (p)		
Genotype frequencies	0,041	0,038	0,05
Allelic frequencies	0,019	0,032	0,035

II. Study of Atypical Familial Mycobacteriosis MIM 209950 (MSMD) in Siberia

Design of study

- Formation of samples group with serious clinical forms of TB
- Screening of “prevailing” mutations of MSMD-genes
- Sequencing of MSMD-genes (*IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2*, *STAT1* and *NEMO*)
- Screening of obtained mutations in studied Siberian populations and in TB patients

1. Formation of samples group



+

14 families with serious primary TB at children

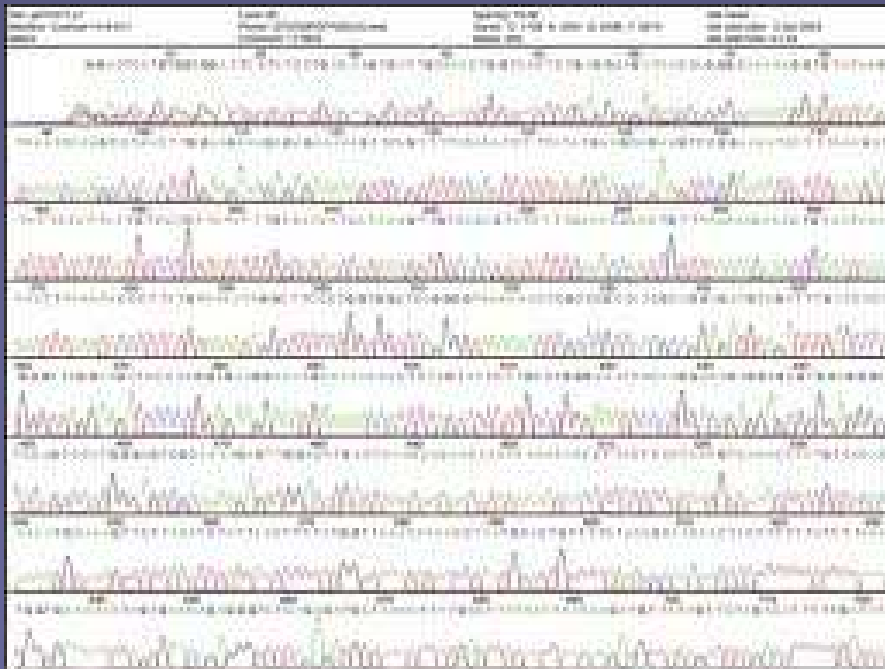
BCG-osteitis – 2 , lymph node TB – 6 (first-year children),
generalized TB – 6

2. Screening of “prevailing” mutations

<i>Gene</i>	mutations
<i>IL12RB1</i>	Gln32Ter, Gln376Ter, Arg213Trp
<i>IFNGR1</i>	Ile87Thr, 4-bp Del, NT818; 1-bp Del, NT818
<i>IFNGR2</i>	2-bp Del, 278A/G, Thr168Asn, 663Del27
<i>STAT1</i>	Leu706Ser, Gln463His, Glu320Gln

3. Sequencing

- Sequencing of 65 translated exons of 6 genes:
- IL12B – 6 exons
- IL12RB1 – 18 exons
- IFNGR1 – 7 exons (10 locy)
- IFNGR2 - 7 exons
- STAT1 – 23 exons
- NEMO – 1 exon



First results of MSMD-genes sequencing

40 exons from 65 planned

Type of mutations	mRNA		Protein	
	Position	Alleles exchange	Position	Alleles exchange
<i>IL12RB1</i>				
synonymous rs11086087	451 (5 exon)	CTG→GTA GTG→GTC	129	V[Val]→V[Val] V[Val]→V[Val]
missence rs11575934	705 (7 exon)	CAG→CGG	214	Q[Gln]→R[Arg]
synonymous rs17852635	748 (7 exon)	CCC→CCT	228	P[Pro]→P[Pro]
missence rs401502	1196 (10 exon)	GGG→CGG	378	G[Gly]→R[Arg]
rs12461312	32041 (13 intron)	A/C	-	-
rs17882555	32018 (13 intron)	C/T	-	-
rs3746190	2087 (3'UTR)	C/T	-	-

First results of MSMD-genes sequencing

(continuance)

Type of mutations	mRNA		Protein	
	Position	Alleles exchange	Position	Alleles exchange
<i>IL12B</i>				
rs919766	14918 (3 intron)	A/C	-	-
<i>IFNGR1</i>				
rs2234711	48 (5'UTR)	C/T	-	-
rs7749390	5198 (1 intron)	A/G	-	-
rs11754268	5233 (1 intron)	C/T	-	-
missence rs11575936	143 (1 exon)	GTG→ATG	14	V[Val] →M[Met]
synonymous rs11914	153 (7 exon)	TCT→TCG	350	S[Ser] →S[Ser]
<i>IFNGR2</i>				
rs17883129	34996 (6 intron)	C/T	-	-

Possible application of new knowledge

- Understanding of infectious diseases pathogenesis and host defense from infectious agents.
- Prediction of disease development risk.
- Design of new methods of TB prevention and treatment.
- Genomic medicine and gene therapy.

Our research collaborations

- Siberian State Medical University
- Ministry of Health of Tuva Republic
- Yakutsk Research Center
- Wellcome Trust Center of Human Genetics (Oxford, UK)
- Novosibirsk Research Institute of Tuberculosis

Research grants

- Grant of the Federal Scientific-Technical Program of the Federal Agency for Education NK-116(5)
- Russian President's Grant MK-2115.2009.7
- Grant of RFBR №09-04-00558-a.

Thank you!