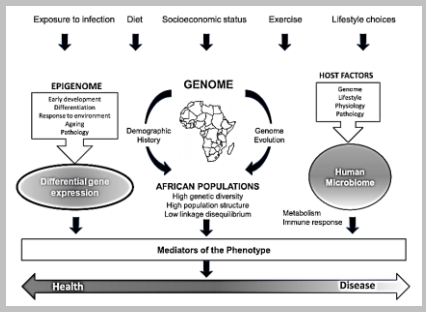
## Molekulární disekce imunity

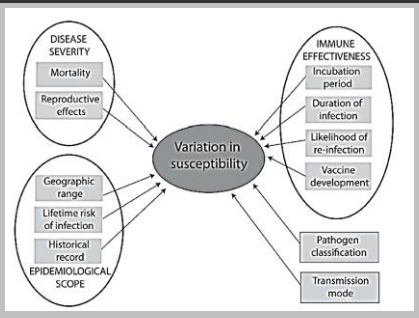
- ✓ Identifikace genů a drah
- ✓ Mechanismy nemocí
- ✓ Farmakogenetika, farmakogenomika
- ✓ Genetika vakcinace, vakcinomika

## Genetika vnímavosti k infekcím



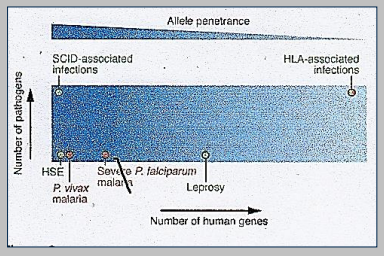

Ramsay FEBS Lett 2012

## Vnímavost k infekci jako komplexní znak



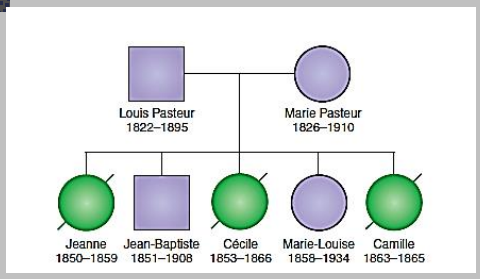

Baker, Antonovics 2012

## Imunogenom a infekce



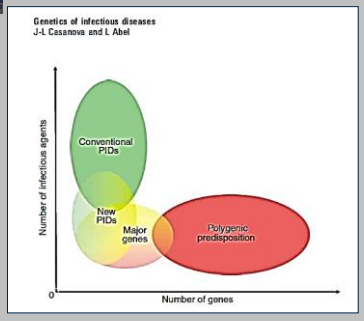

Alcais et al. J Clin Investig 2009

## Dědičnost vnímavosti k infekci




Quintana-Murci et al. 2007

## Typy dědičnosti vnímavosti k infekcím




Casanova, Abel EMBO J 2007

## Mendelistická dědičnost

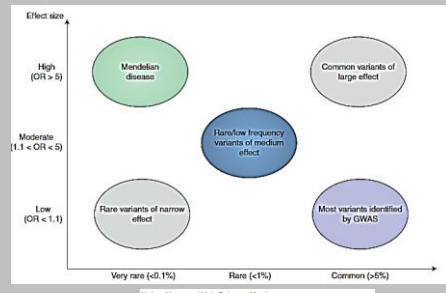
### Nízkočetné varianty s velkým účinkem

**Mendelian disorders of immunity to infection associated with predisposition or resistance to specific infections**

| Infectious agent | Clinical phenotype | Immunological phenotype | Gene |
|---|---|---|---|
| *Neisseria* | Invasive disease | MAC deficiency | *C5, C6, C7, C8A, C8B, C8G, C9* |
| | Invasive disease | Properdin deficiency | *PFC* |
| *Mycobacteria* | MSMD | IL-12/23–IFN-γ deficiency | *IFNGR1, IFNGR2, STAT1, NEMO, IL12B, IL12RB1* |
| | Disseminated tuberculosis | | |
| *Streptococcus pneumoniae* | Invasive disease | IRAK-4 deficiency | *IRAK4* |
| Epstein-Barr virus | X-linked lymphoproliferative disease | SAP deficiency | *SH2D1A* |
| Human papillomavirus | Epidermodysplasia verruciformis | EVER1 or EVER2 deficiency | *EVER1, EVER2* |
| *Plasmodium vivax* | Natural resistance | Lack of receptor for pathogen | *DARC* |
| Human immunodeficiency virus-1 | Natural resistance | Lack of receptor for pathogen | *CCR5* |
| Norovirus | Natural resistance | Lack of receptor for pathogen | *FUT2* |

Picard et al Curr Opin Immunol 2006

## Power of GWAS




Jérémy Manry and Lluis Quintana-Murci
*Cold Spring Harb Perspect Med* 2013; doi: 10.1101/cshperspect.a012450

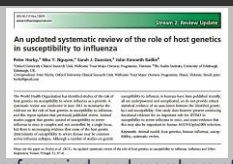
# Komplexní dědičnost: GWAS a infekce u lidí

**Table 1 Genetic loci identified by genome-wide association studies for host susceptibility to infectious diseases**

| Disease | Pathogen | Gene or locus | Biological mechanism |
|---|---|---|---|
| AIDS[1] | Human immunodeficiency virus-1 | Major histocompatibility complex, class I (*HLA-B–HLA-C*), *CCR5* | Acquired immunity, deletion of viral co-receptor |
| Hepatitis B[2] | Hepatitis B virus (HBV) | Major histocompatibility complex, class II (*HLA-DP*) | Acquired immunity |
| Hepatitis C[3,4] | Hepatitis C virus (HCV) | *IL28B* | Innate immunity |
| Leprosy[5] | *Mycobacterium leprae* | Major histocompatibility complex, class II (*HLA-DR–DQ*), *NOD2*, *TNFSF15*, *RIPK2*, *CCDC122* and *C13orf31*) | Acquired and innate immunity, and unknown mechanisms |
| Tuberculosis[6] | *Mycobacterium tuberculosis* | 18q11.2 (*GATA6*, *CTAGE1*, *RBBP8*, *CABLES1*) | Unknown |
| Meningococcal disease[7] | *Neisseria meningitidis* | *CFH*, *CFHR3*, *CFHR1* | Innate immunity |

De Bakker, Telenti 2010

# Příklady kandidátních genů: *chřipka a Mx*


An updated systematic review of the role of host genetics in susceptibility to influenza

- ✓ Interferon-induced members of the dynamin superfamily of large GTPases
- ✓ Key mediators of innate antiviral resistance induced in cells by type I (a/b) and type III (l) interferons
- ✓ They inhibit a wide range of viruses by blocking an early stage of the replication cycle
- ✓ Present in most vertebrate species

(Haller et al. Microbes Infect 2007)

# Příklady kandidátních genů: *flaviviry a OAS1*

- ✓ Coding for 2' - 5' - oligoadenylate synthase, activated by dsRNA
- ✓ Identified in mice as flavivirus resistance gene
- ✓ Exon 4 mutation leading to a STOP codon causes susceptibility to infection
- ✓ In the horse, SNPs within the gene associated with anti-WNV antibody production (Rios et al., 2009)

# Cave: Příklady kandidátních genů: *NRAMP1 a TB*

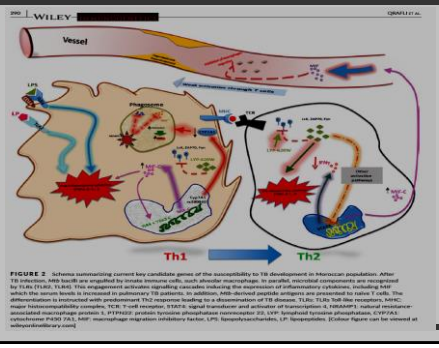



FIGURE 2 Schema summarizing current key candidate genes of the susceptibility to TB development in Moroccan population. After TB infection, Mtb bacilli are engulfed by innate immune cells, such alveolar macrophage. In parallel, microbial components are recognized by TLRs (TLR2, TLR4). This engagement activates signalling cascades inducing the expression of inflammatory cytokines, including MIF which the serum levels is increased in pulmonary TB patients. In addition, MIB-derived peptide antigens are presented to naive T cells. The differentiation is instructed with predominant Th2 response leading to a dissemination of TB disease. TLRs: TLRs Toll-like receptors, MHC: major histocompatibility complex, TCR: T-cell receptor, STAT4: signal transducer and activator of transcription 4, NRAMP1: natural resistance-associated macrophage protein 1, PTPN22: protein tyrosine phosphatase nonreceptor 22, LYP: lymphoid tyrosine phosphatase, CYP2A13: cytochrome P450 2A13, MIF: macrophage migration inhibitory factor, LPS: lipopolysaccharides, LP: lipopeptides. [Colour figure can be viewed at wileyonlinelibrary.com]

## Příklady: FCoV/FIP

- ✓Coronaviry jako model proměnlivých patogenů
- ✓Coronavirové infekce lidí a zvířat: SARS, MERS
- ✓Kočičí model komplexní etiopatologie

Ke všem příkladům: viz Modelové nemoci

## Molekulární disekce imunity

- ✓ Identifikace genů a drah
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## Molekulární disekce imunity

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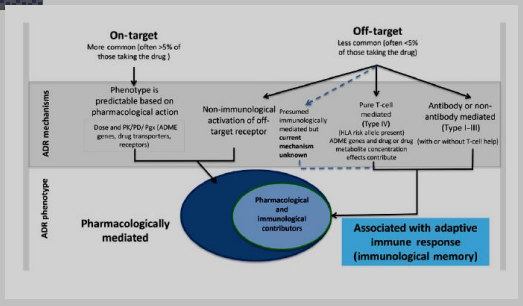
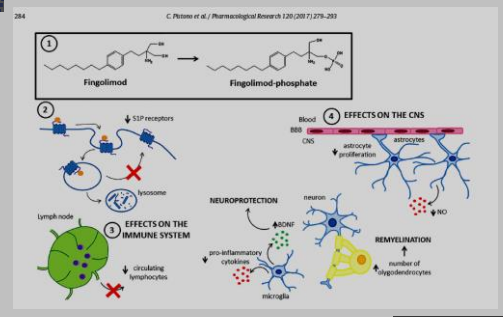
## Farmakogenetika: ADR



| Drug | DHR | HLA risk alleles | PPV | NPV | Populations |
|---|---|---|---|---|---|
| Abacavir | HSS/DIHS | B*57:01[88,115,116] | 59% | 100% | European, African |
| Carbamazepine | SJS/TEN | B*15:02[79-81] | 3% | 100% in Han Chinese | Han Chinese, Thai, Malaysian, Indian |
| | | B*15:11[115,116] | | | Korean, Japanese |
| | | B*15:18, B*59:01 and C*07:04[85] | | | Japanese |
| | | B*15:21[107] | | | |
| | | A*31:01[116,118-120] | | | Japanese, northern European, Korean |
| | HSS/DIHS/ DRESS | 8.1 AH (HLA A*01:01, Cw*07:01, B*08:01, DRB1*03:01, DQA1*05:01, DQB1*02:01)[121] | | | Caucasians |
| | | A*31:01[122] | 0.89% | 99.98% | Europeans |
| | | A*31:01[122] | 0.59% | 99.97% | Chinese |
| | | A*31:01[116,118-120] | | | Northern Europeans, Japanese, and Korean |
| | | A*11 and B*51 (weak)[120] | | | Japanese |
| | MPE | A*31:01[123] | 34.9% | 96.7% | |
| | Any ADR | A*31:01[124] | | | |
| Allopurinol | SJS/TEN/DIHS/DRES S/MPE | B*58:01 (or B*58 haplotype)[81,125-131] | 3% | 100% in Han Chinese | Han Chinese, Thai, European, Italian, Korean |
| Oxcarbazepine | SJS/TEN | B*15:02 and B*15:18[132-134] | 15.02 - 0.73% | 15.02 -99.97 | Han Chinese, Taiwanese |
| Lamotrigine | SJS/TEN | B*15:02 (positive)[135] | | | Han Chinese |
| | | B*15:02 (no association)[135,136] | | | Han Chinese |
| Phenytoin | SJS/TEN | B*15:02(weak), Cw*08:01 and DRB1*16:02[71,72,137] | | | Han Chinese |
| | DRESS/MPE | B*13:01 (weak) | | | Han Chinese |
| | | B*5101 (weak)[127] | | | |
| Nevirapine | SJS/TEN | C*04:01[138] | | | Malawian |
| | HSS/DIHS/DRESS | DRB1*01:01 & DRB1*01:02 (hepatitis and low CD4+)[74,139] | 18% | 96% | Australian, European and South African |
| | | Cw*8 or Cw*8-B*14 haplotype[69,140] | | | Italian and Japanese |
| | | Cw*4[141,142] | | | Blacks: Asians, Whites: Han Chinese |
| | | B*35[71]<br>B*35.01[86]<br>B*35.05[143] | 16% | 97% | Asian |
| | Delayed rash | DRB1*01[144] | | | French |
| | | Cw*04[145] | | | African, Asian, European, and Thai |
| | | B*35.05[143] | | | Thai |
| Dapsone | HSS | B*13:01[146] | 7.8% | 99.8% | |
| Efavirenz | Delayed rash | DRB1*01[144] | | | French |
| Sulfamethoxazole | SJS/TEN | B*38[84] | | | European |
| Amoxicillin-clavulanate | DILI | DRB1*15:01<br>A*02:01<br>DQB1*06:02,<br>and rs3135388, a tag SNP of DRB1*15:01-DQB1*06:02<br>DRB1*07 and HLA-A1 (protective)[145,147] | | | European |
| Lumiracoxib | DILI | DRB1*15:01-DQB1*06:02-DRB5*01:01-DQA1*01:02 haplotype[148] | | | International, multi-center |
| Ximelagatran | DILI | DRB1*07 and DQA1*02[149] | | | Swedish |
| Diclofenac | DILI | HLA-A1[150] | | | European |

## MS a ADR




## MS a ADR

Table 2
HLA alleles related to a different response to the injectable drugs employed in MS treatment. Nabs= neutralizing anti-drug antibodies; OR= Odd Ratio.

| Drug | Genes | Alleles | Response to treatment | OR | Studied populations | References |
|---|---|---|---|---|---|---|
| Natalizumab | HLA-DRB1 | DRB1*13<br>DRB1*14 | Higher risk of developing natalizumab-related anaphylactic/anaphylactoid reactions | 8.06 | French, Spanish, German | [141] |
| | HLA-DRB1 | DRB1*15 | Protective effect on the development of anaphylactic/anaphylactoid reactions | 0.2 | | |
| Glatiramer acetate | HLA DRB1<br>HLA-DRB1<br>HLA-DRB1<br>HLA-DQB1<br>HLA-DRB1<br>HLA-DQB1 | DRB1*1501<br>DRB1*1501<br>DR15<br>DQ6<br>DR17<br>DQ2 | Better response<br>Better response<br>Positive response<br>Positive response<br>No response<br>No response | –<br>–<br>–<br>–<br>–<br>– | Italian<br>American<br>American | [144]<br>[145]<br>[146] |
| Interferon β | HLA-DRB1 | DRB1*0401<br>DRB1*0408 | Increased risk to produce NAbs | 5.15\|9.6 | German | [151] |
| | HLA-DRB1 | DRB1*04:01<br>DRB1*04:08<br>DRB1*16:01 | Increased risk to produce NAbs | – | German | [152] |
| | HLA-DRB1 | DRB1*15 | Risk to produce biologically relevant NAb titers following intramuscular IFNβ-1a administration | 4.36 | Swedish | [153] |
| | | | High risk to produce NAbs titres following subcutaneous IFNβ-1a administration | 4.15 | | |
| | | | Risk to produce biologically relevant NAb titres following subcutaneous IFNβ-1a administration | 8.16 | | |
| | HLA-DQA1 | DQA1*05 | Decreased risk to produce biologically relevant NAb titres following subcutaneous IFNβ-1a administration | 0.29 | | |
| | HLA-DRB1 | DRB1*04 | High risk to produce NAbs following subcutaneous IFNβ-1b administration | 3.53 | | |

## Zmatení pojmů

# Farmakogenomika a farmakogenetika

**Table 1** Terminology.

- Pharmacogenetics
  - Differential effects of a drug – *in vivo* – in different patients, dependent on the presence of inherited gene variants
  - Assessed primarily genetic (SNP) and genomic (expression) approaches
  - A concept to provide more patient-/disease-specific health care
  - One drug – many genomes (*i.e.*, differnt patients)
  - Focus: patient variability
- Pharmacogenomics:
  - Differential effects of compounds – *in vivo* or *in vitro* – on gene expression, among the entirety of expressed genes
  - Assessed by expression profiling
  - A tool for compound selection/drug discovery
  - Many "drugs" (*i.e.*, early-stage compounds) – one genome (*i.e.*, "normative" genome [database, technology platform])
  - Focus: compound variability

Lindpaintner, 2003

# Farmakogenomika v produkci léčiv

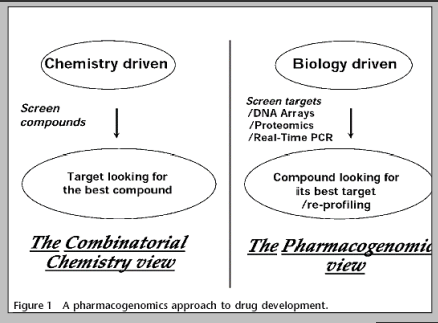



Figure 1 A pharmacogenomics approach to drug development.

Grenet, 2002

# Farmakogenomika a transgenoze

- Rekombinantní produkty mikroorganismů
- DNA vakcíny
- Transgenní savci

# Infekční onemocnění: vakcinace

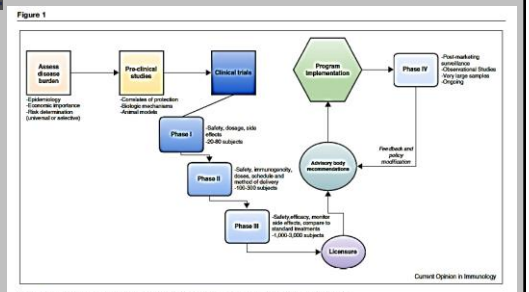



Figure 1

Diagram illustrating vaccine development process from risk assessment to program implementation.

## Vakcinace a genetika

- Využití genetických principů při produkci nových vakcín, farmakogenomika
- Individuální variabilita imunitní odpovědi po vakcinaci

## PRINCIP VAKCÍN

***Odstranění patogenity při zachování imunogenity***

## TYPY VAKCÍN

- *1. generace*
- *2. generace (rekombinantní)*
- *3. generace (DNA)*

## VAKCÍNY 1. GENERACE

- *Živé atenuované*
- *Inaktivované (usmrcené)*

## PRINCIP ATENUACE VAKCÍNY

**MUTACE**

*v genu/genech pro patogenitu*

## VAKCÍNY 2. GENERACE

- *Podjednotkové*
- *Deleční*
- *Deleční markerové*
- *Živé chimérické*
- *Antiidiotypové*

## PODJEDNOTKOVÉ VAKCÍNY

*Gen kódující antigenní protein je vložen do baktérie, která následně produkuje čistý antigen*

## DELEČNÍ VAKCÍNY

*Cílenou mutagenezí je odstraněn gen/genová oblast zodpovědná za patogenní efekt*

## DELEČNÍ MARKEROVÉ VAKCÍNY

*Cílenou mutagenezí je odstraněn/vložen/ nejen gen/genová oblast zodpovědná za patogenní efekt, ale i gen pro dobře zjistitelný antigenní protein (marker)*

*Lze tedy odlišit postinfekční a postvakcinační imunitu*

## ŽIVÉ CHIMÉRICKÉ VAKCÍNY

*Do genomu vektoru je vložen gen/genová oblast kódující antigenní protein(y)*

## VAKCÍNY 3. GENERACE

*DNA vakcíny: do organismu se nevpravuje antigen, ale gen, který jej kóduje*

*DNA ==> RNA ==> Protein (cizí)*
*==> Imunizace in vivo*

## DNA VAKCÍNY

*Indikovány k využití když:*

- *je antigenní protein obtížně purifikovatelný nebo je purifikací zničen*
- *antigenní protein není znám, i když známe gen*
- *protilátky produkované po DNA imunizaci mohou sloužit k purifikaci proteinu*
- *slabě imunogenní proteiny získají imunogenitu po fúzi s genem pro vysoce imunogenní protein*

## MODULACE IMUNITNÍ ODPOVĚDI PŘI DNA IMUNIZACI

- *Geny pro cytokiny*
- *Adjuvantní efekt DNA*

## Farmakogenomika a transgenoze

- Transgenní savci

## TRANSGENNÍ SAVCI

- *Genové konstrukty*
- *Genové konstrukty s tkáňově specifickými promotory*

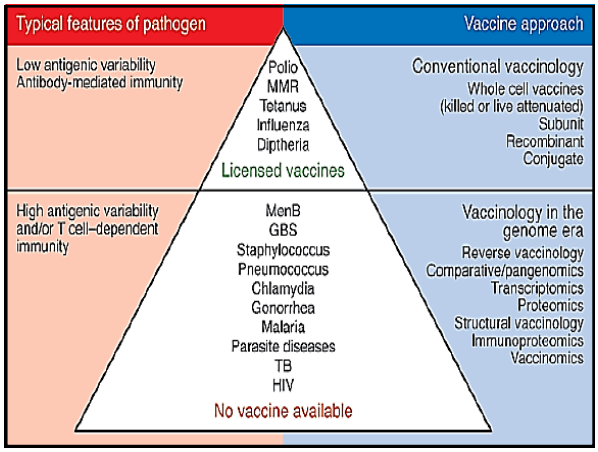
## TRANSGENNÍ SAVCI

- 
- *Genové konstrukty s tkáňově specifickými promotory*

# TRANSGENNÍ SAVCI

***s tkáňově specifickými promotory:***

## *BIOREAKTORY*

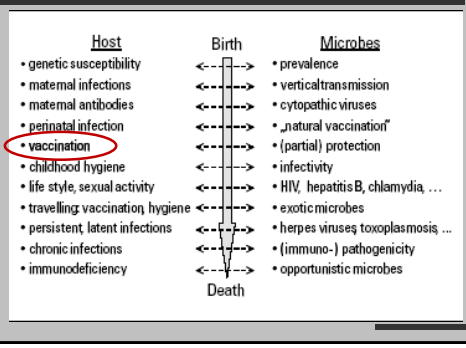



# Vakcinace a genetika

- Využití genetických principů při produkci nových vakcín, farmakogenomika

- **Individuální variabilita imunitní odpovědi po vakcinaci**

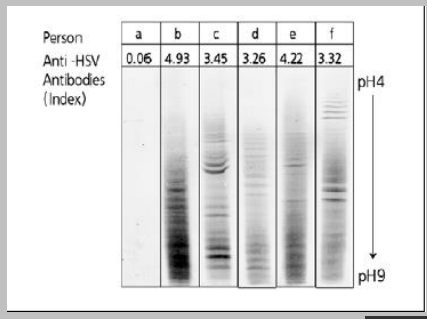
# Člověk a infekce



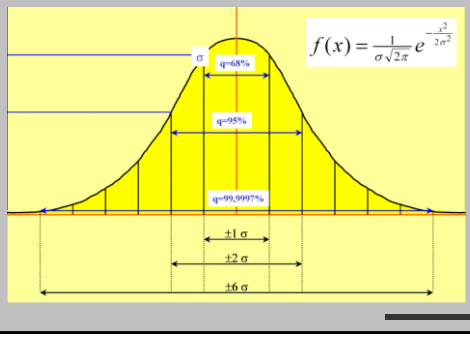
## *Příčiny selhání účinku vakcinace proti chřipce*

- **Druh vakcíny a její kvalita**
- **Změna viru**
- ***Nereaktivita hostitele***

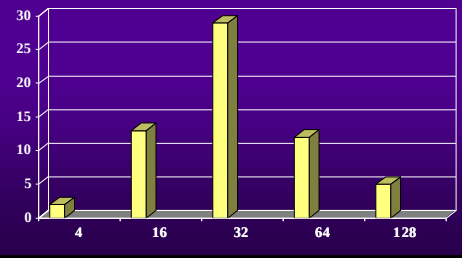
## Variabilita antiinfekční imunitní odpovědi u člověka

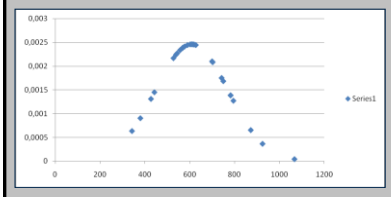



## Výsledek: Gaussovská distribuce

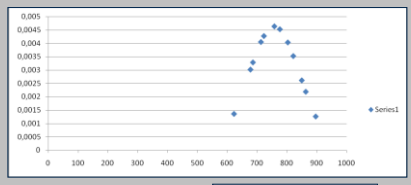



## POSTVAKCINAČNÍ TITRY ANTI-EHV-1 NEUTRALIZAČNÍCH PROTILÁTEK (N=61)






ODCHOV 3. ROČ.




Kmenový plemeník

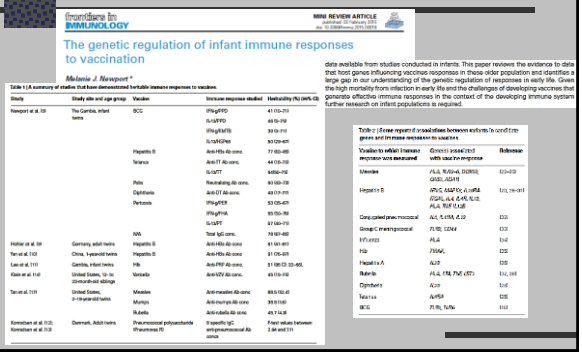
## Genetics of vaccination

**Table 1.** Host mechanisms involved in vaccine-induced immune responses

| Function | Genes (examples) |
|---|---|
| Attachment, entry, replication (live vaccines) | CD150/SLAM |
| Antigen recognition | Toll-like receptors |
| Antigen uptake by and activation of innate immune system | HLA class III genes (complement proteins C3 and C4), costimulatory molecules (CD80, CD86), CD21, CD35, killer Ig-like receptors |
| Antigen processing and presentation | HLA class I/II genes, TAP, CD21, CD35 |
| B/T lymphocyte function | CD40, CD40 ligand, B/T cell receptors, G proteins |
| Immune regulation | Cytokines, monokines, C-C chemokines (and receptors) |
| Effector and accessory cell function | Fc receptors |

SLAM = Signaling lymphocyte activation molecule, identified as measles virus receptor.

## Infekční onemocnění: vakcinace



frontiers in IMMUNOLOGY

MINI REVIEW ARTICLE

The genetic regulation of infant immune responses to vaccination

*Melanie J. Newport **

## Genetics of vaccination

**Table 3.** Heritability estimates of vaccination responses in twin studies

| Vaccine | Parameter | DZ[a] | MZ[a] | Population | Age | Study | Heritability, % | 95% CI % | References |
|---|---|---|---|---|---|---|---|---|---|
| Measles | antibody | 55 | 45 | USA[b] | 2–18 years | cross-sectional | 89 | ≥ 52[c] | 18 |
| Mumps | antibody | 55 | 45 | USA[b] | 2–18 years | cross-sectional | 39 | ≥ 2[c] | 18 |
| Rubella | antibody | 55 | 45 | USA[b] | 2–18 years | cross-sectional | 46 | ≥ 5[c] | 18 |
| HAV | antibody | 95 | 96 | Germany | 18–65 years | prospective | 36 | –2–73 | 15 |
| HBsAg | antibody | 95 | 96 | Germany | 18–65 years | prospective | 61 | 41–81 | 15 |
| HBsAg | antibody | 159 | 48 | Gambia | 5 months | prospective | 77 | 63–85 | 12[d] |
| Polio | antibody | 159 | 48 | Gambia | 5 months | prospective | 60 | 43–73 | 12 |
| Tetanus | antibody | 159 | 48 | Gambia | 5 months | prospective | 44 | 16–70 | 12 |
| Tetanus | IL-13 | 159 | 48 | Gambia | 5 months | prospective | 64 | 50–75 | 12 |
| Diphtheria | antibody | 159 | 48 | Gambia | 5 months | prospective | 49 | 17–77 | 12 |
| Hib | antibody | 147 | 43 | Gambia | 5 months | prospective | 51 | 32–66 | 14 |
| Pertussis | | | | | | | | | |
| Pertactin | IFN-γ | 159 | 48 | Gambia | 5 months | prospective | 53 | 35–67 | 12 |
| FHA | IFN-γ | 159 | 48 | Gambia | 5 months | prospective | 65 | 50–76 | 12 |
| Toxin | IL-13 | 159 | 48 | Gambia | 5 months | prospective | 57 | 40–71 | 12 |
| BCG | | | | | | | | | |
| PPD | IFN-γ | 159 | 48 | Gambia | 5 months | prospective | 41 | 10–71 | 12 |
| KMTB | IFN-γ | 159 | 48 | Gambia | 5 months | prospective | 39 | 3–71 | 12 |
| PPD | IL-13 | 159 | 48 | Gambia | 5 months | prospective | 46 | 5–75 | 12 |
| Hsp65 | IL-13 | 159 | 48 | Gambia | 5 months | prospective | 50 | 29–67 | 12 |

## HLA a spalničky

Associations between HLA gene polymorphisms and humoral (IgG antibody) and cellular (lymphoproliferation) immune responses to measles vaccine

| HLA gene | Variant | Effect | Reference |
|---|---|---|---|
| Class I allele | B*8, B*13, B*44 | Decreased antibody (single dose) | [14] |
| Class I allele | B*7 | Increased antibody (single dose) | |
| Class II allele | DRB1*03, DQA1*0201 | Decreased antibody (single dose) | [15] |
| Class II allele | DRB1*08, DQA1*0104, DPA1*0202 | Increased antibody (single dose) | [16] |
| Class I supertype | B7 | Increased antibody (two doses) | [17] |
| Class I supertype | B44 | Decreased antibody (two doses) | |
| Class I supertype | B58 | Decreased antibody (two doses) | |
| Class I haplotype | A*24-C*03-B*15 | Decreased antibody (two doses) | [18] |
| Class II haplotype | DRB1*07-DQB1*03-DPB1*04 | Decreased antibody (two doses) | [18] |
| Class II haplotype | DRB1*07-DQB1*02-DPB1*02 | Decreased antibody (two doses) | |
| Class II haplotype | DRB1*15/16-DQB1*06-DPB1*04 | Increased antibody (two doses) | |
| Class I haplotype | A*26-C*12-B*38 | Increased cellular (two doses) | [18] |
| Class II haplotype | DRB1*04-DQB1*03-DPB1*03 | Increased cellular (two doses) | [18] |
| Class II haplotype | DRB1*03-DQB1*02-DPB1*04 | Increased cellular (two doses) | |

Cytokine and cytokine receptor SNP associations with humora (IgG antibody) and cellular (lymphoproliferation) immune responses to measles vaccine

| Gene | SNP ID | Genotype | Phenotype |
|---|---|---|---|
| IL-2 | rs2069762<br>rs2069763 | GG/TG/TT<br>GG/TG/TT | Antibody$^{high}$Cellular$^{high}$<br>Antibody$^{high}$Cellular$^{high}$ |
| IL-10 | rs1800890<br>rs1800871<br>rs1800872* | AA/TA/TT<br>AA/GA/GG<br>AA/CA/CC | Antibody$^{low}$Cellular$^{low}$<br>Antibody$^{low}$Cellular$^{low}$<br>Antibody$^{low}$Cellular$^{low}$ |
| IL-12RB | rs3790567<br>rs3729889 | AA/GA/GG<br>AA/AG/GG | Antibody$^{low}$Cellular$^{low}$<br>Antibody$^{low}$Cellular$^{low}$ |

| No. of measles vaccine doses (MMR-II) | HLA class I alleles | HLA class II alleles |
|---|---|---|
| 1 dose | B*8, B*13, B*44 | DRB1*03, DQA1*0201 |
| 2 doses | B*4403 | None |