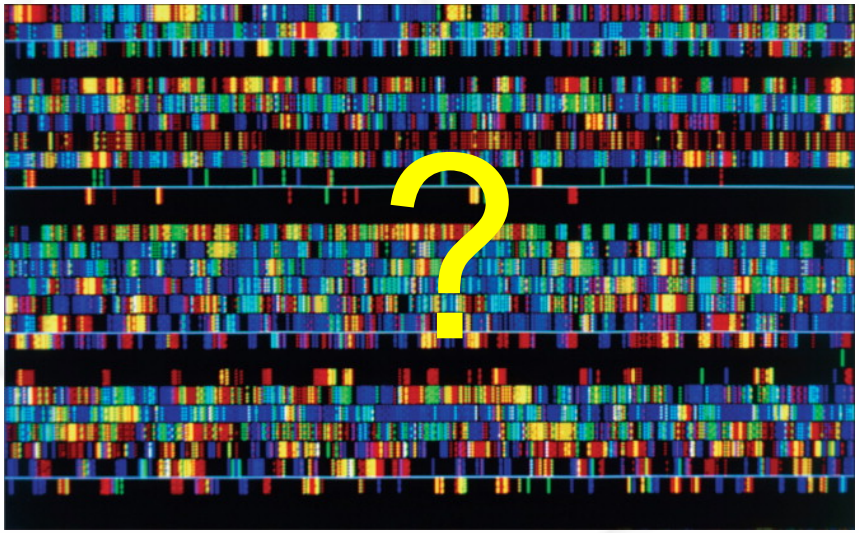
Sigurd K. Thoresen Foundation Seminar, August 26, 2008

Novel variants in multiple sclerosis



Human DNA sequence, IMSGC 2008

Åslaug R. Lorentzen MD

Department of Neurology,
Faculty Division
Ullevål University Hospital,
and
Institute of Immunology,
Rikshospitalet University Hospital

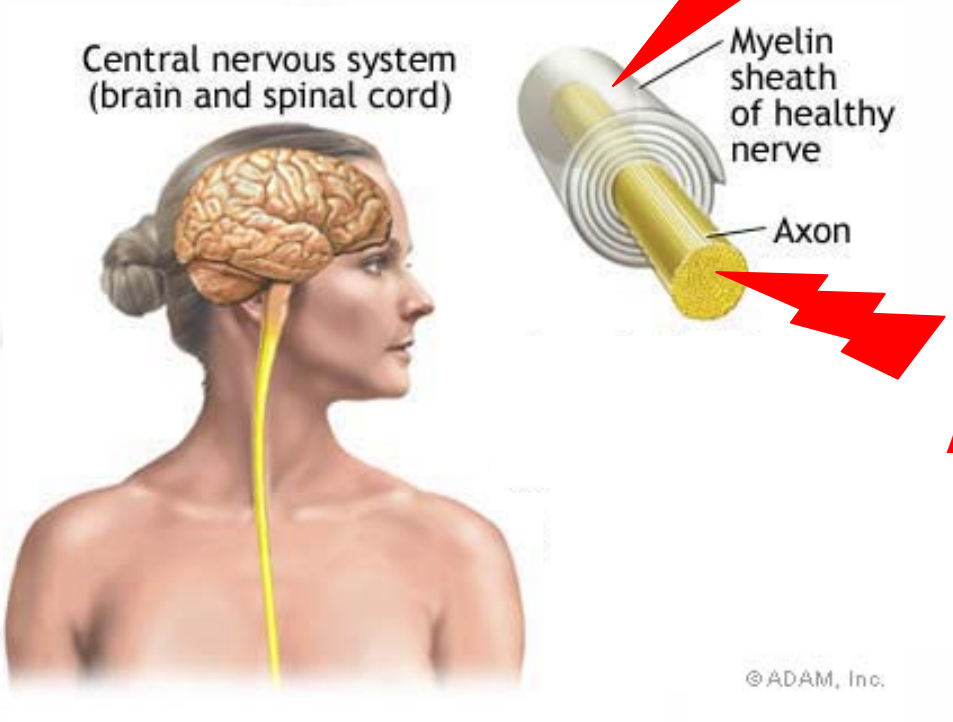
ULLEVÅL universitetssykehus

Bevegelsesdivisjon

Multiple sclerosis

Inflammation (relapsing-remitting MS)

Demyelination



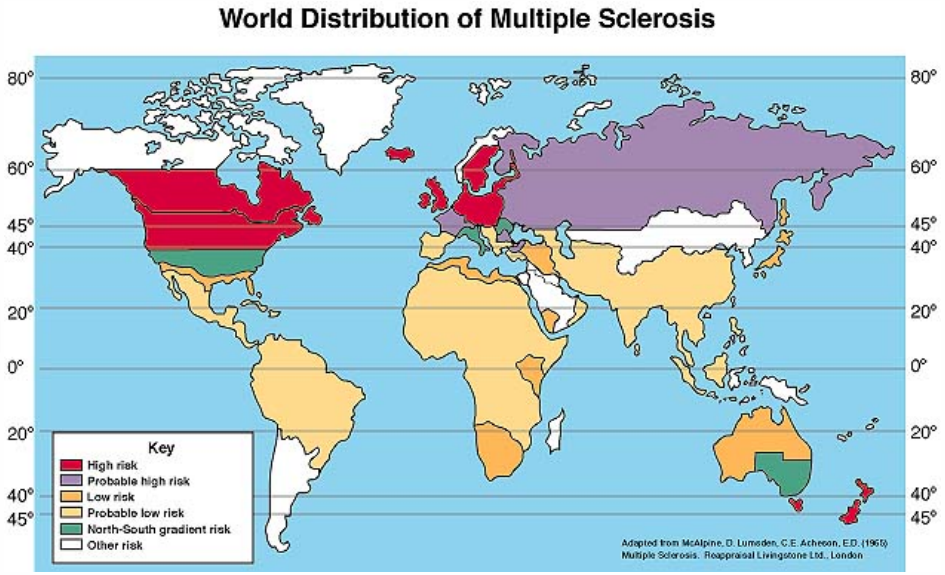
Degeneration (progressive MS)

Axonal damage

Environmental influences in MS

North-south gradient

Oslo: 170/ 100 000
(Smestad et al. 2007)



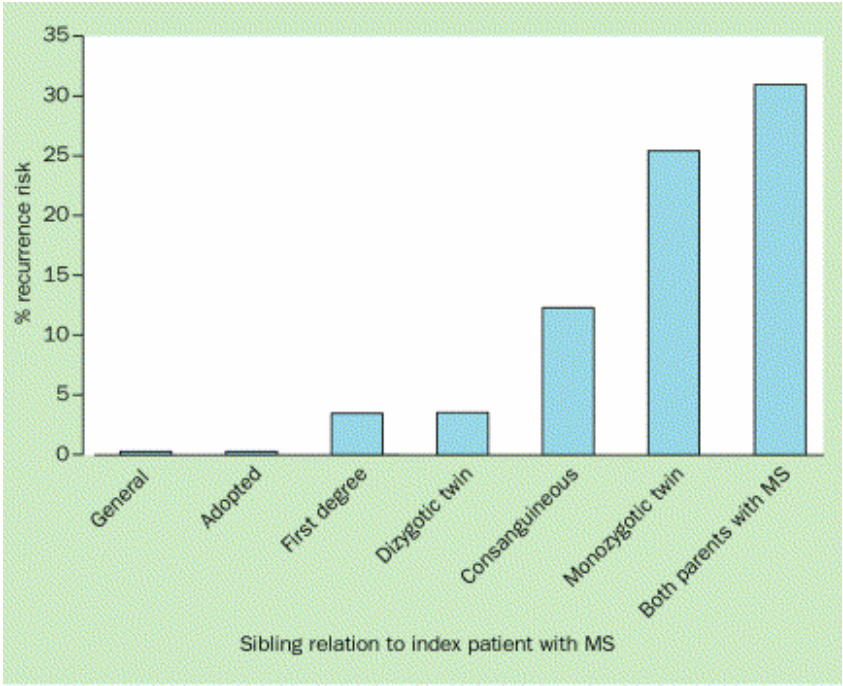
- Infectious agents?
  - EBV? *(Serafini et al. 2007)*

- Importance of life style, diet, sun exposure, climate?
  - Vitamin D? *(Ascherio, 2007)*
  - Smoking? *(Ascherio, 2008)*

Genetic influences: Familial clustering

- There is a genetic basis for familial aggregation in MS
- λ_s = the risk seen in sibling / general population

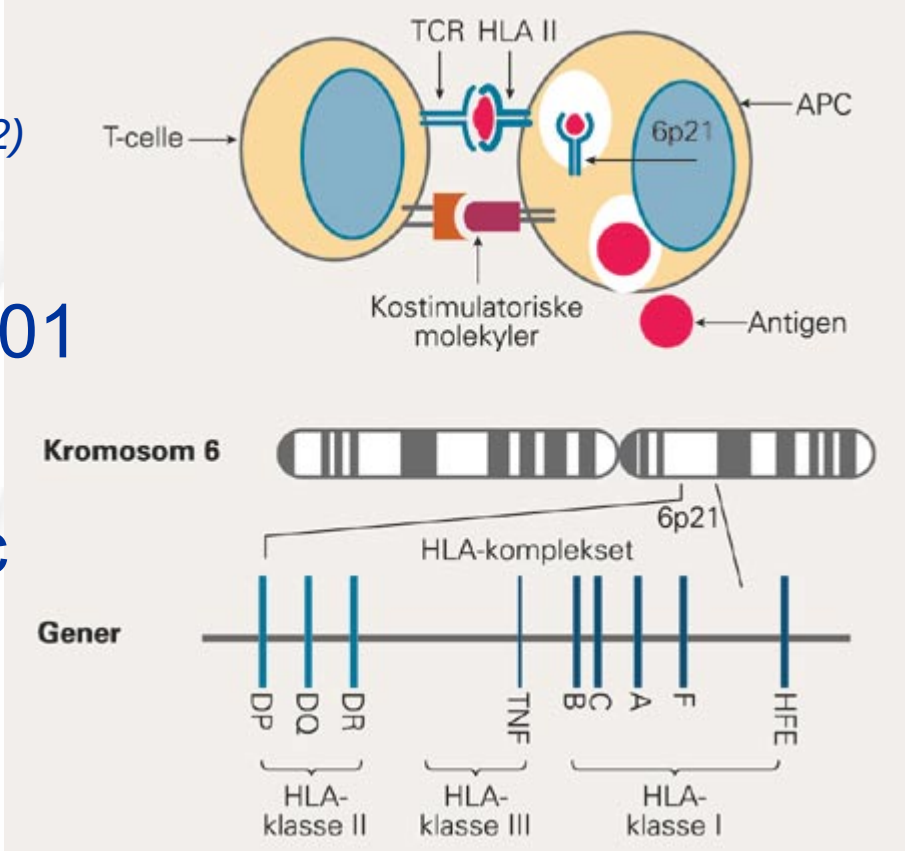
  In MS = 15-20



Dyment et al. 2004

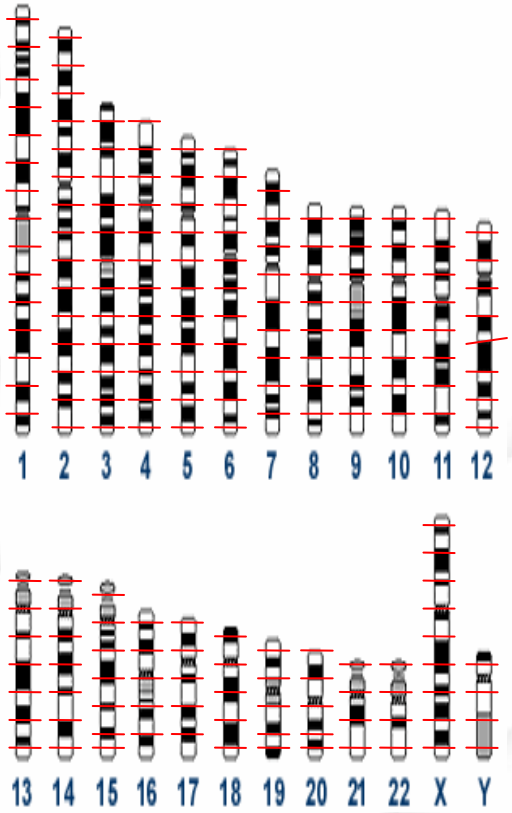
The early success - the HLA association

- Strongest genetic risk
  (Jersild et al. 1972) (Naito et al. 1972)
- DQB1*0602-DRB1*1501
- 20-60 % of the genetic susceptibility

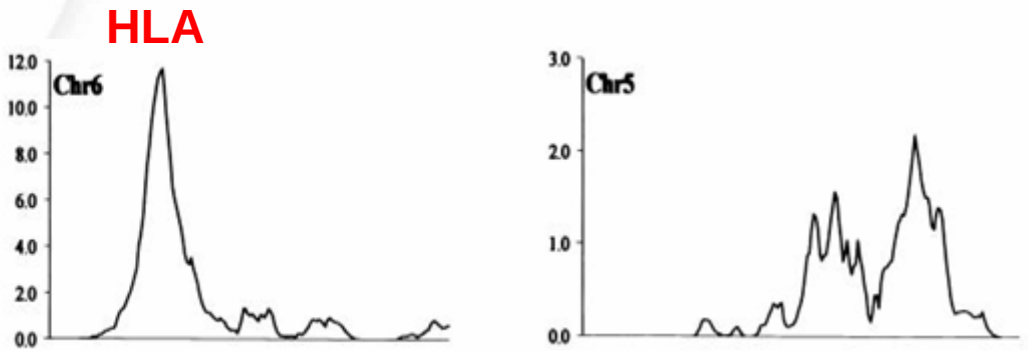


KM Myhr & HF Harbo, 2003

Up and down a rollercoaster



- Linkage screens
  - Collaboration studies *(GAMES, 1996-2003)*
  - High density linkage screen *(Sawcer et al. 2005)*



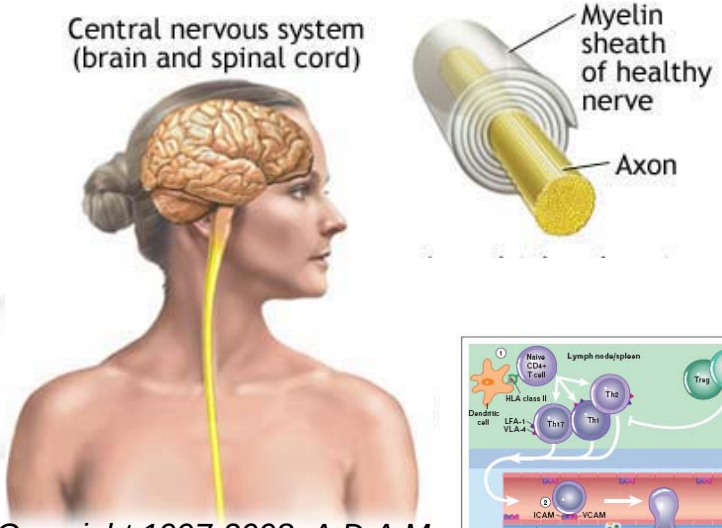
Candidate gene approach - Where to start?

Myelin components

PLP1, MAG, MOG

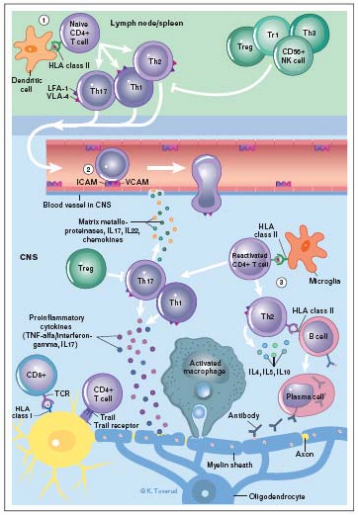
Neuroprotective and growth factors

TGFB1/2, BAX, BCL2, p53, ERBB, ApoE



Cytokines and immune molecules

HLA, TCR, Interleukin and receptors, STAT, CTLA4, SH2D2A, PTPN22, MPO, ICAM-1

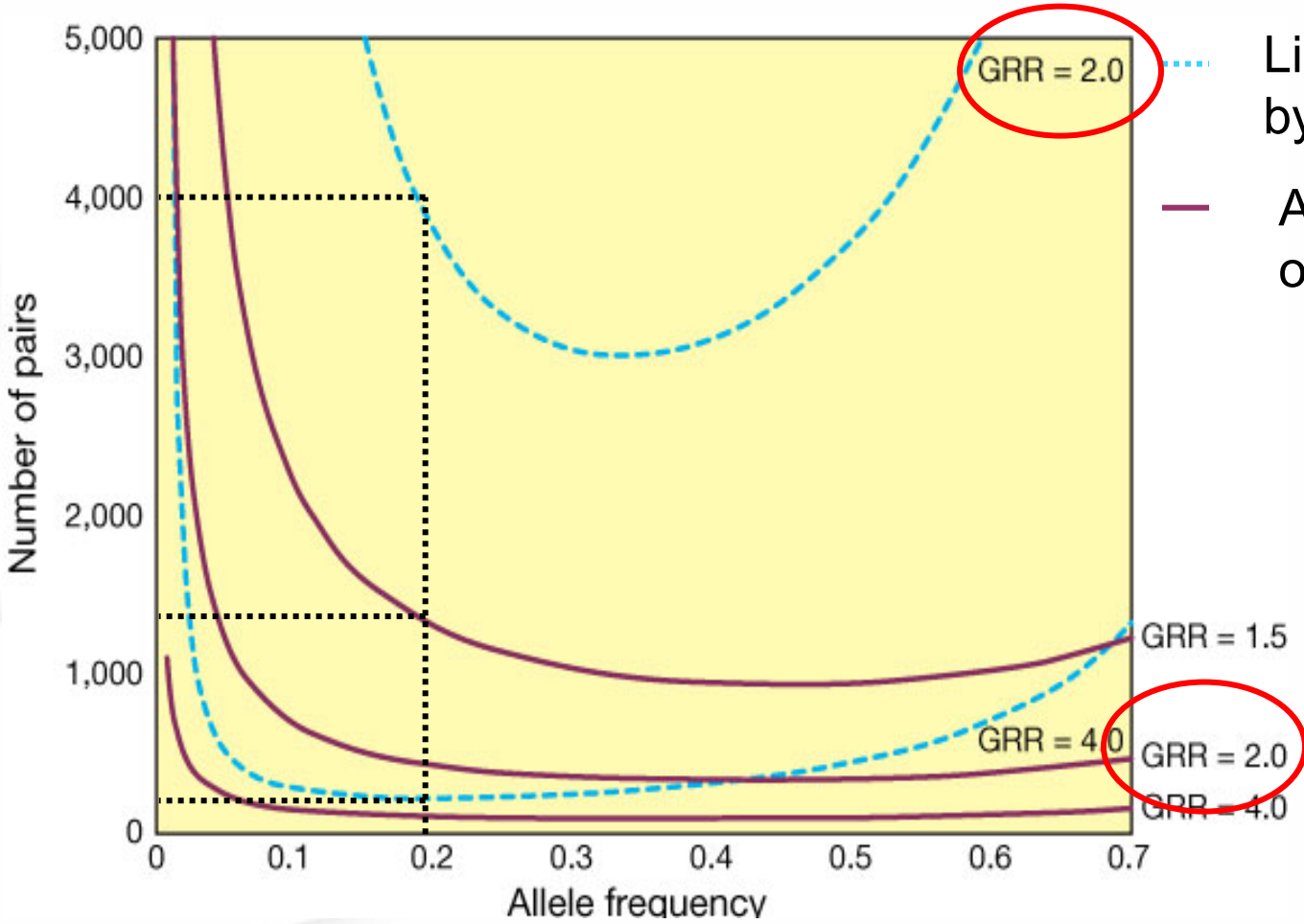


Holmøy & Hestvik 2008

Why so hard to find susceptibility genes in MS?

- Genes of modest risk
- Common disease / common variants
  - 20-100 genes (⇧ risk 1.2-1.5)
- Common disease / rare variants
  - 100 – 1000 (⇧ risk 10-20)

The importance of large sample sizes



Linkage analysis by use of sib-pairs

Association based on case-controls

Genotypic Relative Risk; The risk of disease for one genotype versus another at a locus

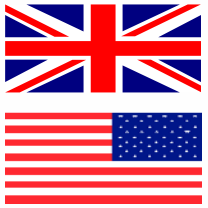
Risch, Nature, 2000

The NEW ENGLAND JOURNAL of MEDICINE

29th July 2007

Risk Alleles for Multiple Sclerosis Identified by a Genomewide Study

The International Multiple Sclerosis Genetics Consortium*



- 334,923 SNPs
- 931 TRIOs
- Replication: 609 TRIOs, 2322 cases and 789 controls

Top non-HLA results in MS GWA screen

Gene (NCBI ID)	Chromosomal position	Biological function(s)	GWA screen	Validation		Overall	
			Family[‡]	*Case–control*[§]	*Combined*[‖]	*Combined*[¶]	*Odds ratio*
IL2RA, interleukin 2 receptor, alpha (3559)	10p15	Apoptosis, immune response	1×10^{-3}	1×10^{-3}	5×10^{-4}	3×10^{-8}	1.25
IL7R, interleukin 7 receptor (16197)	5p13	Cell survival, immune response	6×10^{-3}	2×10^{-2}	3×10^{-5}	3×10^{-7}	1.18
CLEC16A, C-type lectin domain family 16, A (23274)	16p13	Sugar-binding, C-type lectin	3×10^{-2}	7×10^{-3}	2×10^{-5}	4×10^{-6}	1.14
RPL5, ribosomal protein L5 (6125)	1p22	Ribosomal protein, chaperone for the 5S rRNA	4×10^{-4}	2×10^{-4}	9×10^{-4}	8×10^{-6}	1.15
DBC1, deleted in bladder cancer 1 (1620)	9q33	Cell-cycle arrest, apoptosis	1×10^{-4}	2×10^{-4}	1×10^{-3}	8×10^{-6}	1.17
CD58, lymphocyte function-associated antigen 3 (965)	1p13	Cell–cell adhesion, immune response	1×10^{-3}	3×10^{-5}	2×10^{-3}	2×10^{-5}	1.24
ALK, anaplastic lymphoma receptor tyrosine kinase (238)	2p23	Tyrosine kinase receptor, brain development	1×10^{-4}	1×10^{-2}	3×10^{-3}	7×10^{-5}	1.37
FAM69A, family with sequence similarity 69, A (388650)	1p22	Protein binding	2×10^{-5}	2×10^{-2}	2×10^{-3}	9×10^{-5}	1.12

*Listed are the eight non-MHC SNPs showing the highest statistical evidence of association after replication as reported by the International Multiple Sclerosis Genetics Consortium. For additional results, consult REF 6. [‡]931 MS trios. [§]931 cases, 2,431 controls. [‖]609 MS trios, 2,322 MS cases, 2,987 controls. [¶]1,540 MS trios, 2,322 MS cases, 5,418 controls. GWA, genome-wide association; MHC, major histocompatibility complex; MS, multiple sclerosis; NCBI, National Center for Biotechnology Information.

HLA association (6p21) ($p=10^{-81}$, OR= 1.99) *Oksenberg et al. Review 2008*

Top non-MHC results in MS GWA screen

	Gene (NCBI ID)	Chromosomal position	Biological function(s)	GWA screen	Validation		Overall	
				Family[‡]	*Case–control*[§]	*Combined*[‖]	*Combined*[¶]	*Odds ratio*
★	*IL2RA*, interleukin 2 receptor, alpha (3559)	10p15	Apoptosis, immune response	1×10^{-3}	1×10^{-3}	5×10^{-4}	3×10^{-8}	1.25
★	*IL7R*, interleukin 7 receptor (16197)	5p13	Cell survival, immune response	6×10^{-3}	2×10^{-2}	3×10^{-5}	3×10^{-7}	1.18
★	*CLEC16A*, C-type lectin domain family 16, A (23274)	16p13	Sugar-binding, C-type lectin	3×10^{-2}	7×10^{-3}	2×10^{-5}	4×10^{-6}	1.14
★	*RPL5*, ribosomal protein L5 (6125)	1p22	Ribosomal protein, chaperone for the 5S rRNA	4×10^{-4}	2×10^{-4}	9×10^{-4}	8×10^{-6}	1.15
	DBC1, deleted in bladder cancer 1 (1620)	9q33	Cell-cycle arrest, apoptosis	1×10^{-4}	2×10^{-4}	1×10^{-3}	8×10^{-6}	1.17
★	*CD58*, lymphocyte function-associated antigen 3 (965)	1p13	Cell–cell adhesion, immune response	1×10^{-3}	3×10^{-5}	2×10^{-3}	2×10^{-5}	1.24
	ALK, anaplastic lymphoma receptor tyrosine kinase (238)	2p23	Tyrosine kinase receptor, brain development	1×10^{-4}	1×10^{-2}	3×10^{-3}	7×10^{-5}	1.37
	FAM69A, family with sequence similarity 69, A (388650)	1p22	Protein binding	2×10^{-5}	2×10^{-2}	2×10^{-3}	9×10^{-5}	1.12

*Listed are the eight non-MHC SNPs showing the highest statistical evidence of association after replication as reported by the International Multiple Sclerosis Genetics Consortium. For additional results, consult REF 6. [‡]931 MS trios. [§]931 cases, 2,431 controls. [‖]609 MS trios, 2,322 MS cases, 2,987 controls. [¶]1,540 MS trios, 2,322 MS cases, 5,418 controls. GWA, genome-wide association; MHC, major histocompatibility complex; MS, multiple sclerosis; NCBI, National Center for Biotechnology Information.

HLA association (6p21) ($p=10^{-81}$, OR= 1.99) *Oksenberg et al. Review 2008*

★ = replicated

ULLEVÅL universitetssykehus

Bevegelsesdivisjon

LETTERS

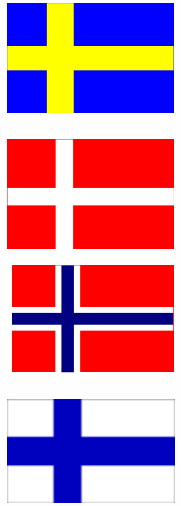
29th July 2007

nature genetics

Variation in interleukin 7 receptor α chain (*IL7R*) influences risk of multiple sclerosis

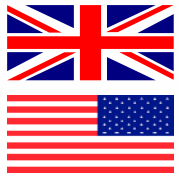
Frida Lundmark[1], Kristina Duvefelt[2], Ellen Iacobaeus[3], Ingrid Kockum[1,3], Erik Wallström[3], Mohsen Khademi[3], Annette Oturai[4], Lars P Ryder[5], Janna Saarela[6], Hanne F Harbo[7,8], Elisabeth G Celius[8], Hugh Salter[9], Tomas Olsson[3] & Jan Hillert[1]

nature genetics



Interleukin 7 receptor α chain (*IL7R*) shows allelic and functional association with multiple sclerosis

Simon G Gregory[1,9], Silke Schmidt[1,9], Puneet Seth[2], Jorge R Oksenberg[3], John Hart[1], Angela Prokop[1], Stacy J Caillier[3], Maria Ban[4], An Goris[5], Lisa F Barcellos[6], Robin Lincoln[3], Jacob L McCauley[7], Stephen J Sawcer[4], D A S Compston[4], Benedicte Dubois[5], Stephen L Hauser[3], Mariano A Garcia-Blanco[2], Margaret A Pericak-Vance[8] & Jonathan L Haines[7], for the Multiple Sclerosis Genetics Group



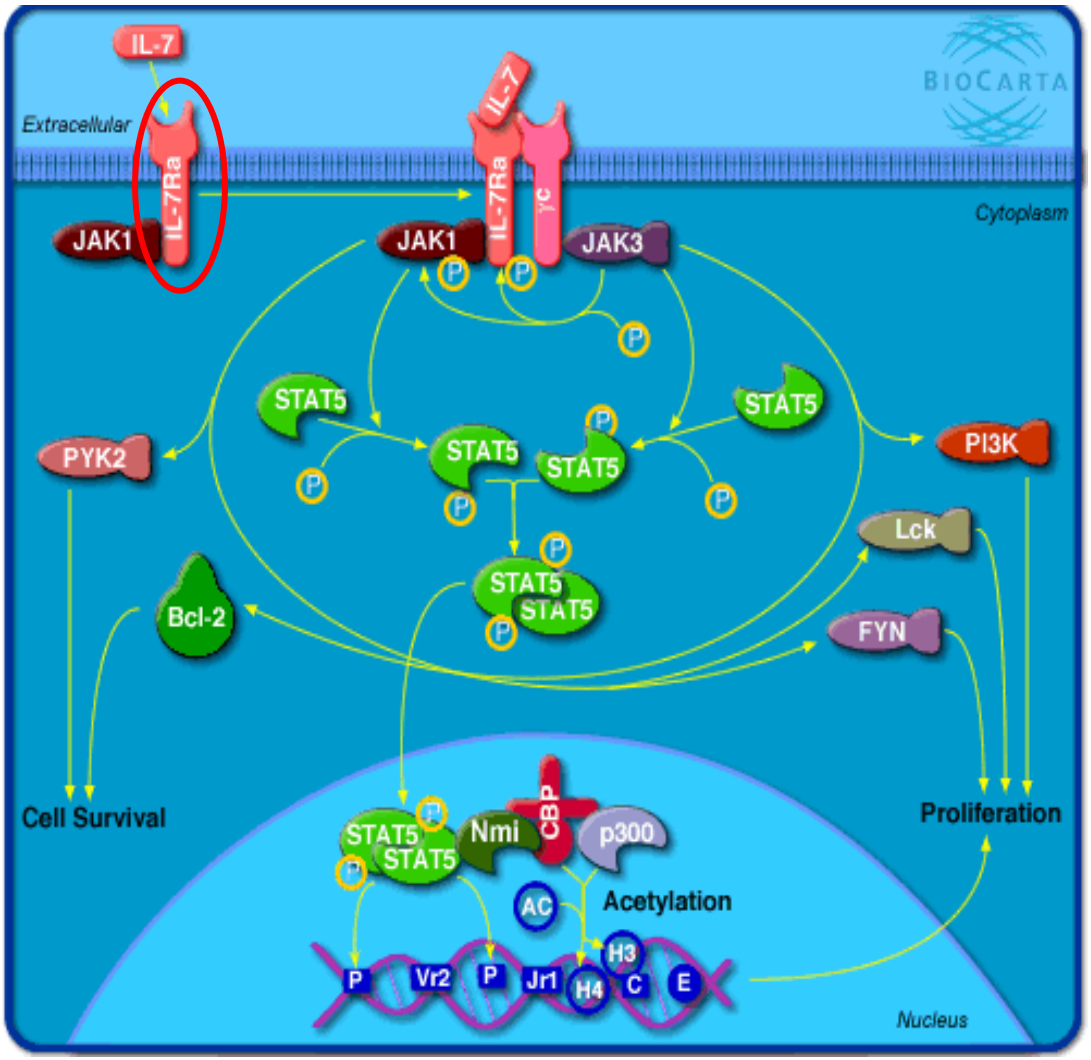
The Interleukin-7 receptor gene

- IL7Rgene:
  - Located at chromosome 5q13 (total 8 exons)
  - Causal SNP, rs6897932 (C/T), a ns coding SNP(T244I) located in exon 6
- A transmembrane protein, expressed in T cells
- Alternative splicing
  - Skip exon 6 -> soluble form of the protein
  - Include exon 6 -> membrane -bound IL7R
- “MS-associated” C-allele results in increased soluble form of the protein -> reduced function of the protein

The IL-7 receptor

- IL7R- mediated signalling is essential for the development and survival of T-lymphocytes

- More soluble IL7R may influence both innate and adaptive immune responses



http://www.biocarta.com/genes/index.asp

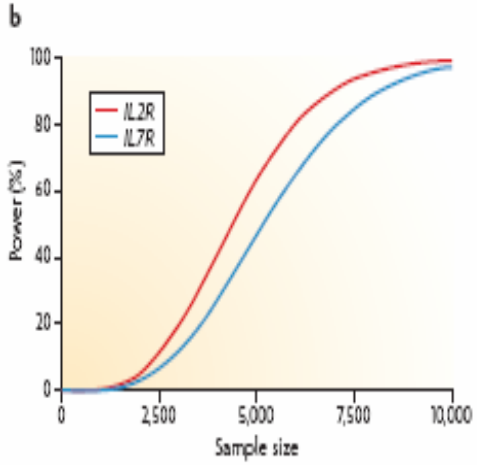
The Interleukin-2 receptor A gene

- IL2RA gene:
  - Located at chromosome 10p15 (total 8 exons)
  - Two associated SNPs
    - rs12722489, rs2104286 (both in intron 1)

- IL2R-mediated susceptibility effect is shared among other autoimmune diseases:
  - T1D, Graves disease, RA

Common disease mechanisms underlie different autoimmune conditions?

IL7R and IL2RA: the first definitely confirmed non-HLA susceptibility genes



Small effects need large sample size
Power for replication OR 1.2 and p= 5 x 10^{-7}
(Oksenberg et al. Review 2008)

Study by IMSGC:
Total 33 068 individuals (cases, controls, TRIOs)

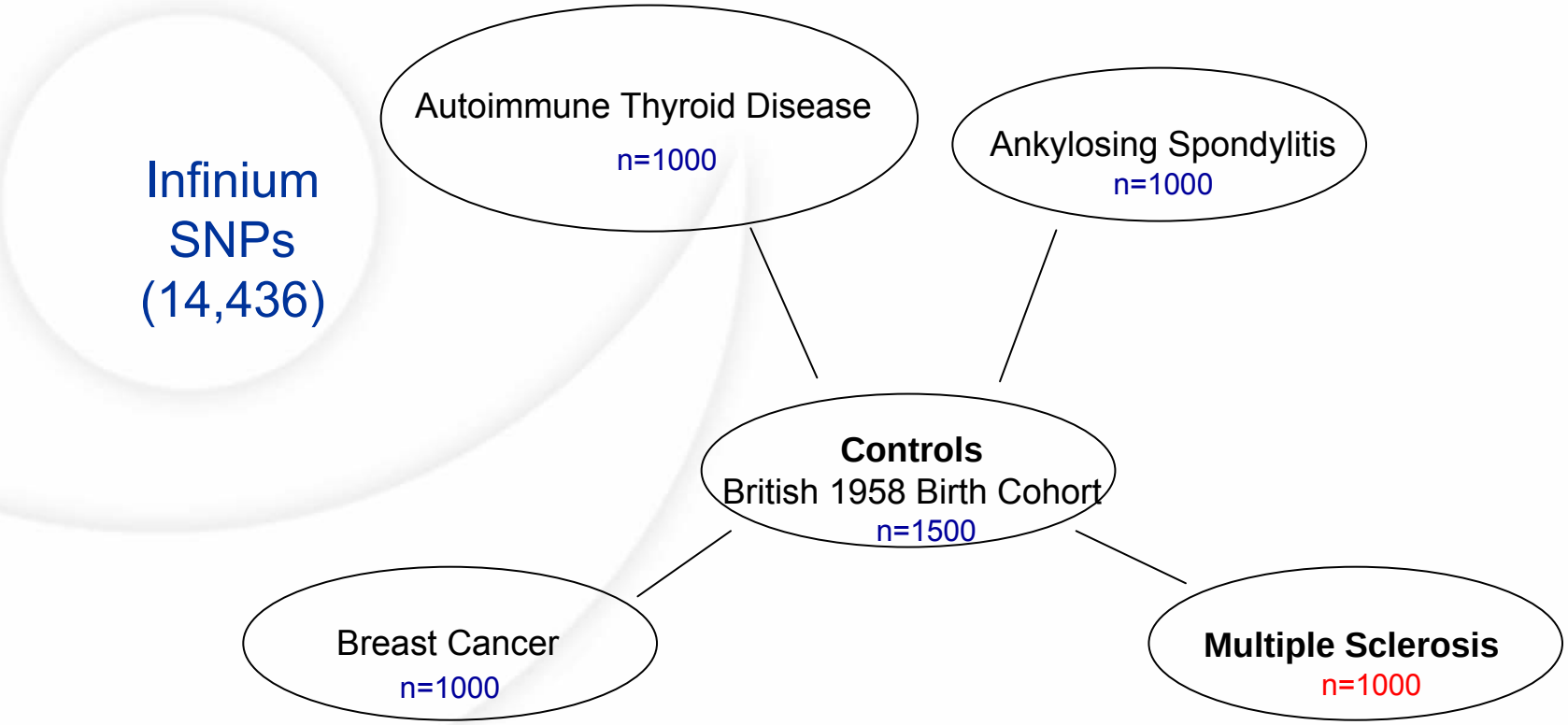
	χ^2	p	Odds ratio (95% CI)
C allele of rs6897932 (*IL7R*)			
Case-control*	73·14	$1{\cdot}21\times10^{-17}$	1·200 (1·151–1·252)
Triost	10·33	$1{\cdot}31\times10^{-03}$	1·153 (1·057–1·258)
T allele of rs2104286 (*IL2RA*)			
Case-control*	99·12	$2{\cdot}38\times10^{-23}$	1·247 (1·194–1·302)
Triost	24·67	$6{\cdot}80\times10^{-07}$	1·278 (1·160–1·409)
C allele of rs12722489 (*IL2RA*)			
Case-control*	62·84	$2{\cdot}24\times10^{-15}$	1·234 (1·172–1·300)
Triost	11.95	$5{\cdot}47\times10^{-04}$	1·232 (1·094–1·387)

IMSGC Lancet Neurology 2008

nature genetics

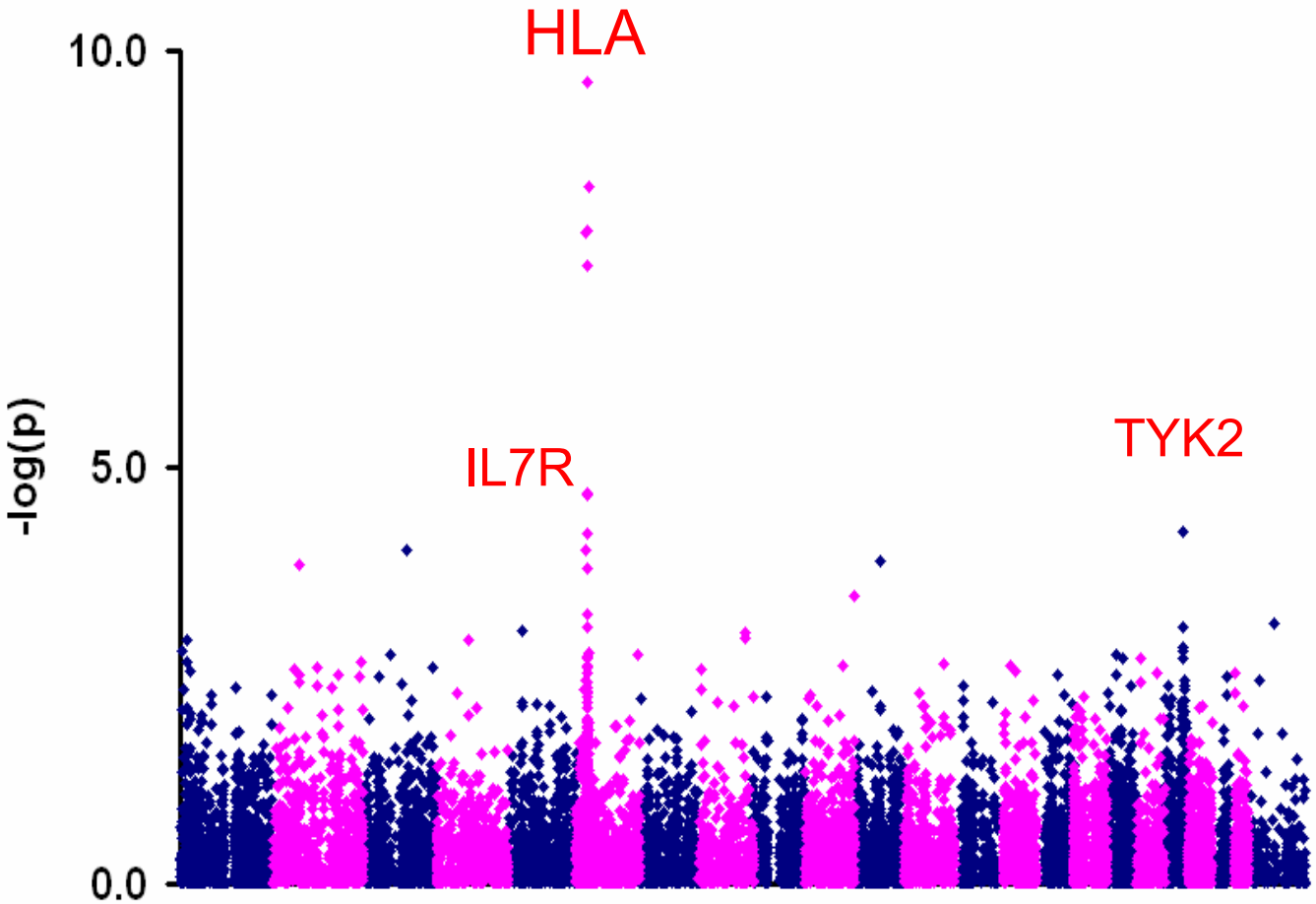
Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants

Wellcome Trust Case Control Consortium[1] & The Australo-Anglo-American Spondylitis Consortium[1]



WTCCC Nat Gen (2007) 39:1329-1337

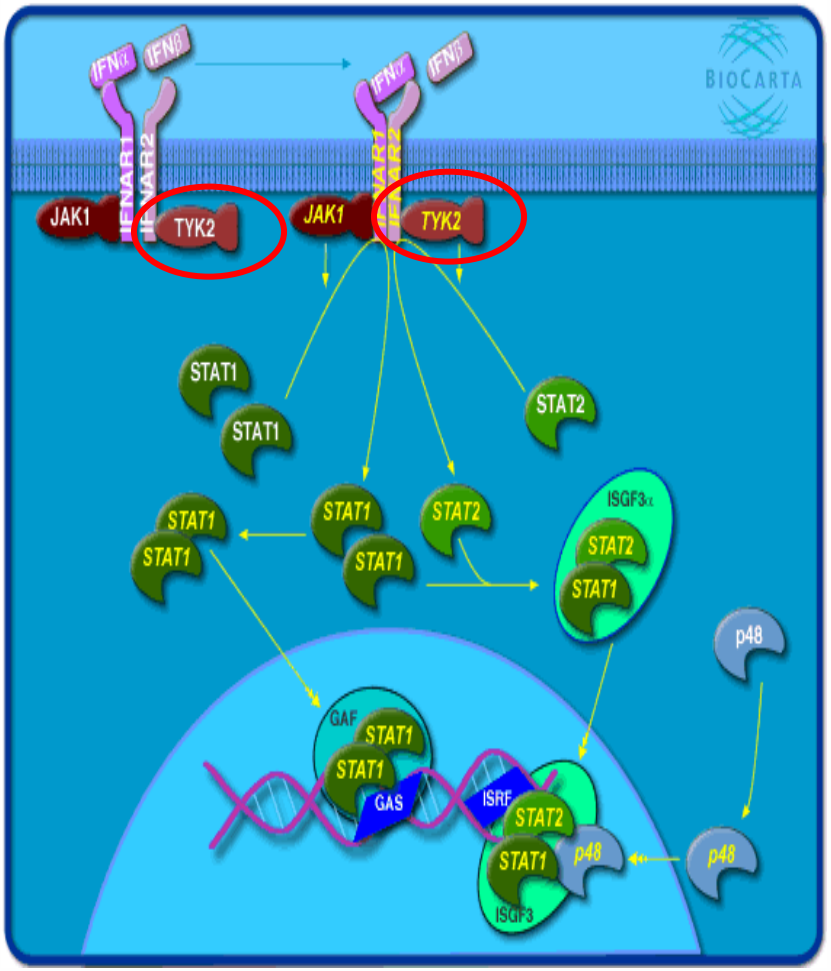
Overall results- replication of IL7R and a novel variant in the TYK2 gene



WTCCC Nat Gen (2007)

The Tyrosine kinase 2 gene

- Located at chr 19p13
- Expressed in lymphocytes and the nervous system
- TYK2 is essential in IFN-α and – β signalling
- TYK2 is activated in response to various cytokines



http://www.biocarta.com/genes/index.asp

The future in MS genetics

GWAS starting now:

500k – 1mill SNPs and CNV

10-15 000 MS cases

Funded by Welcome Trust

performed by IMSGC (International Multiple Sclerosis Genetics Consortium)

Novel variants

Replication studies, fine-mapping and functional studies

- Difficulties

Resequencing approach (detect hotspots (20% of the genome))

Copy number variation *(Beckmann et al. Nature 2007)*

Acknowledgments

- **Oslo MS genetics group**
  - Hanne F Harbo
  - Åslaug R Lorentzen
  - Inger-Lise Mero
  - Cahrine Smestad
  - Elisabeth G Celius
  - Anne Spurkland
- **Nordic MS genetics Network**
- **Immunogenetics of autoimmune diseases**
  - Benedicte A Lie and colleagues
- **IMSGC= International MS Genetic Consortium**
  - Stephen Sawcer, UK

UNIVERSITY OF CAMBRIDGE

wellcome trust

MRC Medical Research Council

University of California San Francisco

UCSF

RIKSHOSPITALET

VANDERBILT UNIVERSITY

University of Miami

ULLEVÅL universitetssykehus

KEELE UNIVERSITY

CARDIFF UNIVERSITY

PRIFYSGOL CAERDYDD

ULLEVÅL
universitetssykehus

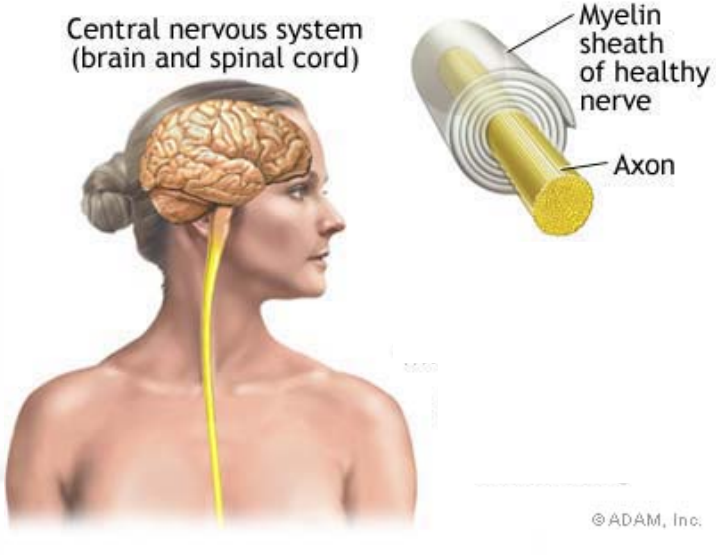
UNIVERSITY OF
CAMBRIDGE

RIKSHOSPITALET

UNIVERSITAS OSLOENSIS
MDCCCXI

? ? ? ? ?

Candidate gene approach



Copyright A.D.A.M.

Sample size

GWAS

Novel variants

Finmapping
resequencing
functional studies

ULLEVÅL universitetssykehus

Bevegelsesdivisjon