Fattori di rischio genetici ed ambientali nella sclerosi multipla

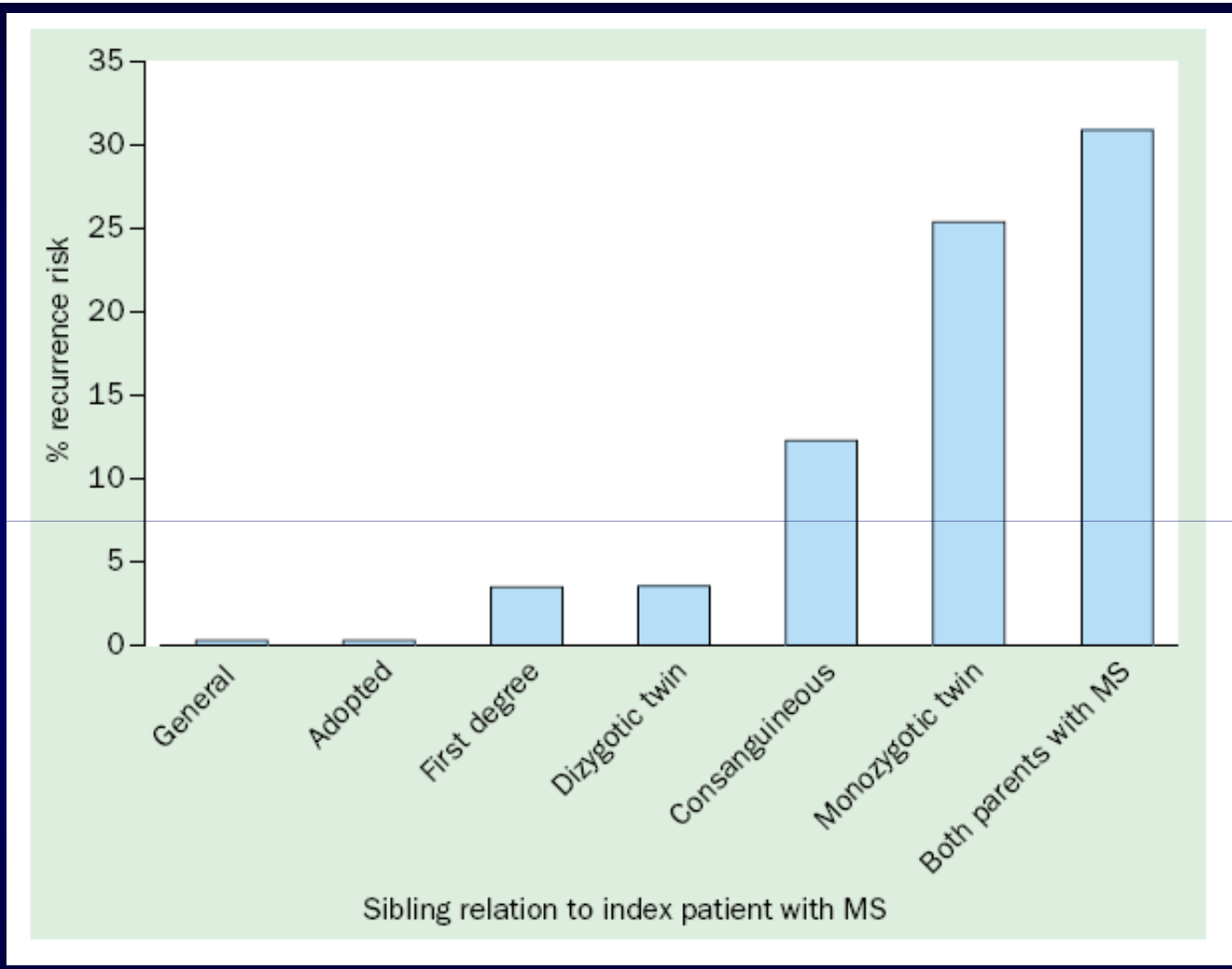
Filippo Martinelli Boneschi

Dalla chiarezza diagnostica all'efficacia terapeutica

Monza, 2 Febbraio 2012

CENTRO ANALISI MONZA

Fattori di rischio genetici



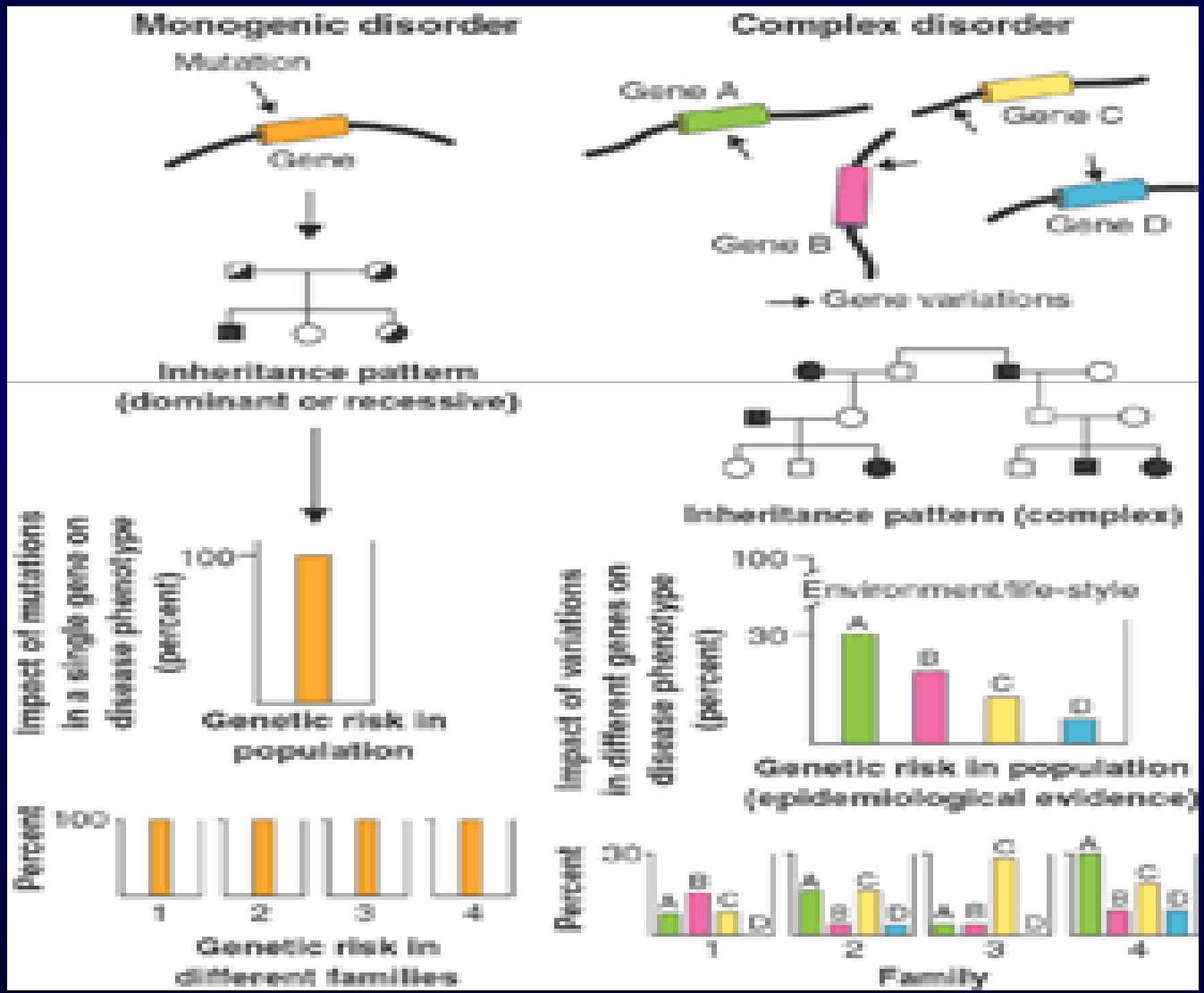
Il rischio di malattia nei familiari di individui con sclerosi multipla si modifica con il grado di parentela

Dyment et al; The Lancet Neurology 2004

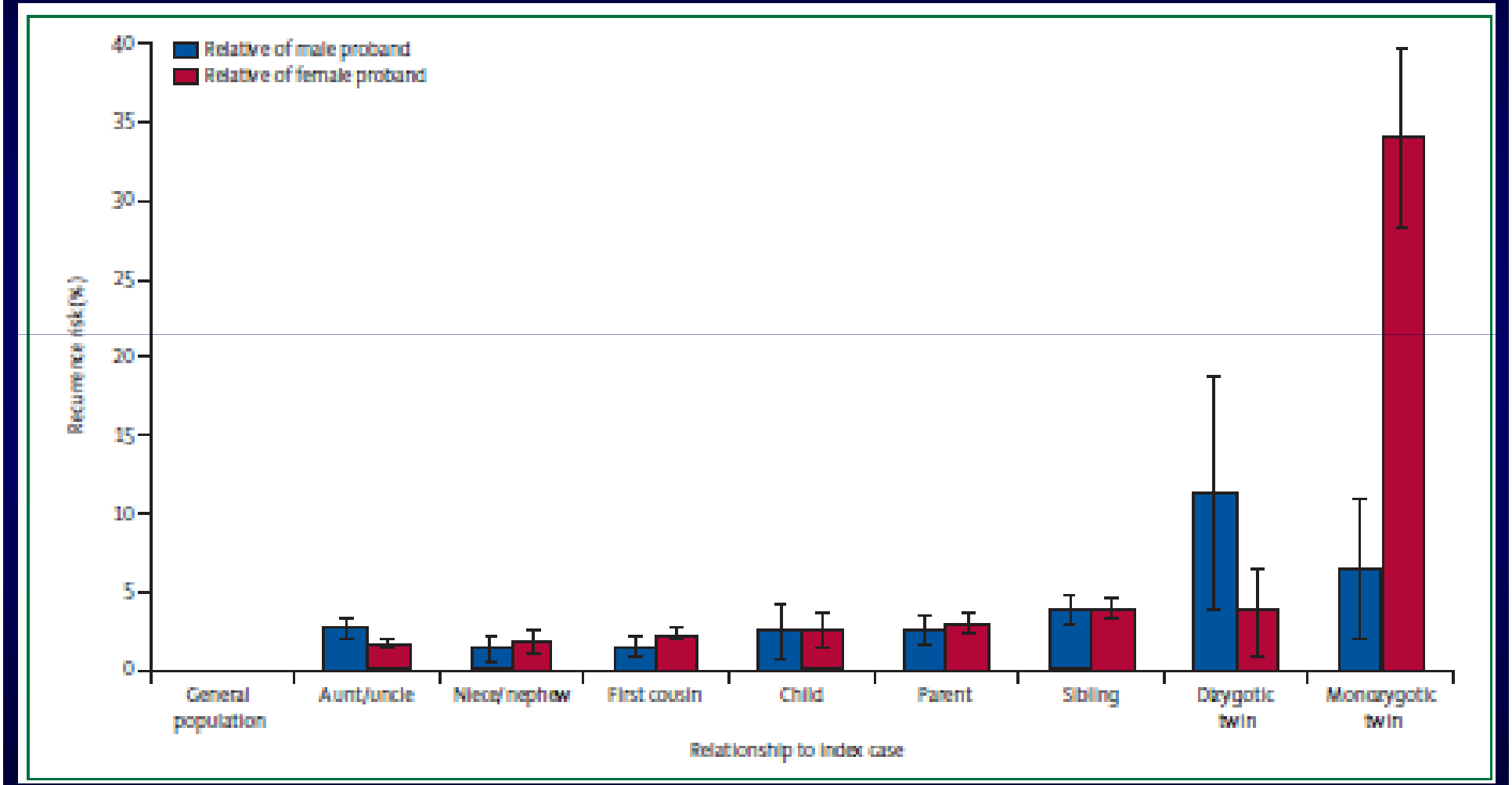
Fattori di rischio genetici
Rischio di malattia in fratelli di probandi affetti

Malattia	**Lambda$_s$**
Malattia di Huntington	5000
Fibrosi cistica	500
Sclerosi Multipla	**34.6**
Diabete tipo 1	15
Scizofrenia	10.6
Artrite reumatoide	8
Malattia di Alzheimer	4-5

Contributo genetico nelle malattie monogeniche ed in quelle complesse



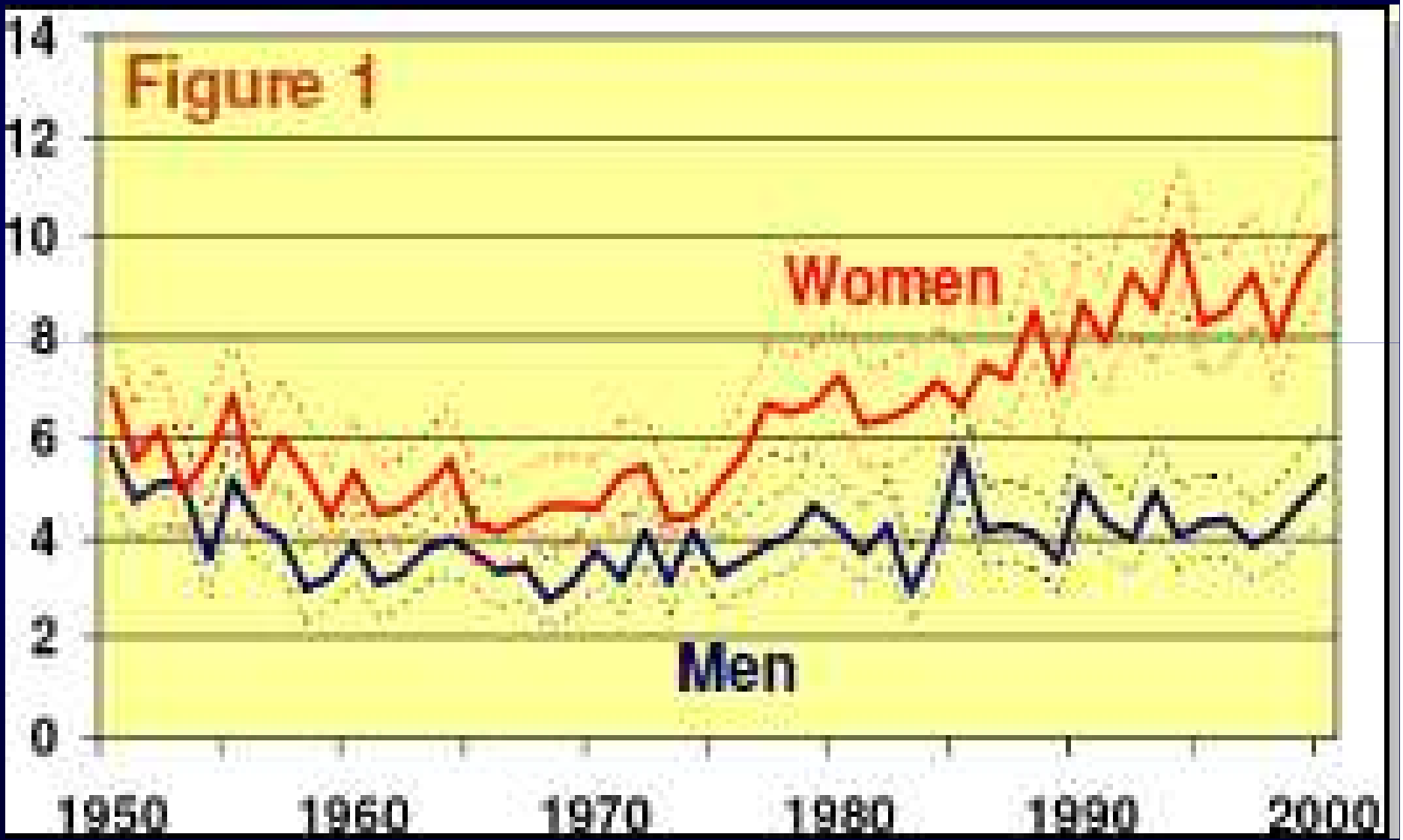
Fattori di rischio genetici
Stratificazione per sesso



Willer CJ et al – *Proc Natl Acad Sci USA 2003*

Frequenza di malattia nelle donne

Incidenza di malattia stratificato x sesso ed anni



Koch-Henriksen N; Lancet Neurology 2010

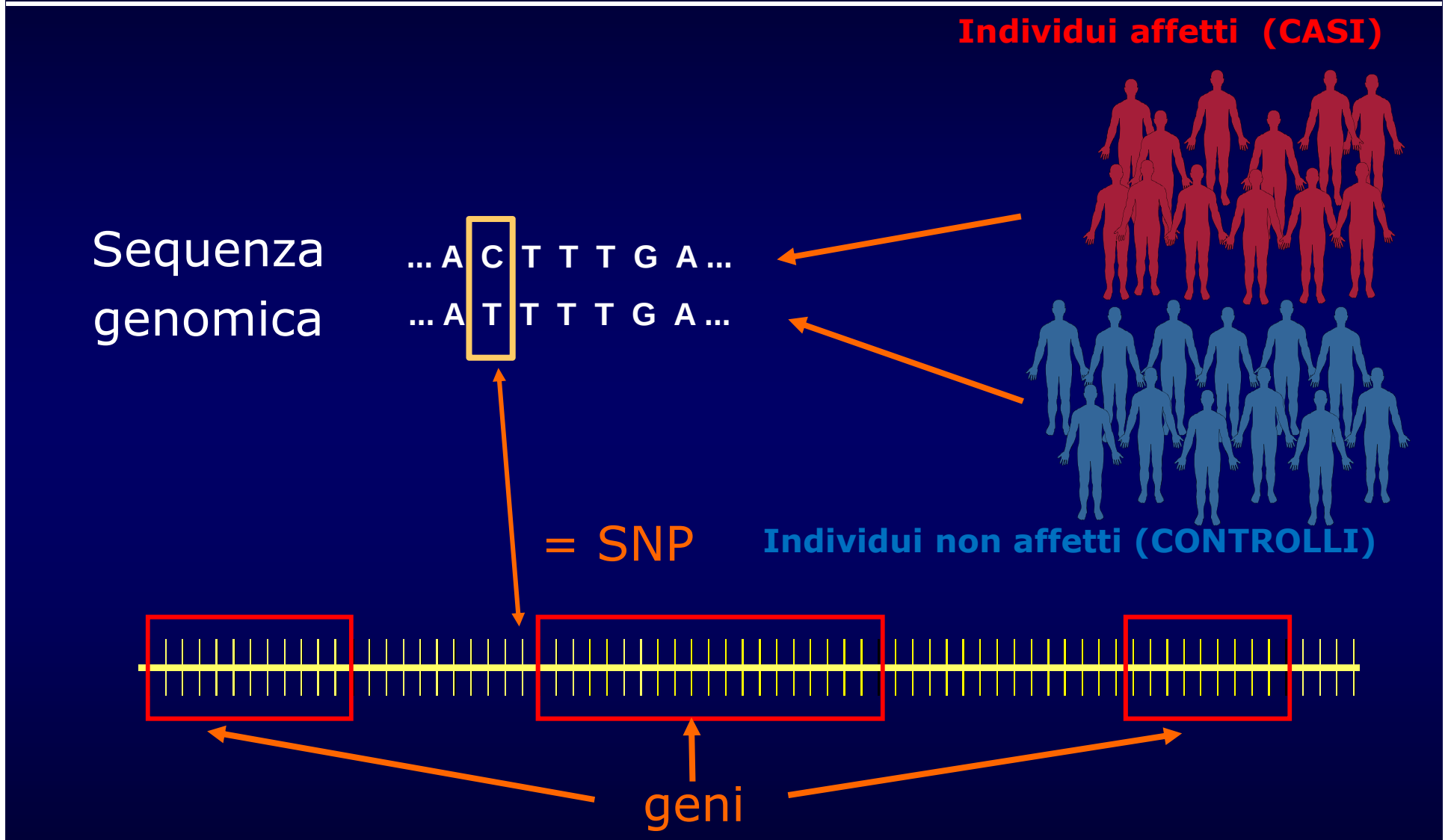
Effetto familiare di origine

- I fratellastri per parte materna di malati di SM hanno un rischio quasi doppio di sviluppare la SM rispetto ai fratellastri per parte paterna (2.35% vs 1.31%, p=0.04).
- Il rischio di malattia nei fratellasti per parte materna non è significativamente diverso rispetto ai fratelli (2.35% *vs 3.11%, p=0.1).*
- Meccanismi epigenetici?

Etnicità

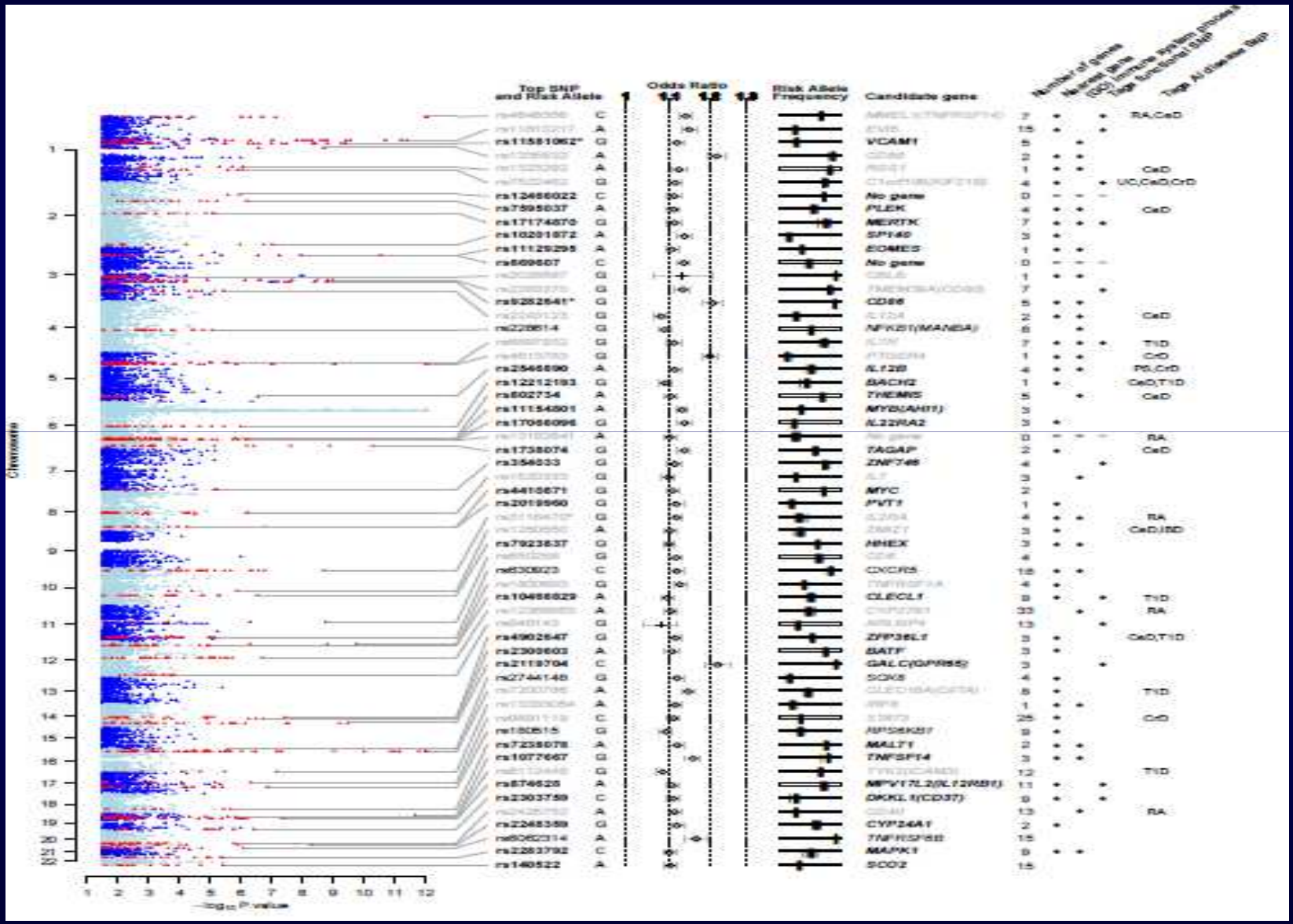
Ridotta frequenza di malattia negli Afro-Americani (gli afro-Americani hanno il 40% di rischio in meno di sviluppare la SM rispetto ai caucasici), nativi Americani, Messicani, PortoRicani e Giapponesi, e la malattia è quasi assente nei Cinesi e nei Filippini.

Studi di associazione caso-controllo



→ Confrontare la frequenza degli SNPs nei due gruppi

Loci genetici associati alla Sclerosi Multipla



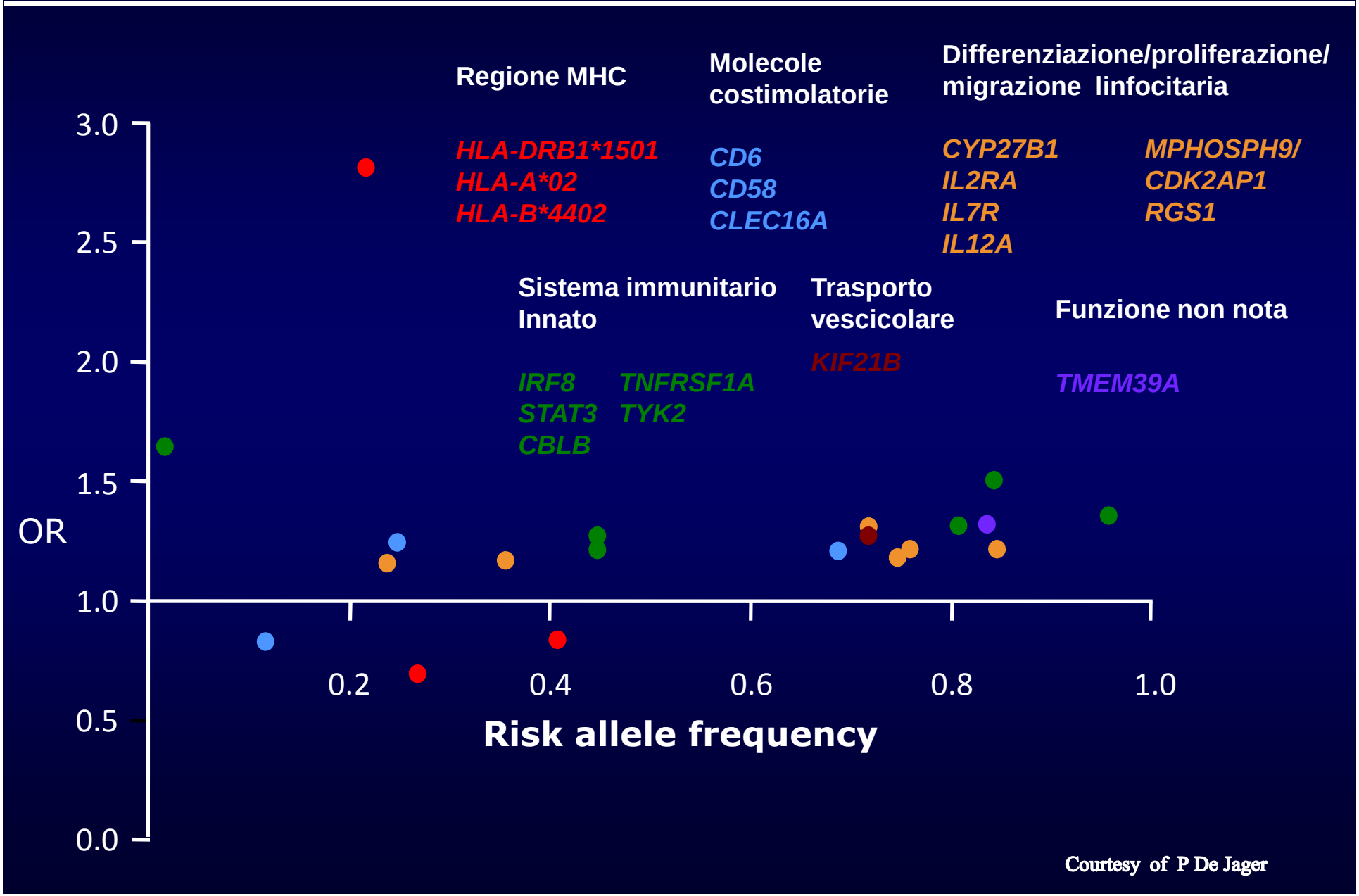
23/26 loci genetici confermati
29 nuovi loci genetici

IMSGC & WTCCC2 Nature (2011) 476; 214-9

La genetica aiuta a curare le malattie?

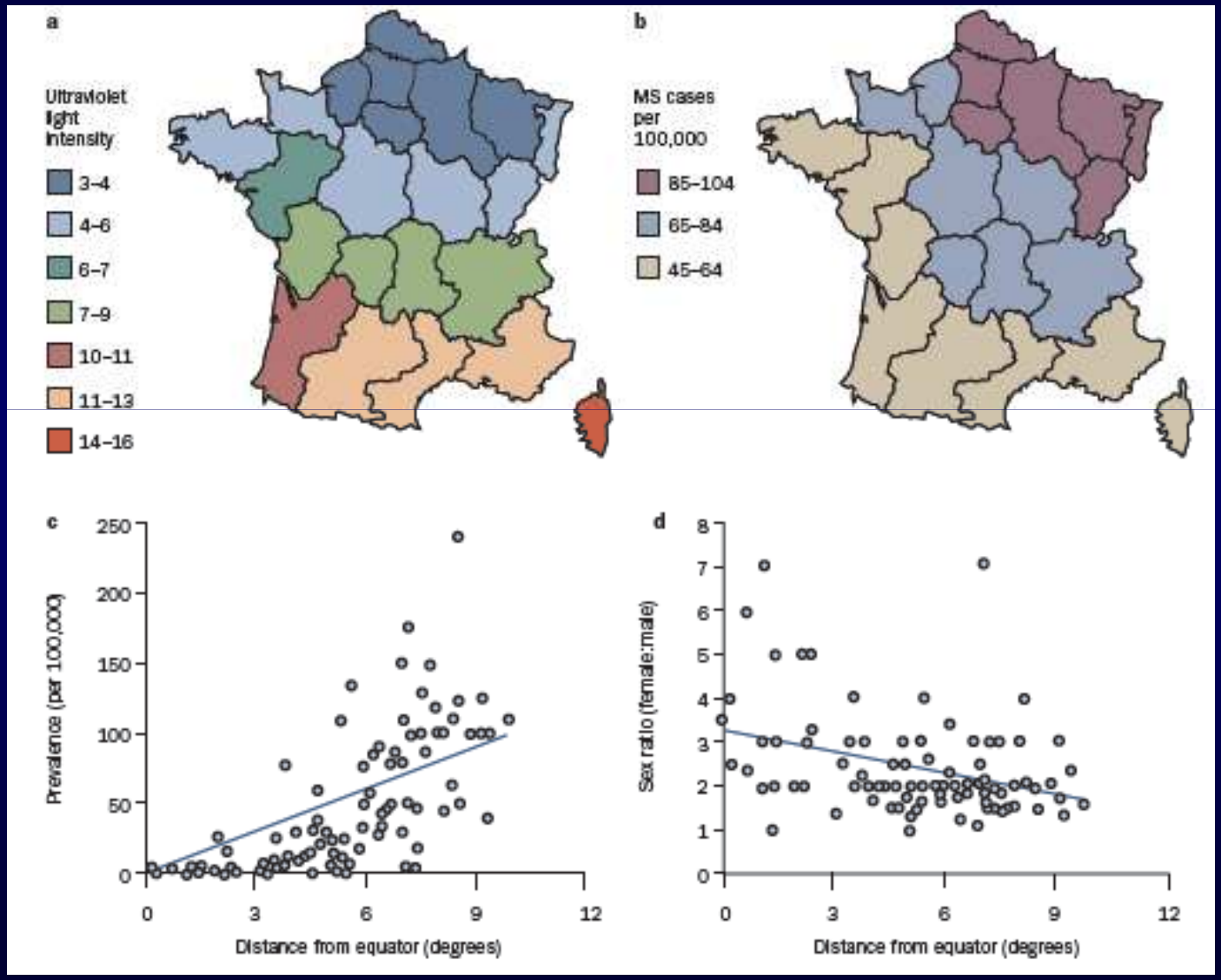
- Sviluppo di tests diagnostici e prognostici
- Migliorare la conoscenza delle cause della malattia
- Sviluppo di nuovi farmaci efficaci a curare la malattia [es. vitamina D (*CYP27B1, CYP24A1*), natalizumab (*VCAM1*) e daclizumab (*IL2RA*)]

Loci genetici e funzione



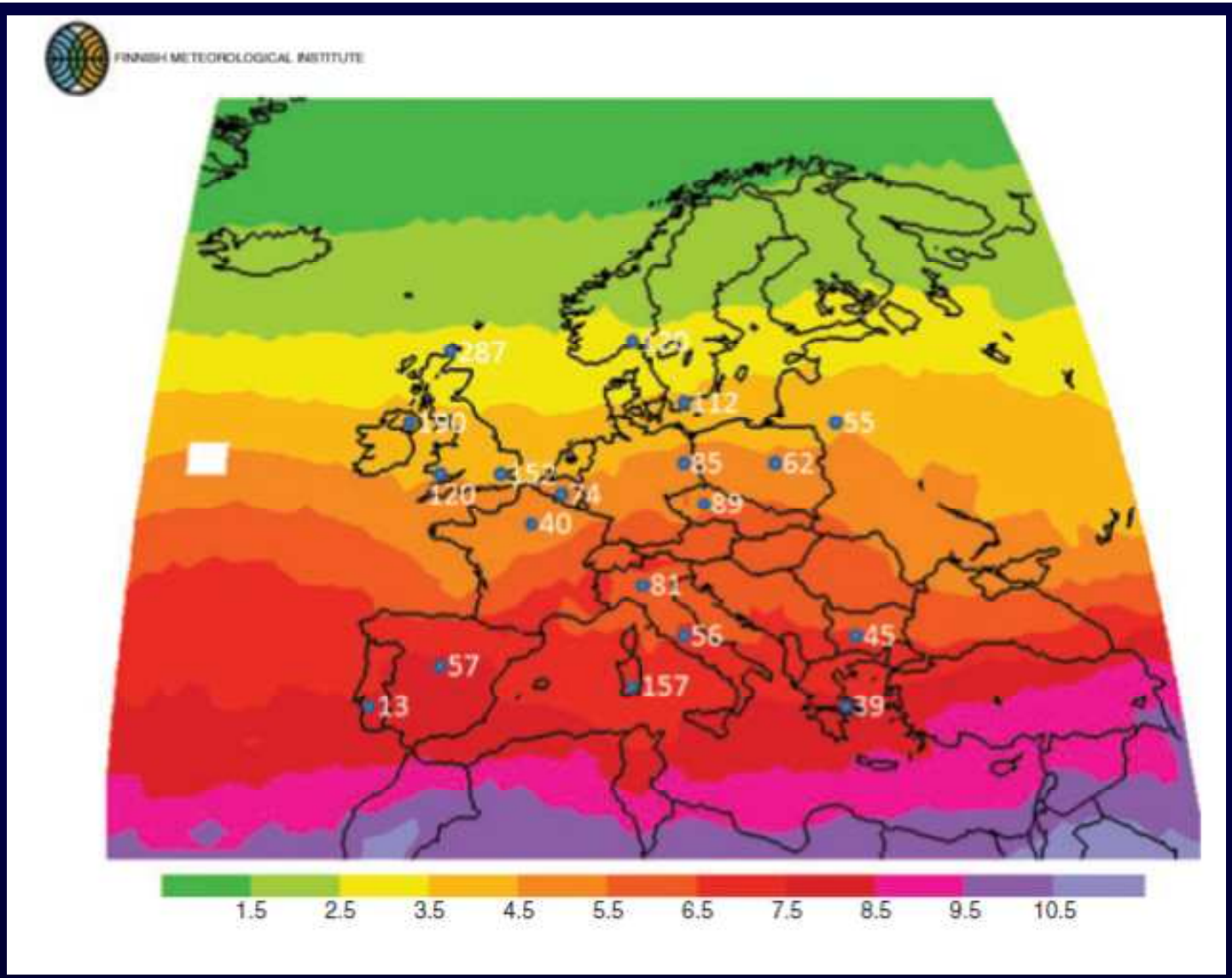
Courtesy of P De Jager

Fattori di rischio ambientali
Esposizione ai raggi solari



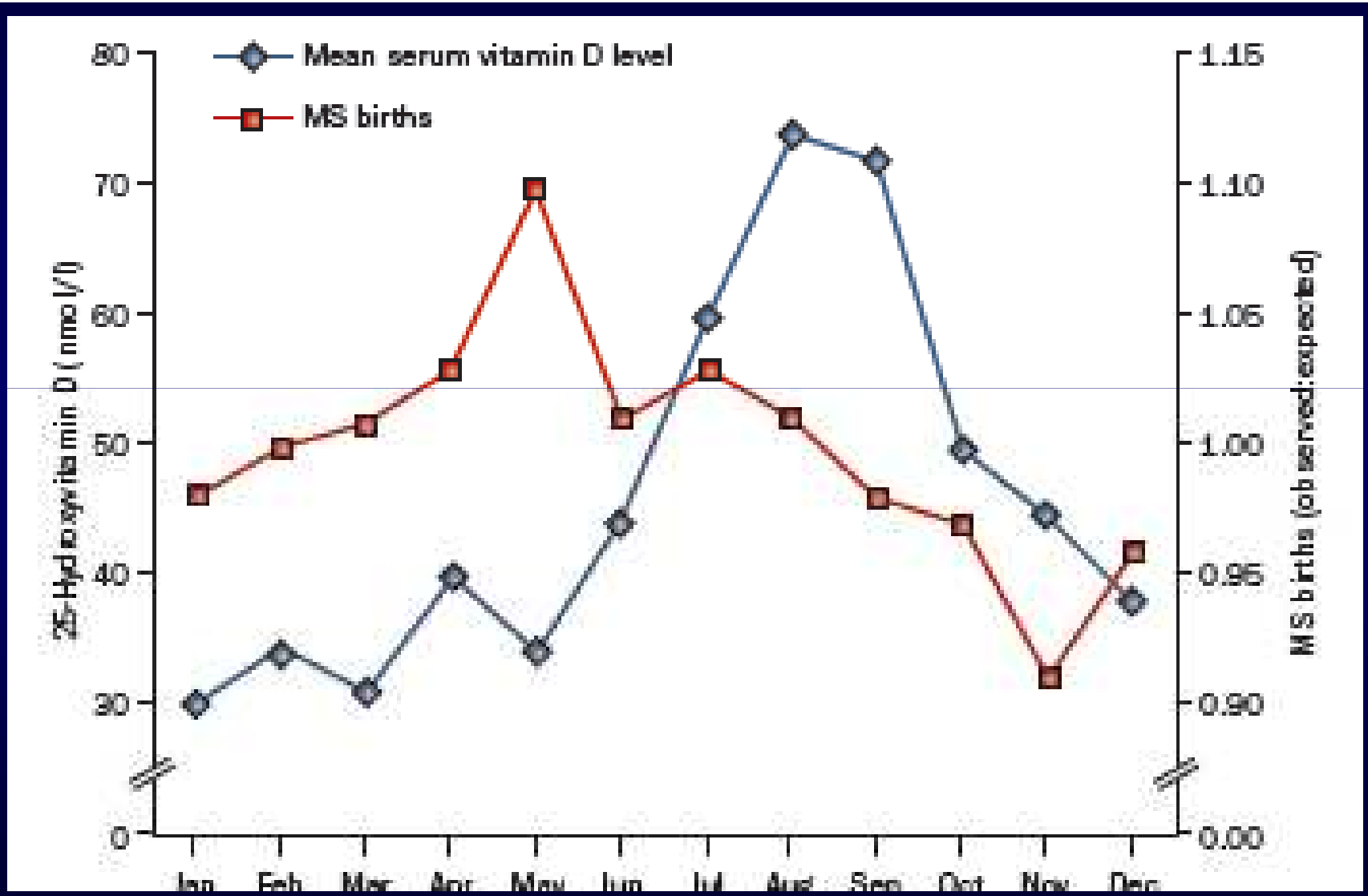
Handel AE, Nat Rev Neurol 2010

Fattori di rischio ambientali
Esposizione ai raggi solari - rischio SM in Europa



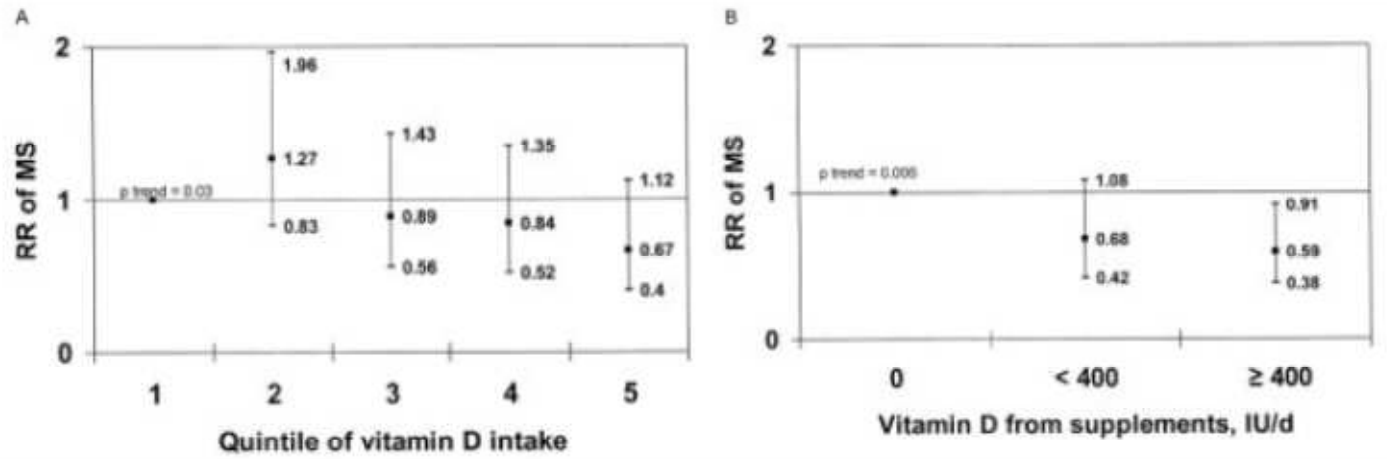
Handel AE, Europ J Neurol 2010

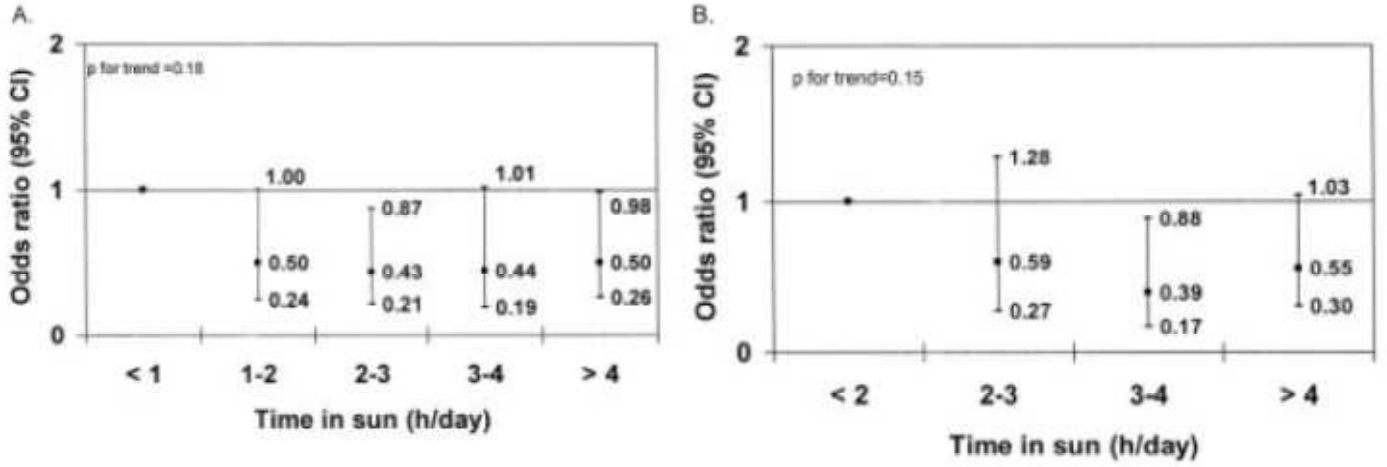
Fattori di rischio ambientali
Livelli sierici vitamina D e rischio di SM



Handel AE, Nat Rev Neurol 2010

Fattori di rischio ambientali
Vitamina D, esposizione al sole e rischio di SM





Ascherio A, Ann Neurol 2007

Fattori di rischio ambientali
Livelli di vitamina D ed SM

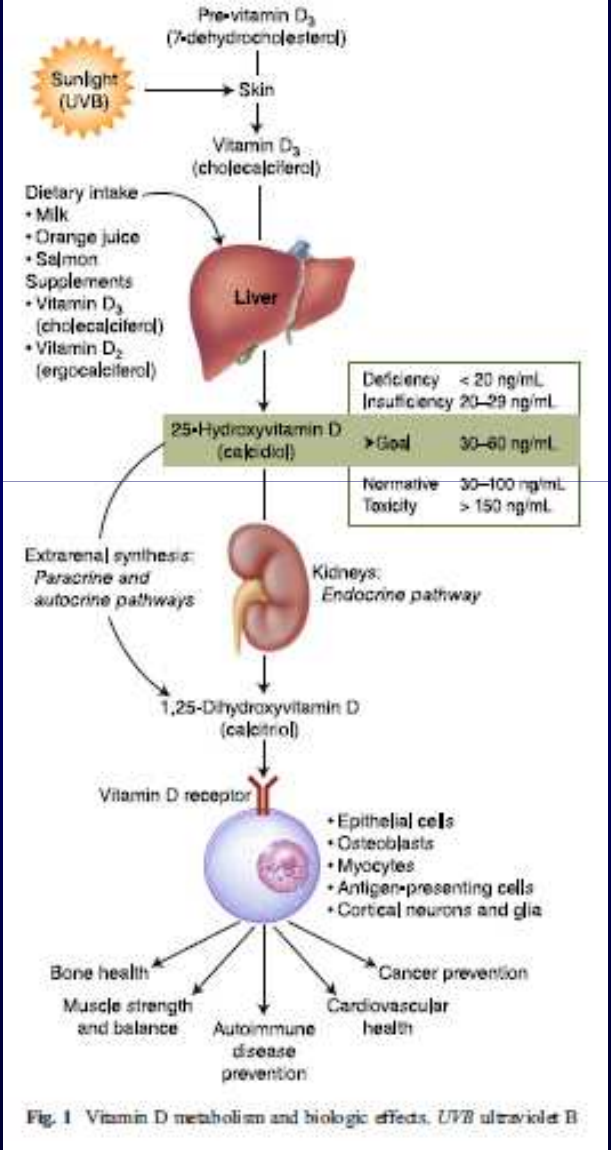


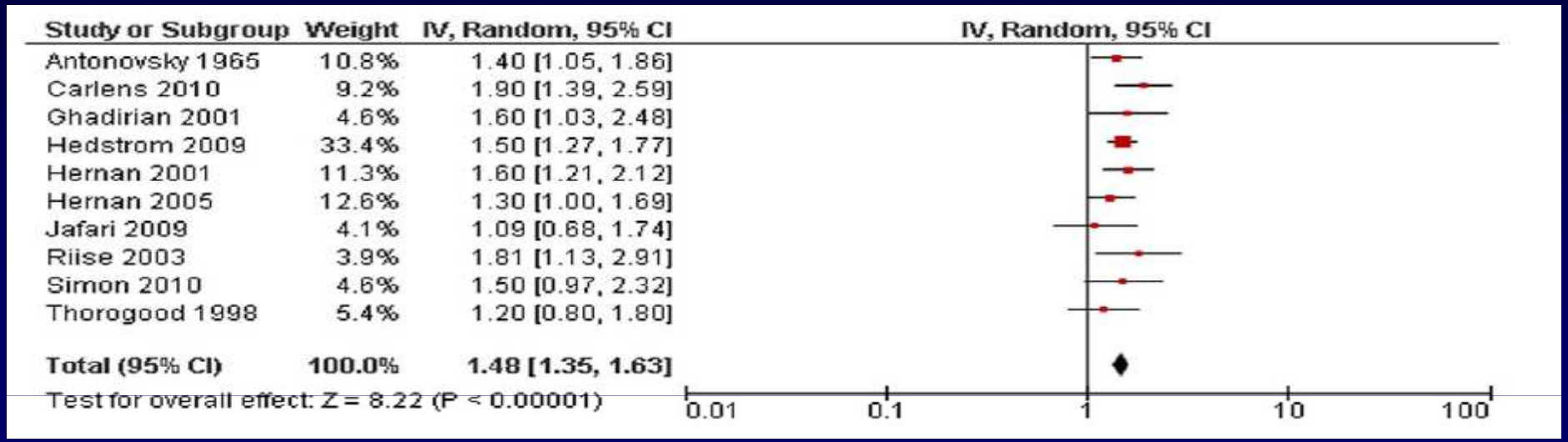
Fig. 1 Vitamin D metabolism and biologic effects. *UVB* ultraviolet B

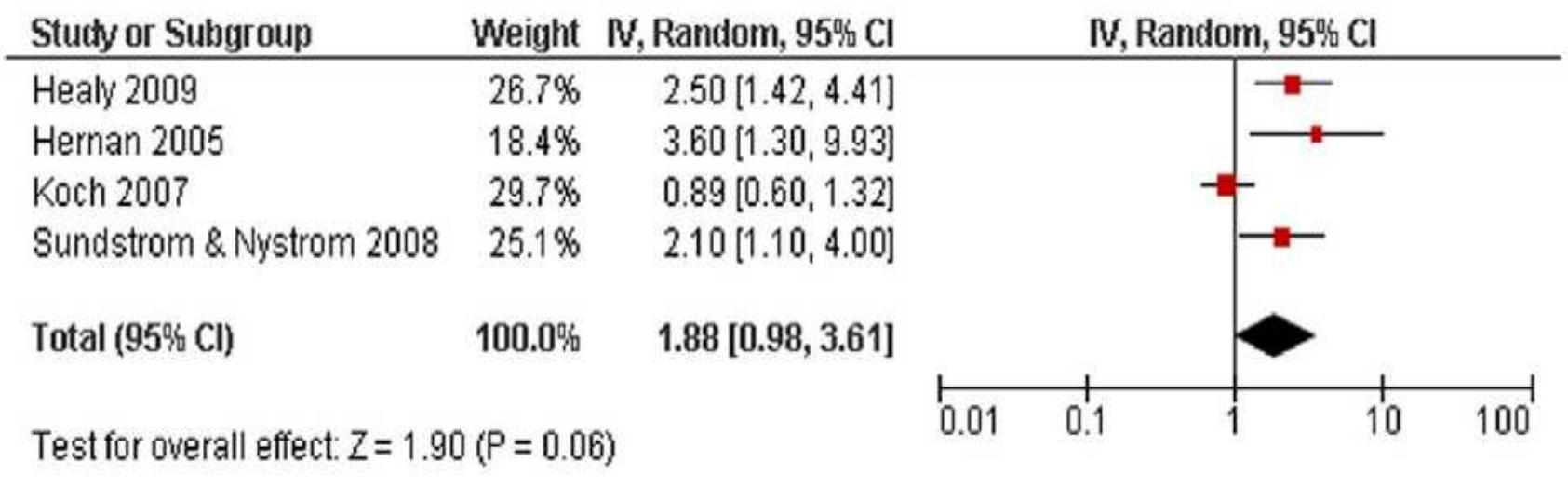
- Livelli sierici di vitamina D $\geq$ 30 ng/mL sono raccomandati, ma non è ancora chiaro il livello ottimale benefico per la SM.
- Per individui con forme CIS o SM associati ad una carenza di vitamina D (<20ng/mL) >> supplementazione con 50.000 IU/wk di vitamina D2 per 8 settimane e successiva rivalutazione serologica per continuare il trattamento fino a che I livelli di vitamina D sono > 30 ng/mL.
- Per individui con una lieve insufficienza (20-29 ng/mL), una supplementazionee di mantenimento con vitamina D2 da 1.000 a 2000 IU/d puo' essere consigliata fino alla correzione del difetto.

Studio su 23 RRMS randomizzati per 6 mesi a 6000 IU vitamina D2> non efficace nel ridurre le lesioni T2 e captanti gadolinio (Stein MS et al, Neurology 2011) .

Solomon AJ et al , Curr Neurol Neurosci Rep 2010

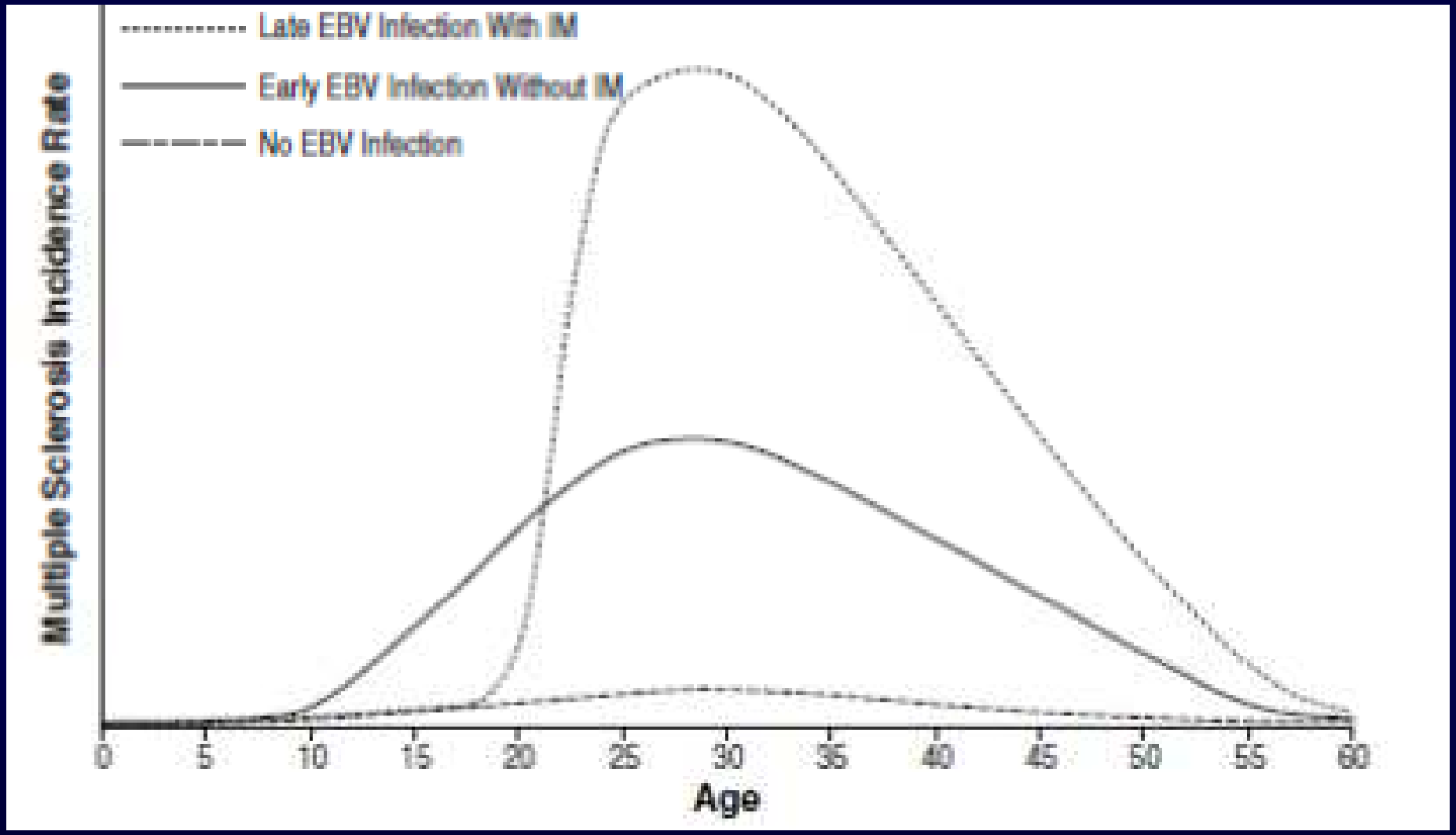
Fattori di rischio ambientali
Fumo e rischio di SM e SPMS





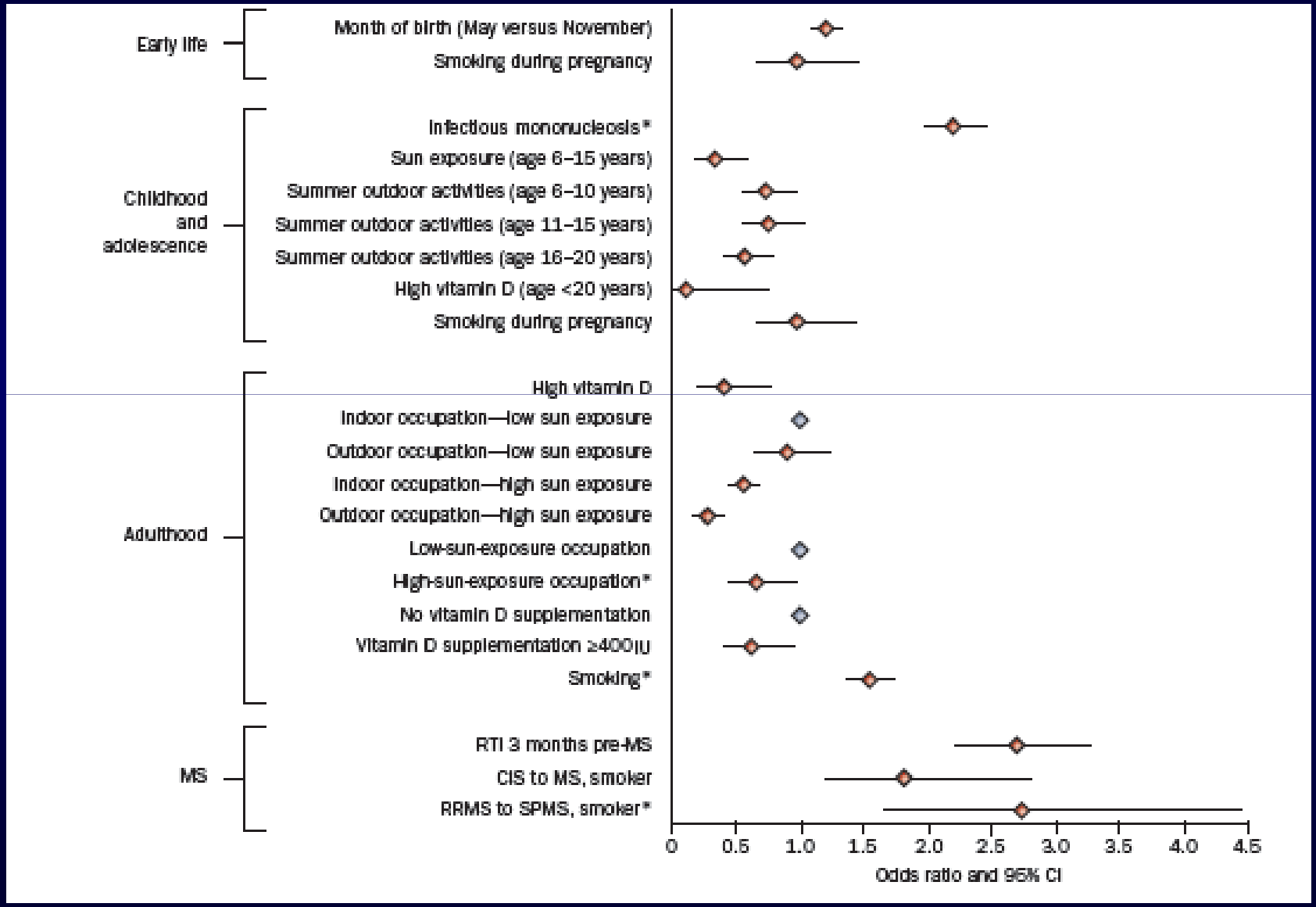
Handel AE, PLOS one 2011

Fattori di rischio ambientali
Infezione da virus di Epstein-Barr



Ascherio A, J Neuroimmune Pharmacol 2010

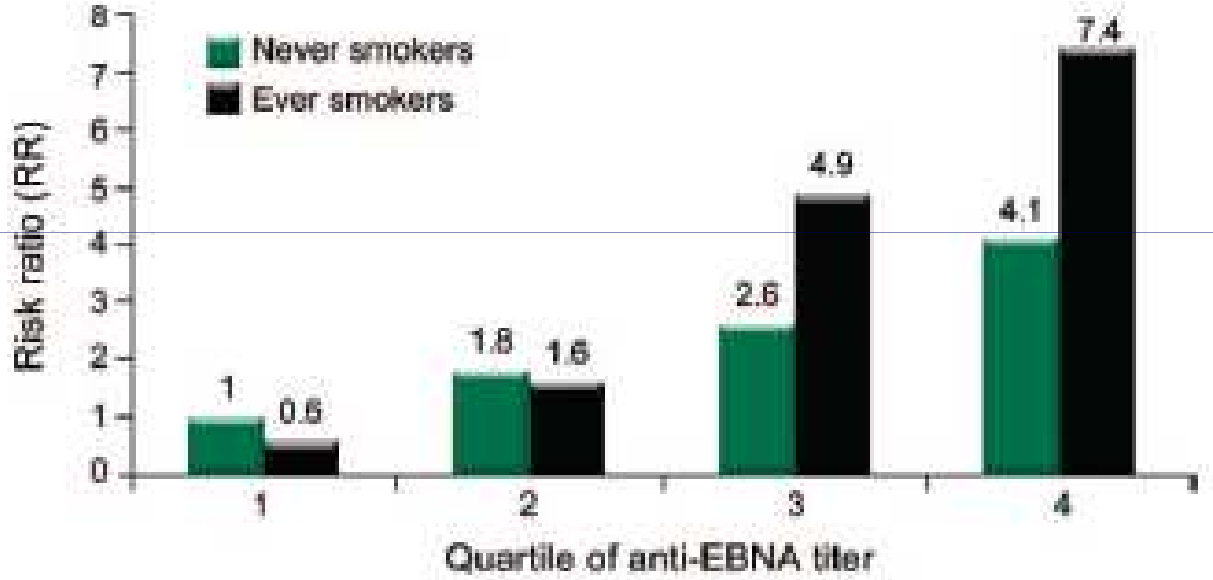
Riassunto fattori di rischio ambientali



Handel AE, Nat Rev Neurol 2010

Fattori di rischio ambientali
Modelli di Interazione

Figure Risk of multiple sclerosis (MS) associated with increasing quartile of antibody titers to the Epstein-Barr virus nuclear antigens (anti-EBNA)* according to smoking status†



†Smoking history at time of diagnosis. In the Swedish study, smoking history at time of diagnosis was estimated from smoking at time of blood draw as described in Methods. *EBNA1 for Nurses' Health Studies and Swedish study; total EBNA for Tasmanian study. Number of cases and controls included for never smokers: Q1 = 30/126, Q2 = 48/107, Q3 = 35/68, Q4 = 46/56; and ever smokers: Q1 = 17/100, Q2 = 51/126, Q3 = 65/76, Q4 = 85/63. Numbers may not sum to total number of cases and controls due to missing values.

Simon KC et al, Neurology 2010

Modelli di interazione fra fattori di rischio genetici ed ambientali

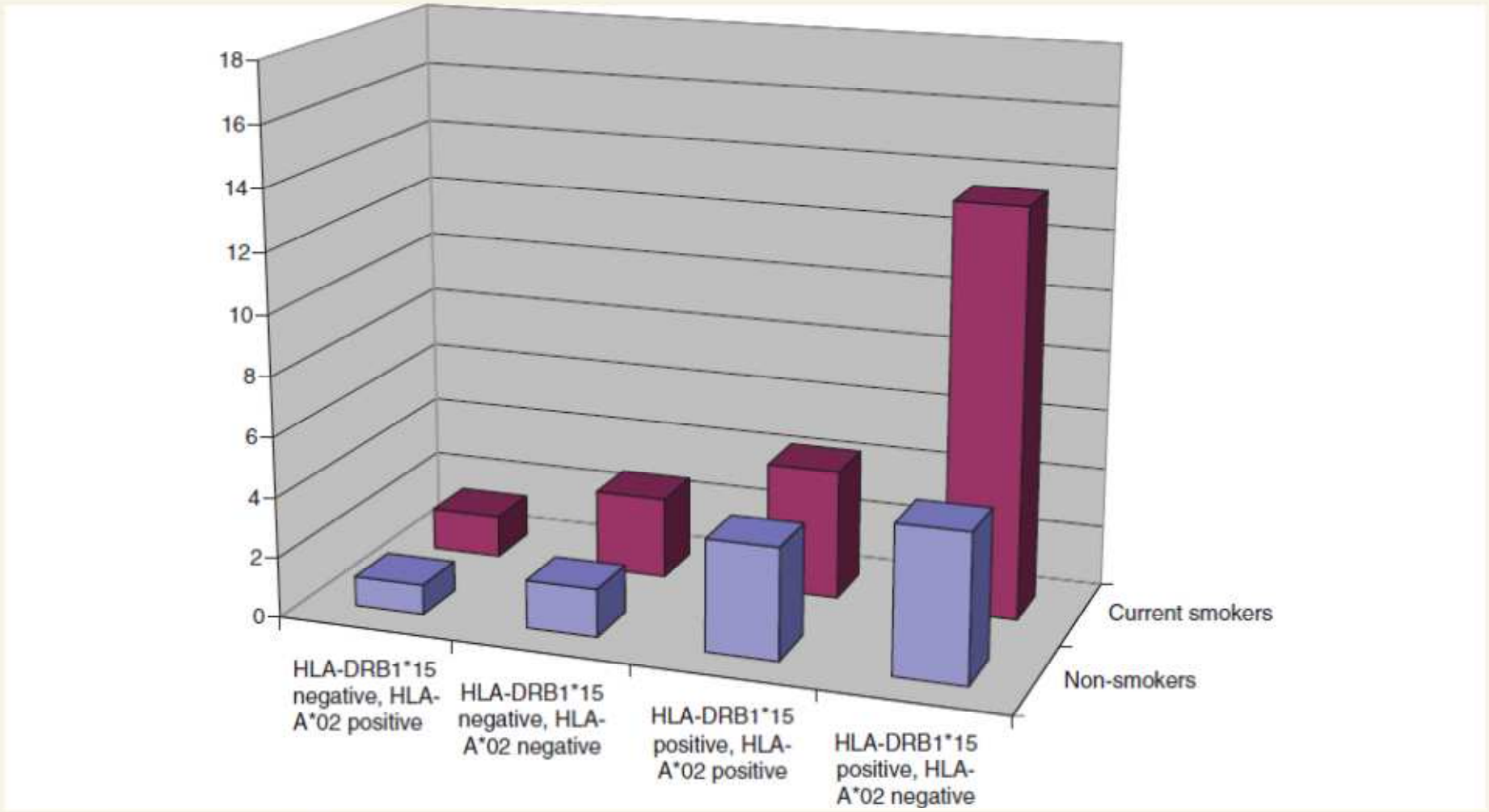
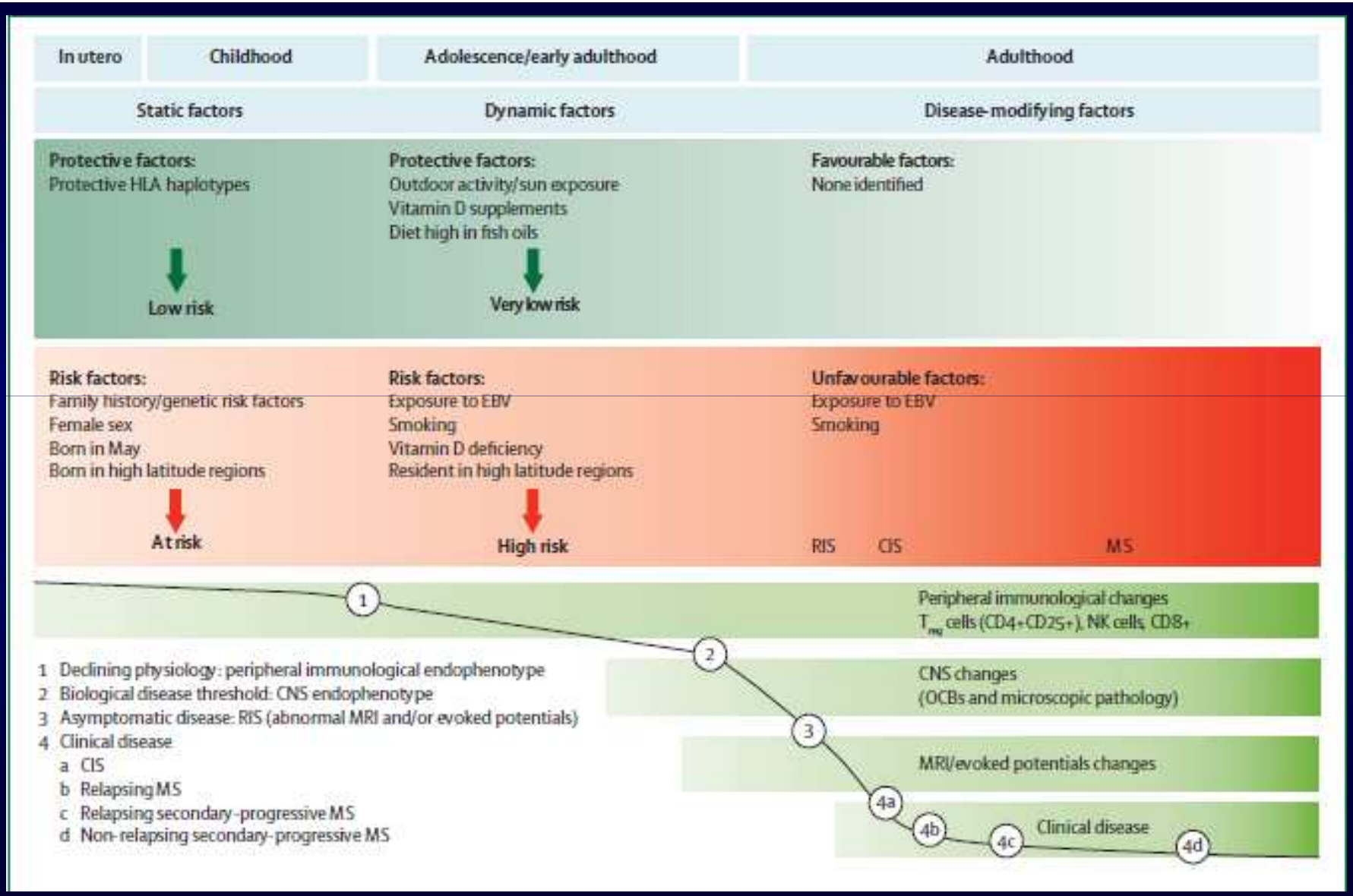


Figure 1 Odds ratios for different combinations of two genetic risk factors (absence of HLA-A*02 and carriage of HLA-DRB1*15) compared with non-smokers carrying none of the genetic risk factors, among smokers and non-smokers. Statistics are shown in Table 6.

Hedstrom AK et al, Brain 2011

Ipotesi di una pathway causale nella SM



Ramagopalan, Lancet Neurol 2010

Ringraziamenti

- G. Giacalone
- M. Sorosina
- N. Clarelli
- P. Brambilla

-C. Guaschino
-G. Liberatore
- V. Capece
- F. Esposito

COLLABORATORI

- Dipartimento di Neurologia Ospedale San Raffaele di Milano (M Rodegher, B Colombo, P Rossi, M Radaelli, L Moiola, V Martinelli, G Comi)
- PROGRESSO consorzio
- Università di Novara (S D'Alfonso, N Barizzoni, M Leone)
- IMSGC Consorzio
- Università di Milano & Fondazione Filarete (D Cusi, S Lupoli)
- Istituto Mario Negri di Milano (D Albani, G Forloni)

Environmental risk factors
Summary

- Sunlight exposure and vitamin D levels: immunomodulatory effects
- Epstein-Barr virus: clonal expansion of B lymphocytes in the CNS or EBV infection triggers autoimmunity via molecular mimicry
- Smoking: nitric oxide mediated demyelination, axonal loss and epigenetic effects
- Sex hormones: altered antigen reactivity, tolerance and epigenetic effects
- Stressful life events: dysregulation of the hypothalamic-pituitary-adrenal axis
- Respiratory tract infections: immunological trigger for inflammatory demyelination
- Organic solvents: BBB disruption
- Diet: vitamin D supplementation with oily fish

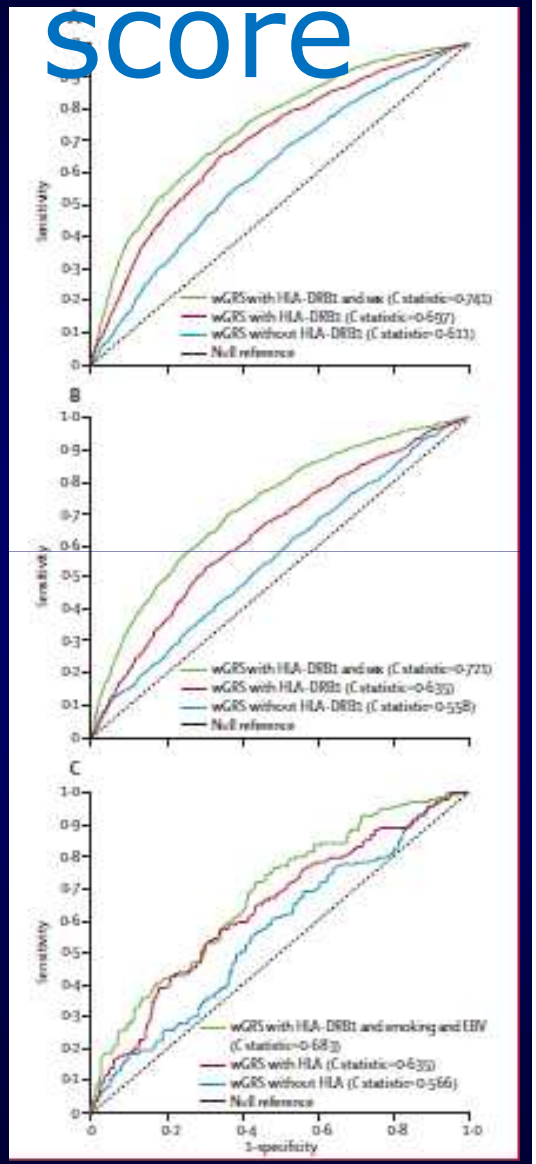
Weighted combined genetic and non-genetic risk score

	SNP	Allele	Base pair	OR	Gene	Weight	% of total weight
1*	rs2300747	A	116905738	1·30	CD58	0·261365	7·8%
1*	rs2760524	G	190797171	1·15	RGS1	0·139262	4·2%
2*	rs883300	C	136692725	1·19	CXCR4	0·174353	5·2%
3	rs4680534	C	161181639	1·12	IL12A	0·113329	3·4%
5*	rs6897932	C	35910332	1·12	IL7R	0·116534	3·5%
5*	rs6896969	C	40460183	1·10	PTGER4	0·094311	2·8%
6*	rs2523393	A	29813638	1·28	HLA B	0·248461	7·4%
6	rs31353888	A	32521029	2·75	HLA DRB1	1·011601	30·3%
6*	rs9321619	A	137916101	1·12	OLIG3/TNFAIP3	0·116534	3·5%
10*	rs2104286	T	6139051	1·15	IL2RA	0·139262	4·2%
10	rs1250540	G	80706013	1·12	ZMIZ1	0·113329	3·4%
11	rs17824933	G	60517188	1·18	CD6	0·165514	5·0%
12	rs1800693	C	6310270	1·20	TNFRSF1A	0·182322	5·5%
12	rs1790100	G	122222678	1·11	MPHOSPH9	0·104360	3·1%
16*	rs11865121	C	11074189	1·15	CLEC16A	0·139262	4·2%
16*	rs17445836	G	84575164	1·25	IRF8	0·223144	6·7%

SNPs are listed by chromosome. All ORs used in the algorithm are referenced to the risk allele of each SNP, which can be the minor or major allele. *SNPs have their major allele as the risk allele, as the OR in the initial report[1] is referenced to the minor allele, here we convert the original OR for these SNPs to reflect the use of the major allele as the reference allele using 1/(OR of the minor allele). SNP=single nucleotide polymorphism. OR=odds ratio.

Table 3: SNPs that compose the weighted genetic risk score and weights assigned to each marker

+ HLA-DRB1 + sex
+ smoking + EBV infection



De Jager P et al, Lancet Neurol 200

Summary of interaction model of risk factors

	Risk factors	Study details	Findings
Islam et al[47]	Latitude of birth, familial risk	400 monozygotic twin pairs	Concordance was 1·9 times (95% CI 1·2–3·2) greater among northern-born twins
Haghighi et al[48]	OCBs, familial risk	Case-control study of 47 healthy siblings of patients with MS vs 50 unrelated healthy controls	9 (19%) healthy siblings of patients with MS had OCBs vs 2 (4%) controls
Soderstrom et al[49]	HLA, OCBs, MRI	Population-based study of 147 consecutive patients with acute monosymptomatic optic neuritis	Abnormal MRI and presence of OCBs were strongly associated with MS; of 25 individuals with a normal MRI and no OCBs, none developed MS; presence of HLA-DR2 was related to MS, but did not add to the PPV of MRI and OCBs
Cellus et al[50]	HLA, sex, age	Cohort of 286 Norwegian patients with MS	HLA-DR2, DQ6 was significantly more frequent among women than men ($p=0·025$), and was negatively correlated with age at diagnosis ($p=0·0254$)
Hensiek et al[51]	HLA, sex	Cohort of 229 patients with MS	HLA-DR13 was associated with younger age at diagnosis and female sex
De Jager et al[52]	HLA, EBV	Nested case-control study of 148 women with MS and 296 age-matched healthy women	MS among HLA-DRB1*15-positive women with increased anti-EBNA 1 titres (>1:320) was 9·7-fold (95% CI 3·2–29·2) higher than that of HLA-DRB1*15-negative women with low anti-EBNA 1 titres (≤1:80)
Sundstrom et al[53]	HLA, EBV	Case-control study of 109 individuals with MS and 217 age-matched and sex-matched controls	OR of developing MS in individuals with HLA-DRB1*15 and high EBNA1 reactivity was 16·0 (95% CI 5·1–50·0) compared with HLA-DRB1*15-negative subjects with low titres of EBNA1
Nielsen et al[54]	HLA, EBV	Case-control study of 76 MS patients with IM, 1836 MS patients without IM, and 62 blood donors with history of IM and 484 without a history of IM	HLA-DRB1*15-positive individuals with a history of IM had a 10 times (95% CI 6·0–17·9) greater risk of MS than HLA-DRB1*15-negative individuals without a history of IM
De Jager et al[55]	HLA, non-HLA genes, EBV, smoking, sex	Case-control validation populations: 1340 patients with either MS or CIS matched to 1109 controls, 143 patients with MS and 281 controls from the Nurses' Health Study	A weighted genetic risk score produced for 16 susceptibility loci including HLA-DRB1*15 gave modest prediction of MS, which was slightly enhanced by addition of sex, EBV, and smoking
Kelly et al[56]	HLA, MRI	Cohort of 70 patients with CIS	MS developed in 86% of MRI-positive, *HLA-DRB1*1501*-positive patients vs 55% of MRI-positive, *HLA-DRB1*1501*-negative patients ($p<0·025$)
Hauser et al[57]	HLA, MRI	Cohort of 178 patients with optic neuritis	HLA-DR2 was present in 85 (48%) patients, and was associated with increased odds of probable or definite MS at 5 years (OR 1·92 [95% CI 1·01–3·67], $p=0·04$); the association was most apparent among patients with signal abnormalities on baseline brain MRI

CIS=clinically isolated syndrome. EBV=Epstein-Barr virus. EBNA1=EBV nuclear antigen 1. IM=infectious mononucleosis. MS=multiple sclerosis. OCBs=oligoclonal bands. OR=odds ratio. PPV=positive predictive value.

Ramagopalan, Lancet Neurol 201

Summary of genetic and non genetic risk factors for MS

	Study	OR (95% CI)
Family history		
Non-twin first-degree relative affected[88]	19 615 first-degree relatives of 8205 Danish patients with MS	7·1 (5·8–8·8)
Ethnic origin		
White male compared with black male[89]	US cohort study of 4951 patients with MS and 9378 controls	1·49 (1·09–2·27)
HLA haplotype		
HLA-DRB1*15 homozygote[90]	Canadian cohort study of 2454 patients with MS and 4639 unaffected first-degree relatives	5·42 (4·12–7·16)
HLA-DRB1*15 heterozygote[90]	Canadian cohort study of 2454 patients with MS and 4639 unaffected first-degree relatives	2·91 (2·42–3·51)
HLA-DRB1*15/HLA-DRB1*14 heterozygote[90]	Canadian cohort study of 2454 patients with MS and 4639 unaffected first-degree relatives	1·06 (0·56–2·03)
Immune marker genes		
Interleukin 2 receptor α (IL2RA)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·15 (1·04–1·27)
Interleukin 7 receptor α (IL7RA)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·13 (1·02–1·23)
C-type lectin domain family 16 A (CLEC16A)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·15 (1·04–1·25)
CD58 (CD58)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·30 (1·14–1·47)
Tumour necrosis factor receptor superfamily, member 1A (TNFRSF1A)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·20 (1·10–1·31)
Interferon regulatory factor 8 (IRF8)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·25 (1·12–1·39)
CD6 (CD6)[91]	Case-control study of 4839 patients with MS and 9336 controls	1·18 (1·07–1·30)
Place of birth		
Migration before vs after age 15 years[92]	Cohort study of 76 immigrant patients in the UK	7·07 (1·13–3·77)
Age		
Incidence at age 30 years vs age 55 years[93]	Cohort study of 1099 Canadian patients	4·5 (1·52–13·3)
Clinically isolated syndrome		
Abnormal MRI vs normal[94]	UK cohort study of 107 CIS patients	3·99 (1·65–9·65)
Presence of oligoclonal bands, independent of MRI[94]	Spanish cohort study of 415 CIS patients	1·7 (1·1–2·7)
Sex		
Female[95]	Population-based study of 27 074 Canadian patients with MS	6·62 (6·21–7·13)
EBV infection		
Infectious mononucleosis[96]	Meta-analysis of 11 case-control and 3 cohort studies totalling 1667 patients with MS and 3606 controls	2·30 (1·70–3·01)
Anti-EBNA1 antibody geometric mean titre >320 vs <80[97]	Nested US case-control study of 148 women with MS and 296 healthy female controls	1·66 (1·32–2·08)
Smoking		
Ever vs never[98]	Meta-analysis of case-control studies totalling 1155 patients with MS and 153 182 controls	1·51 (1·32–1·87)
Month of birth		
May[99]	17 874 Canadian, 11 502 British, 6276 Danish, and 6393 Swedish patients with MS compared with population controls	1·10 (1·07–1·13)
Vitamin D		
Serum 25-hydroxycholecalciferol increased in the lower quintile (<63·3 nmol/L) vs the upper quintile (>99·1 nmol/L)[100]	Nested US case-control study of 148 white patients with MS and 296 matched healthy white controls	1·69 (1·03–2·78)

Ramagopalan, Lancet Neurol 201

Fenomeno della "missing heritability"
Contribution of genetic variants in complex disease

Table 1 | Estimates of heritability and number of loci for several complex traits

Disease	Number of loci	Proportion of heritability explained
Age-related macular degeneration[72]	5	50%
Crohn's disease[21]	32	20%
Systemic lupus erythematosus[73]	6	15%
Type 2 diabetes[74]	18	6%
HDL cholesterol[75]	7	5.2%
Height[15]	40	5%
Early onset myocardial infarction[76]	9	2.8%
Fasting glucose[77]	4	1.5%

Multiple Sclerosis (HLA+ GWAS loci): about 20%

Mahrer B. Nature 2008 FMB

Roche

Life Sciences GS-454 ($500,000)

Life Sciences GS Junior ($100,000)

Roche Light Cycler® 480 System

The COBAS® TaqMan® Analyzer

illumina

HiSeq 2000 ($690,000)

Eco Real-Time PCR System

iScan

applied biosystems part of life technologies

SOLiD 4 ($495,000)

ion torrent ($50,000)

3500 (8-capillary) and 3500xL (24-capillary) Sequencers

The 7900HT Fast

GeneAmp® PCR System 9700

Next Gen Sequencing

Sequencing **Genotyping**

Next-Generation Sequencing

- High data volume per run (giga to megabaes)
- Long run time (days to week)
- Low sample throughput
- High instrument and reagent cost (reagent cost ~3000-5000 per human genome)

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Source: Ride the Next-Gen Sequencing Wave. GenomeWeb,com, 2010