Farmacogenomica: estremo traguardo della personalizzazione terapeutica

Federica Esposito, MD PhD
Laboratorio di Genetica Umana delle Malattie Neurologiche
Unità di Malattie Infiammatorie del SNC & INSPE
Istituto Scientifico San Raffaele - Milano

Monza – 5 Giugno 2017

Outline

- Basic concept for pharmacogenetics in MS
  - ✓ Pharmacogenetics of IFN and GA
  - ✓ Preliminary data on FTY
- Gene expression signature
- Markers to reduce PML risk
- Big data and precision medicine

Moving toward a personalized management in MS

The contribution of genetic variants in treatment response

- ✓ Individual **patients responses** to disease-modifying therapies **are highly heterogeneous**
- ✓ There is **a strong need to predict patient response status**, even before treatment start, considering:
  - potential irreversible consequences of partially effective drugs
  - patient exposure to treatment-related side effects
  - high socioeconomic costs of partially effective drugs
  - alternative treatment options which are becoming available

MS is a typical condition where a **more personalized intervention** would be highly beneficial, favorably **impacting long-term clinical outcomes and optimizing treatment costs**

Heterogeneity in treatment response

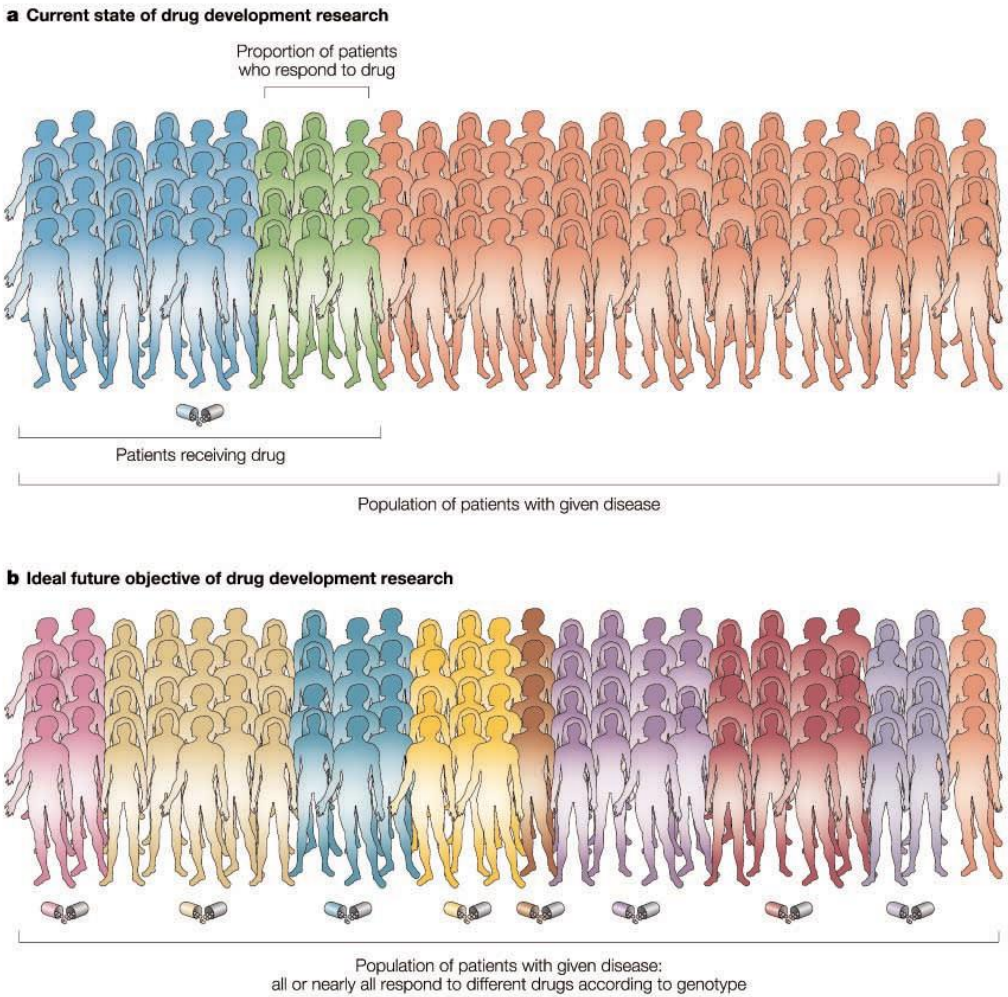
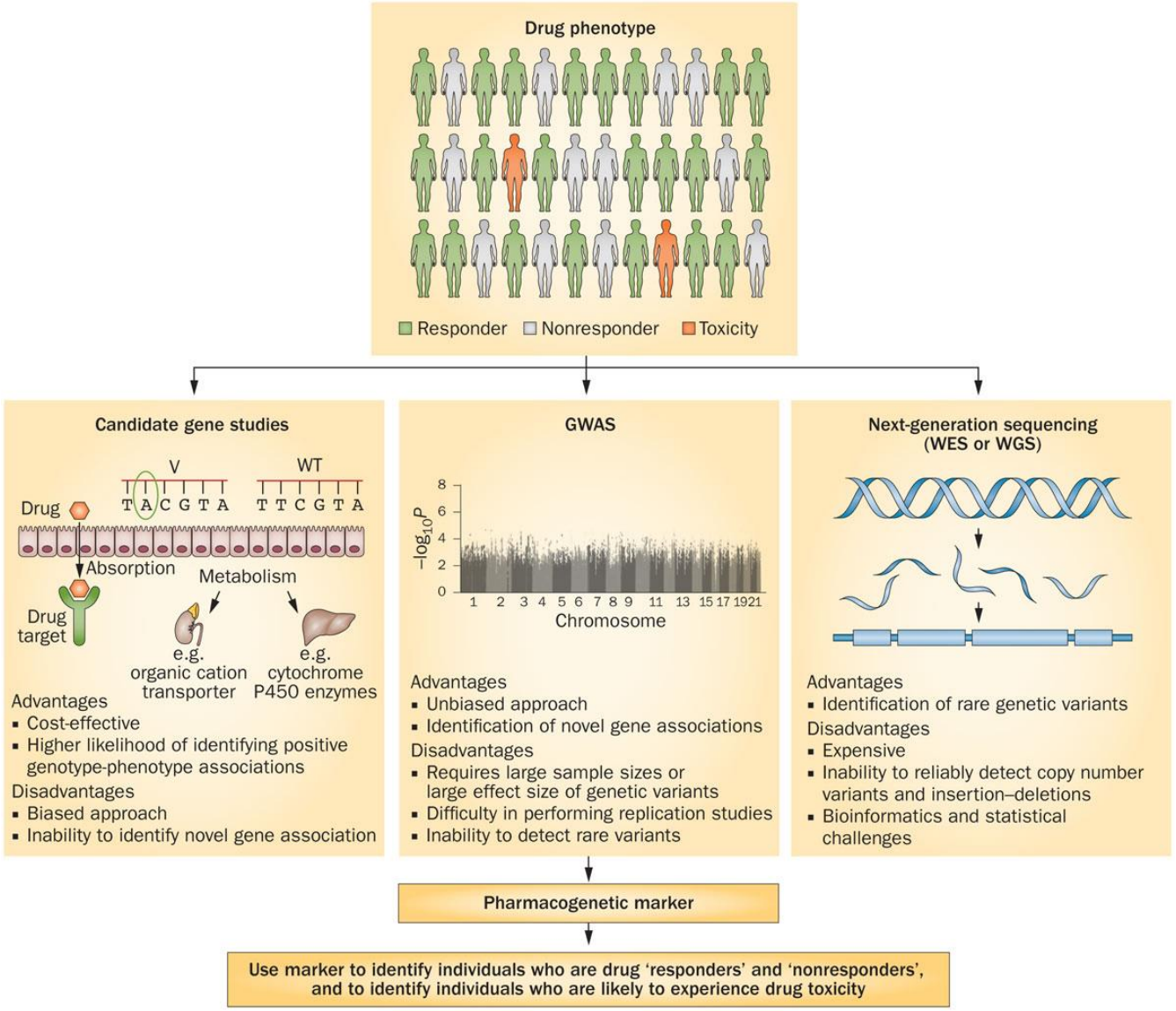


Figure 1 | **An ultimate clinical goal of pharmacogenomics. a** | At present, only a limited number of patients are treated with a specific drug for any given disease due to adverse events. Of those patients who are receiving the drug, not all respond. **b** | One ideal goal that is anticipated from pharmacogenomics is to personalize or 'tailor' therapies, so that, on the basis of pre-genotyping, all patients who have a given disease will receive different drugs and respond to therapy with less risk of adverse events.

Issa – *Nat Rev Drug Discov 2002*

Strategies to identify pharmacogenetic markers



Nature Reviews | Cardiology

Pharmacogenetic studies in MS

✓Most of the studies have been performed on **interferon (IFNβ) and glatiramer acetate (GA) treated patients**

✓The **candidate gene approach** has been mainly used

✓The **results** are generally **poorly replicated** across independent datasets

✓The **heterogeneity in treatment response definition** contribute to inconsistency across studies

✓Most of the **studies** are **underpowered**

✓A **polygenic model** is supposed to account for heterogeneity in treatment response

How to assess treatment response?

Table 2 | Effect of definition on the proportion of non-responders

	Non-responder definitions	Proportion of non-responders (%)
A	An increase of at least 1 EDSS point confirmed at 6 months	18
B	Presence of any relapse	45
C	Presence of two or more relapses	20
D	A decrease in relapse rate of less than 30% compared with the 2 years before therapy	16
E	A decrease in relapse rate of less than 50% compared with the 2 years before therapy	20
F	No decrease in or identical relapse rate compared with the 2 years before therapy	15
G	Definition A or B	49
H	Definition A or E	30
I	Definition A and B	13
J	Definition A and E	7

Results from a study that evaluated various criteria for treatment response in 393 patients with relapsing–remitting multiple sclerosis who had been treated with IFNβ for at least 2 years.[10] Abbreviations: EDSS, Expanded Disability Status Scale. Permission obtained from John Wiley & Sons, Inc. © Río, J. *et al.* Defining the response to interferon β in relapsing–remitting multiple sclerosis patients. *Ann. Neurol.* 59, 344–352 (2006).

Is NEDA classification a possible solution?

Rio et al – *Nat Rev Neurol 2009*

Pharmacogenetic studies for IFNβ

Candidate-gene approach

Table 1 Significant results on the association of genetic polymorphous loci with IFNβ treatment response in multiple sclerosis patients (candidate-gene studies)

Gene	Polymorphous locus (i)	Allele/genotype/haplotype associated with response (+) or nonresponsiveness (−)	*P*-value	OR/HR [95% CI]	MS patients: number, ethnicity	References
IFNAR1	rs1012334	A (+)	0.030	0.9 [0.2–1.2]	147, Spanish	Sriram *et al.* [43]
	rs55884088	GT_n repeat (+)	0.036	NA	162, Irish	Cunningham *et al.* [44]
LMP7	rs2071543	C (+)	0.002	6.4 [1.8–24.1]		
CTSS	rs1136774	C (+)	0.020	0.4 [0.2–0.8]		
MXA	rs2071430	G (+)	0.015	3.4 [1.1–11.4]		
	rs17000900	G/G (+)	0.018	2.4 [1.1–5.4]		
IRF5	rs2004640	T/T (−)	0.01[a]	NA	73, Spanish/the Netherlands (test group)	Vosslamber *et al.* [45]
			0.037[b]	NA	261, Americans/the Netherlands (validation group)	
IRF8	rs17445836	A/A (−)	0.017/0.05[c]	0.45 [0.2–0.9]/0.5 [0.3–1.0][c]	424, Americans (test group)	Gross *et al.* [5]
			NS	NS	211, Germans (replication cohort)	
USP18	rs2542109	A/A (+)	0.041	1.8 [1.0–3.1]	225, Spanish	Malhotra *et al.* [46]
IFNG	Polymorphic microsatellites in the first intron	$(CA)_{12}$ (−)	0.013	0.5 [0.3–0.9]	110, Spanish	Martinez *et al.* [47]
		$(CA)_{13}$ (−)	0.04	1.8 [1.0–3.3]		
		$(CA)_{14}$ (−)	0.009	NA		
		$(CA)_{15}$ (+)	0.005	0 [0–0.6]		
IL10	rs1800896/rs1800871/rs1800872	Non-GCC haplotypes (+)	0.04	NA	25, Norwegians	Wergeland *et al.* [48]
CCR5	rs333	32 bp deletion (+)	0.036	1.9 [1.0–3.6]	253, Russians	Kulakova *et al.* [49]
TGFB1	rs1800469	C (+)	0.042[d]	9.2 [0.2–70.4]		
TRAILR1	rs20576	C/C (+)	NS	NS	509, Spanish (original cohort)	López-Gómez *et al.* [50]
			0.005	0.2 [0.1–0.7]	226, Spanish (validation cohort)	
			0.048[d]	0.3 [0.1–0.6]	Combined analysis	
CD58	rs12044852	C/C (−)	< 0.05	NA	120, Iranian	Torbati *et al.* [51]
CD46	rs2724385	T/T (+)	0.006	3.5 [1.4–8.9]	163, Spanish	Alvarez-Lafuente *et al.* [52]
GPC5	rs10492503	A/A (+)	0.018[d]	3.0 [1.3–6.6]	199, Spanish	Cenit *et al.* [53]
	rs1411751	G/G (+)	0.012[d]	3.7 [1.5–9.4]		

Tsareva et al – *Parmacogenet Genomics 2016*

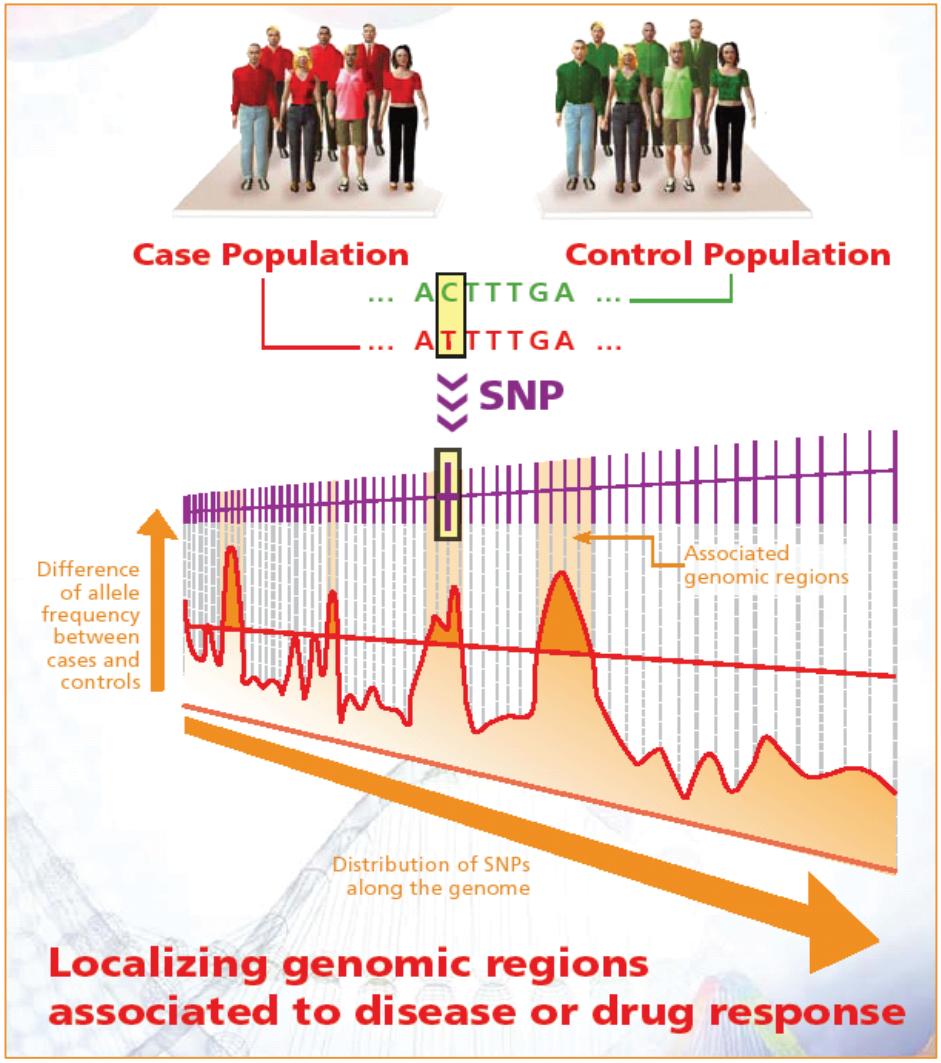
Pharmacogenetic studies for GA
Candidate-gene approach

Table 3 Significant results on the association of genetic polymorphous loci with GA treatment response in multiple sclerosis patients (candidate-gene studies)

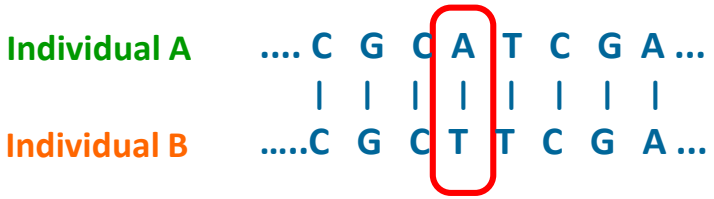
Gene	Polymorphous locus	Allele/genotype/haplotype associated with response (+) or nonresponsiveness (−)	*P*-value	OR/HR [95% CI]	MS patients: number, ethnicity	References
HLA-*DRB1*		1501 (+)	0.008	NA	44, Italians	Fusco *et al.* [37]
HLA-*DRB1*	rs3135388	A/A[a] (+)	0.015/0.048[b]	2.7 [1.2–6.0]/2.2 [1.0–4.9][b]	332, Americans	Gross *et al.* [5]
HLA-*DRB1*		DR15 (+)	0.02	NA	64, Americans	Dhib-Jalbut *et al.* [77]
HLA-*DQB1*		DQ6 (+)	0.014			
		DR17 (−)	0.012			
		DQ2 (−)	0.002			
		Haplotypes:	0.044[c]			
		DR15-DQ6(+)	0.046[c]			
		DR17-DQ2(−)				
HLA-*DRB1*		DR4 (+)	0.027	1.9 [1.0–3.5]	285, Russians	Tsareva *et al.* [78]
CCR5	rs333	Wild type/wild type (+)	0.046	1.7 [1.0–3.1]		
TCRB	rs71878	C (+)	0.015[d]	6.8 [1.5–32.0][d]	73, USA Caucasians (discovery cohort)	Grossmann *et al.* [4]
IL12RB2	rs946685	G (+)	0.027[d]	0.24 [0.07–0.85][d]		
MBP	rs470929	T (+)	0.04[d]	5.3 [1.1–25.9][d]	101, Europeans/ Canadians (replication cohort)	
IL1R1	rs956730	A (+)	0.025[c,d]	5.8 [1.2–27.1][d]		
CD86	rs1129055	C (−)	0.022[c,d]	6.3 [1.3–30.3][d]		
CTSS	rs2275235	G (+)	0.014[c,d]	11.6 [1.6–81.9][d]		
	rs1415148	A (+)	0.009[c,d]	6.9 [1.6–29.2][d]		
FAS	rs982764	C (+)	0.05[d]	3.0 [1.0–8.8][d]		

Tsareva et al – *Parmacogenet Genomics 2016*

Genome-wide association studies (GWAS)



Single Nucleotide Polymorphism (SNP)



- Naturally occurring variants that affect a single nucleotide
- They account for the vast majority of variations in our genome

GWAS for IFNβ response

ORIGINAL CONTRIBUTION

Genome-wide Scan of 500 000 Single-Nucleotide Polymorphisms Among Responders and Nonresponders to Interferon Beta Therapy in Multiple Sclerosis

Manuel Comabella, MD; David W. Craig, BSc; Carlos Morcillo-Suárez, BSc; Jordi Río, MD; Arcadi Navarro, PhD; Marta Fernández, BSc; Roland Martin, MD; Xavier Montalban, MD, PhD

Arch Neurol. 2009;66(8):972-978

ORIGINAL CONTRIBUTION

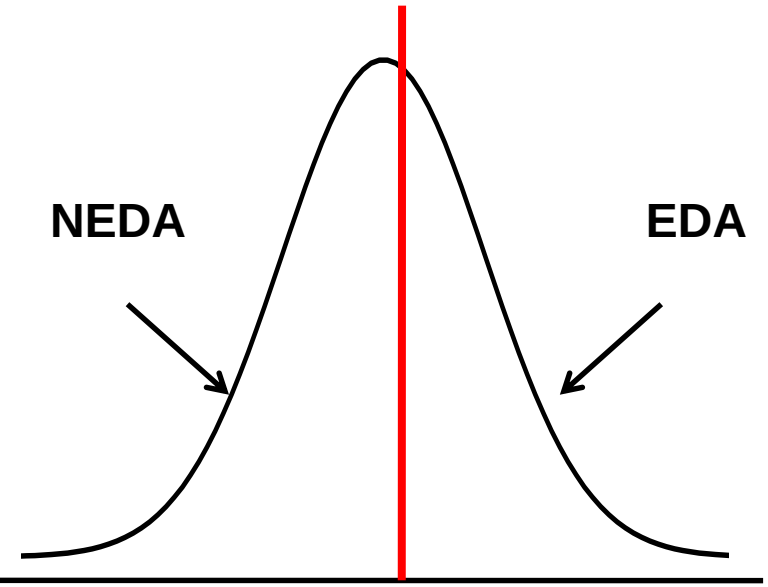
Genome-Wide Pharmacogenomic Analysis of the Response to Interferon Beta Therapy in Multiple Sclerosis

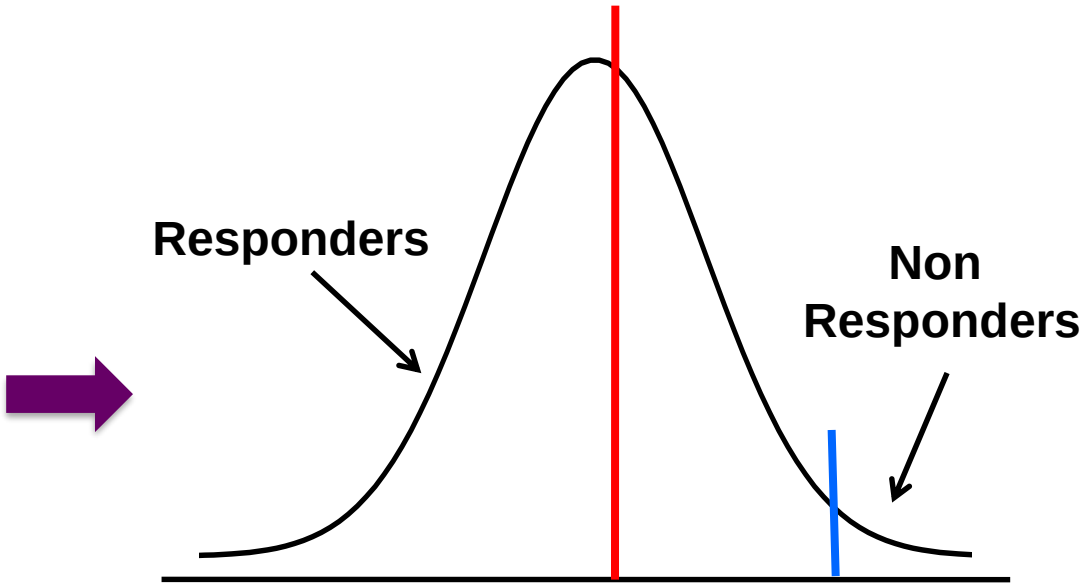
Esther Byun, MD; Stacy J. Caillier, BSc; Xavier Montalban, MD; Pablo Villoslada, MD, PhD; Oscar Fernández, MD; David Brassat, MD; Manuel Comabella, MD, PhD; Joanne Wang, MPH; Lisa F. Barcellos, PhD; Sergio E. Baranzini, PhD; Jorge R. Oksenberg, PhD

Arch Neurol. 2008;65(3):337-344

No clear replication of the findings in independent populations

Extremes phenotypes for pharmacogenetic studies





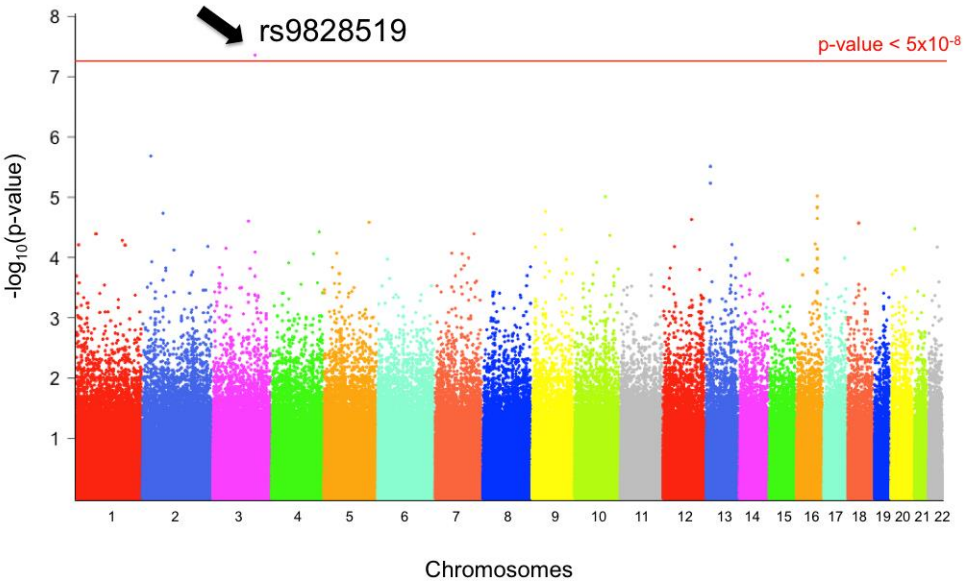
The EDA group is very heterogeneous

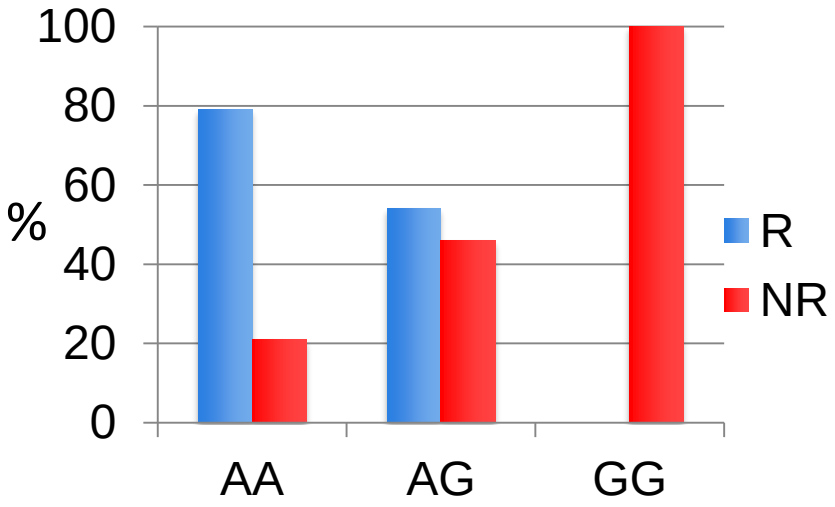
In order to increase the power to detect a signal in the genetic analyses we should try to select the extremes of our distribution

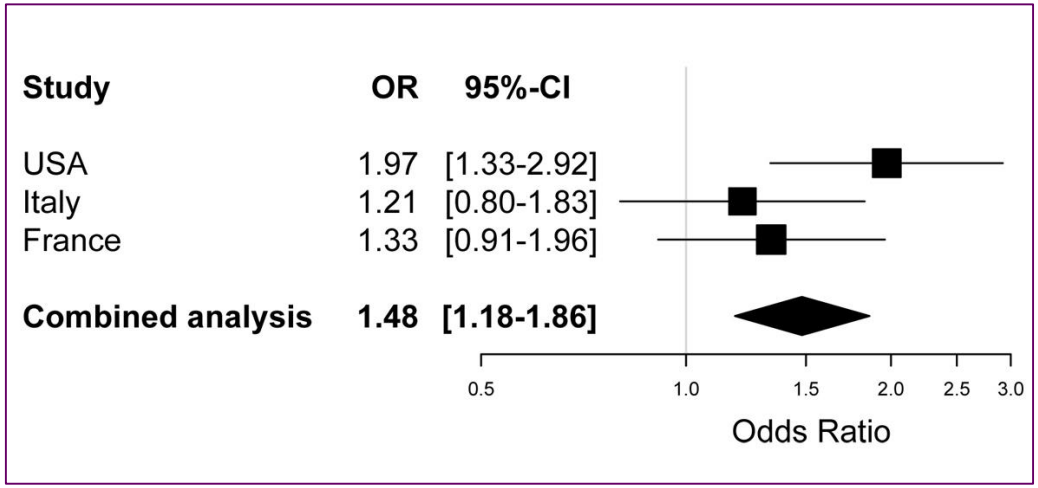
An Italian GWAS study for IFNβ

The *SLC9A9* story

- GWAS in 73 responders (R) and 42 non-responders (NR) to IFNβ
- Replication in 483 R and 398 NR
- Identification of a **genetic variant intronic to SLC9A9 associated with increased risk of non-response to IFNβ**







Esposito et al – *Ann Neurol 2015*

Open questions

Is rs9828519 a marker of treatment response or a marker of disease severity?

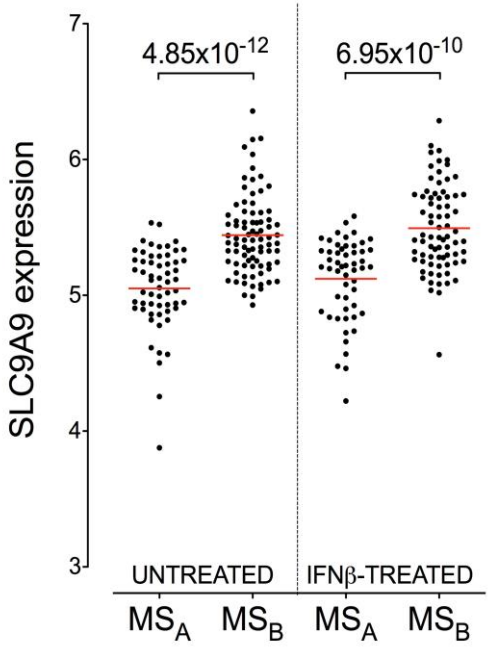
American Glatiramer Acetate (GA) treated patients (R=98, NR=91)

rs9828519 doesn't affect the response to GA (p-value=0.14, OR=1.13)

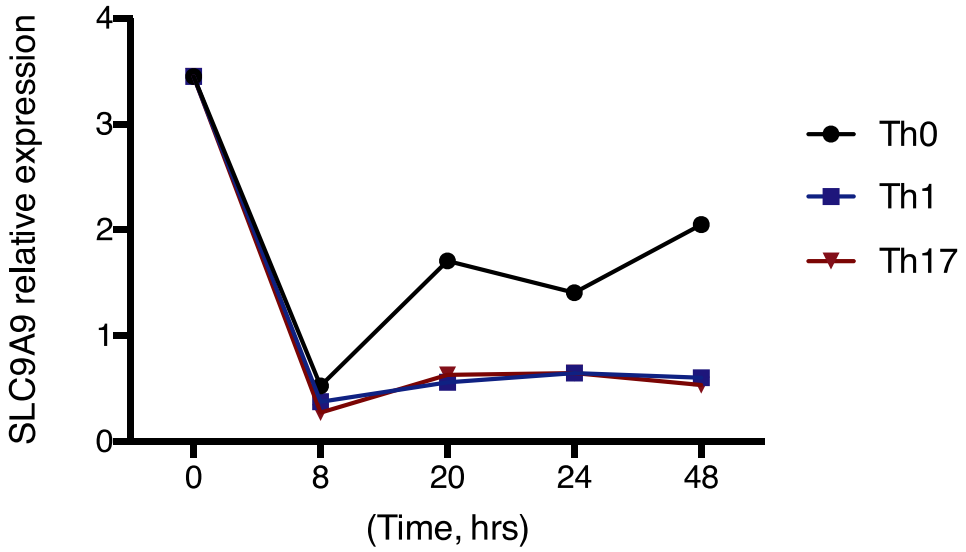
This genetic variant seems to be a **predictive marker for non-response to IFNβ**

How rs9828519 can affect treatment response?

- ✓ rs9828519 is intronic to *SLC9A9,* which encodes a sodium/hydrogen exchanger expressed also in the brain
- ✓ Functional studies are ongoing, e.g. impact of genotype on:
  - expression level of SLC9A9 splice variants
  - mRNA expression profile, cytokine profile, cellular subtypes
  - *ex vivo* cytokine profiling with patient PBMC after IFNβ stimulus



SLC9A9 expression is lower in more active MS patients (MS_A), suggesting an involvement of *SLC9A9* in the inflammatory component of MS



SLC9A9 is downregulated after the induction of differentiation in both Th1 and Th17, suggesting that *SLC9A9* may provide an inhibitory signal of T cell activation

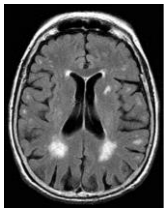
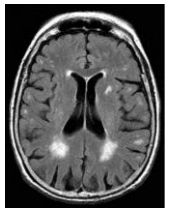
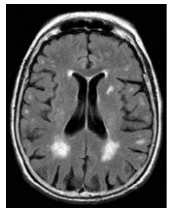
✓*SLC9A9* influences the differentiation of T cells to a pro-inflammatory fate and may have a broader role in MS disease activity, outside of IFNβ-treatment

✓These discoveries may yield insights for novel therapeutic approaches for the inflammatory component of MS

Esposito et al – *Ann Neurol 2015*

A pharmacogenetic study for fingolimod

Study design

30 to 60 days from FTY start

0 3 6 9 12 15 18 21 24

✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓ ✓

RNA profile

RNA profile


* **Assessment of response** according to clinical and MRI data

✓ Scheduled clinic visit

For most of patients, MRI performed at baseline and every 12 months

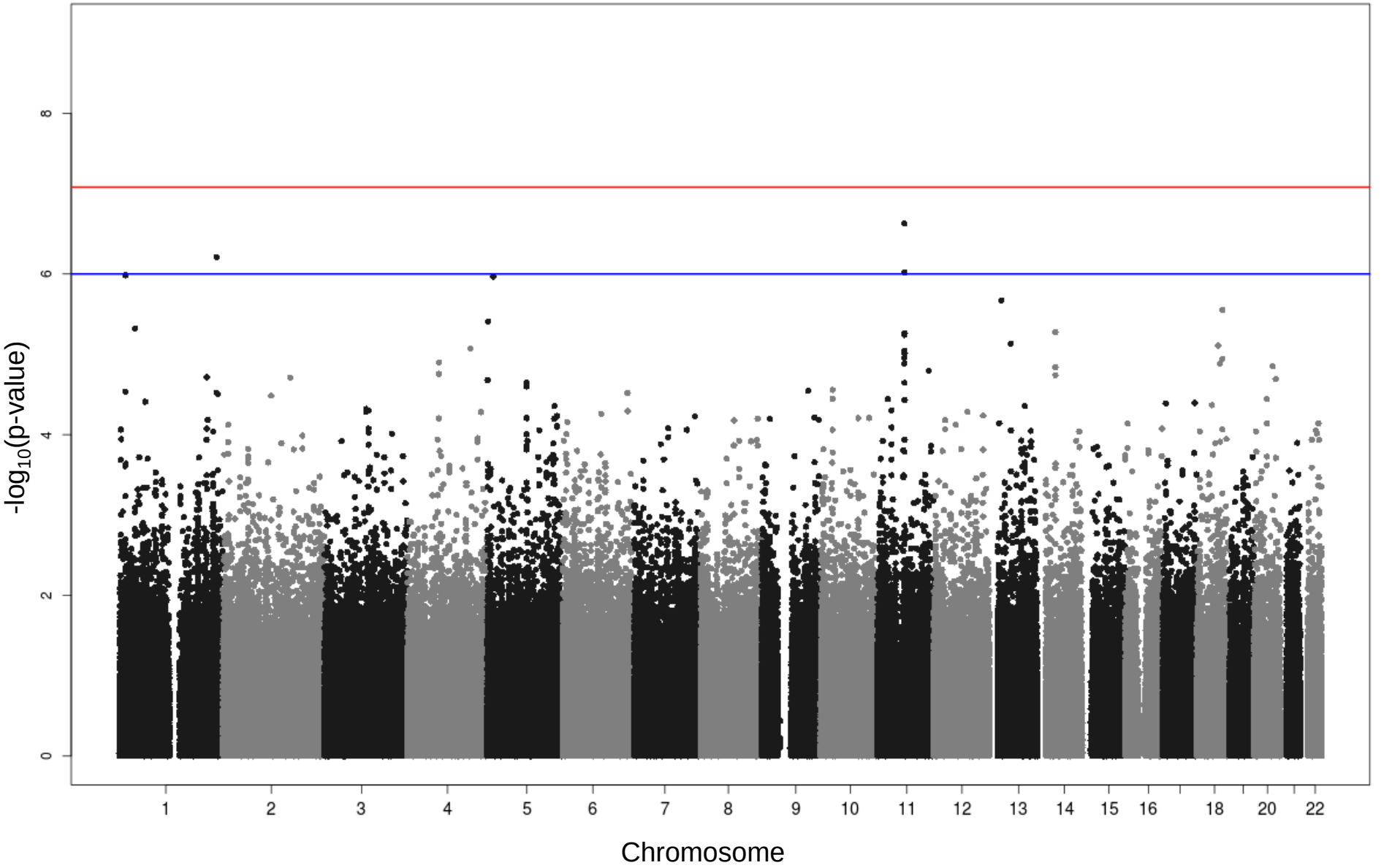
RNA samples at month 0 and 6 in 48 subjects

DNA samples at any time during study

SCLEROSI MULTIPLA ONLUS associazione italiana

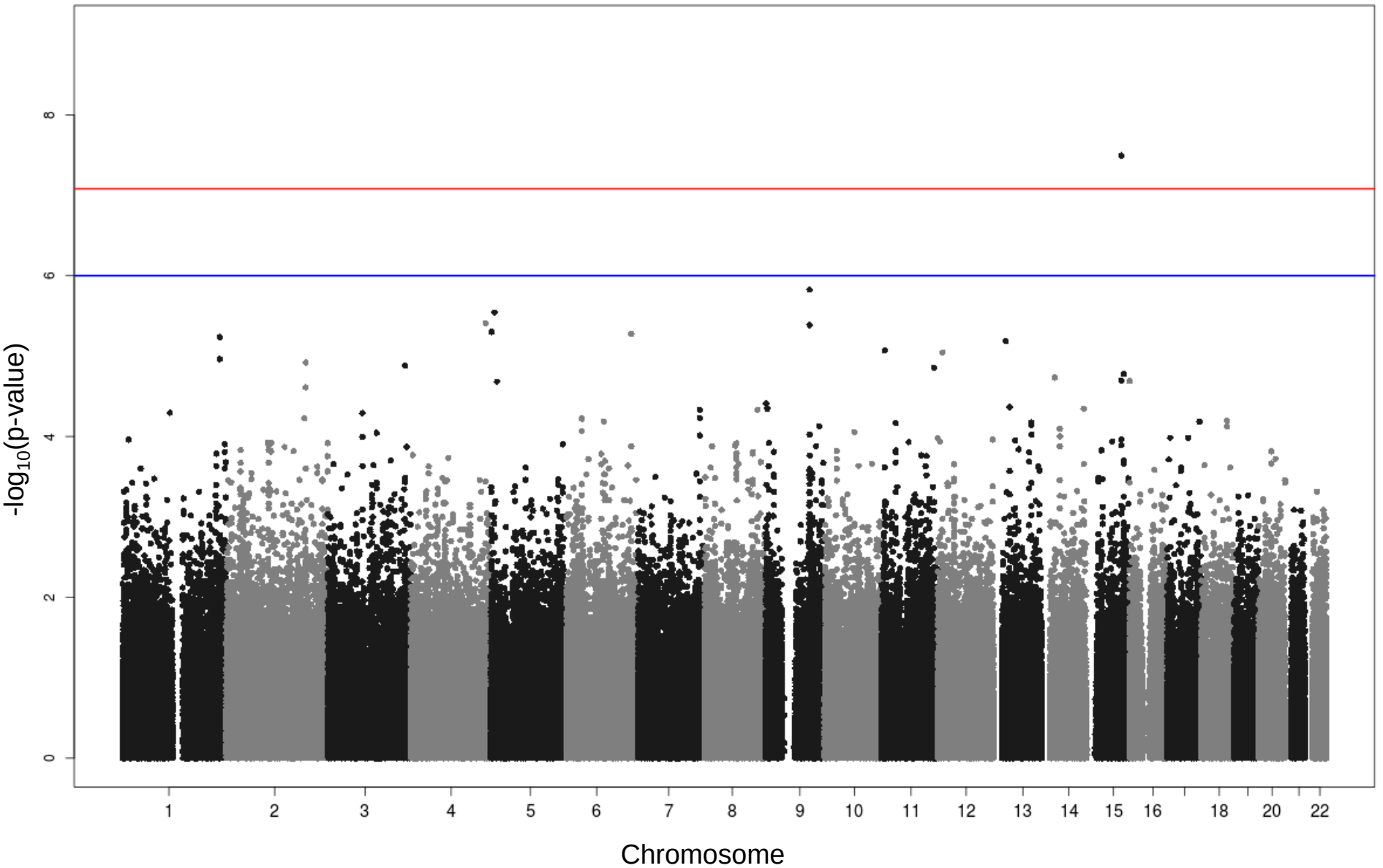
Genome-wide association study

Time to first relapse analysis (n=255)



Genome-wide association study

ARR analysis (n=255)



Candidate gene study design

Curated pathway (Hecker et al. 2016)

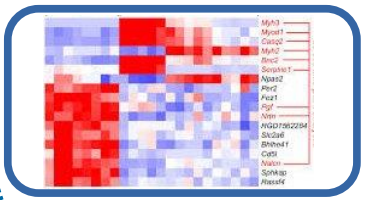
KEGG map 04071

Knowledge-driven selection

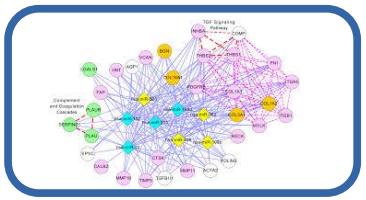
ADME genes

Transcriptomic Assay RNA-seq
6-months induced change

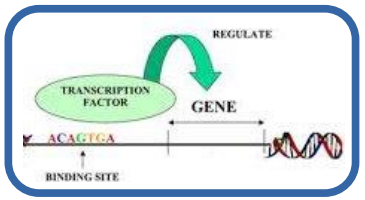
DEGs



Network



Transcription factors



Data-driven selection

Candidate genes + eSNPs from GTEx database

gene-level

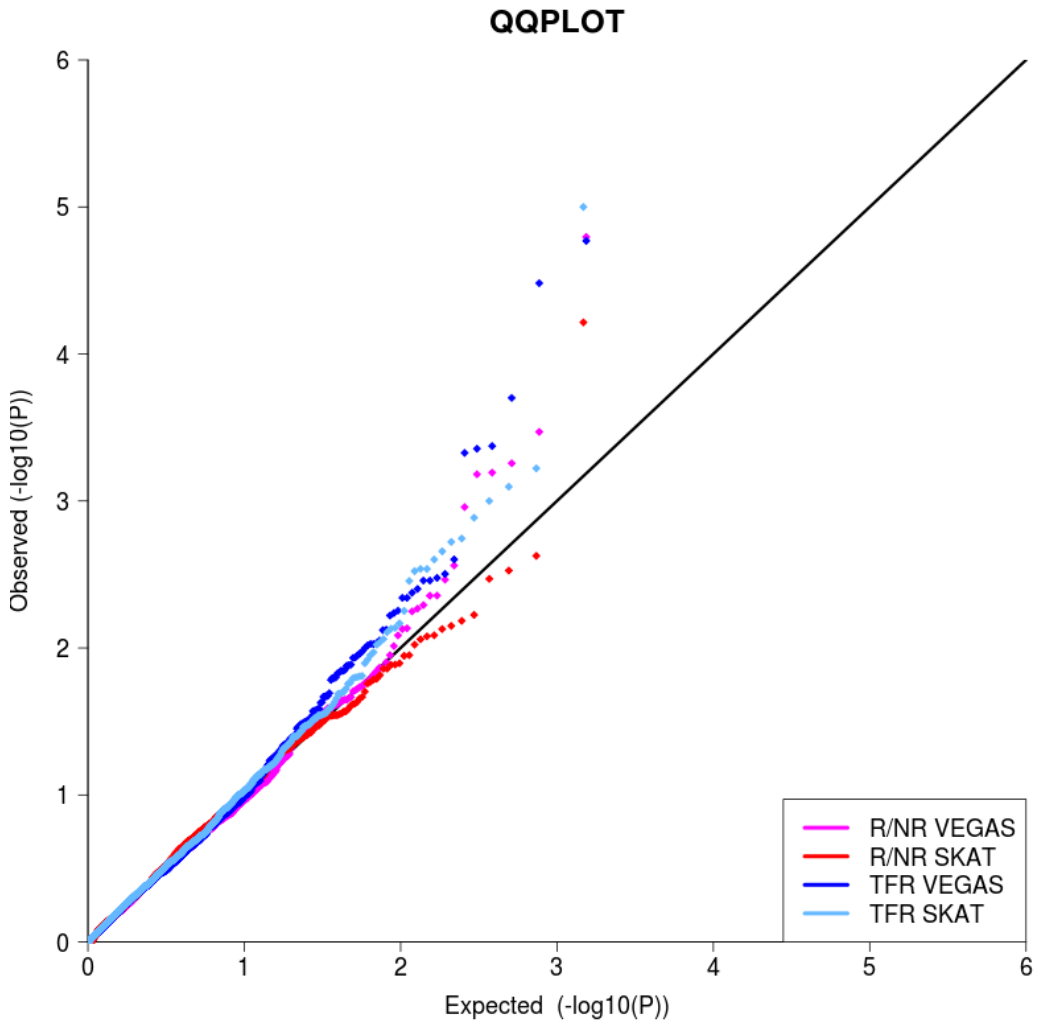
SKAT

VEGAS

SNP-level

Logistic models
Cox models

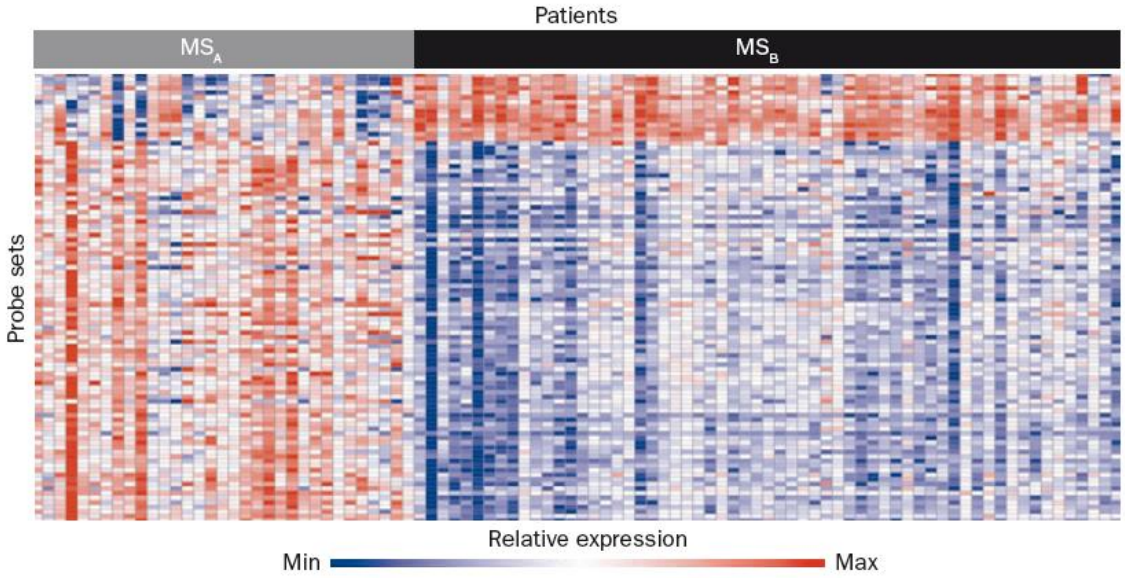
Gene-level associations – QQ plot



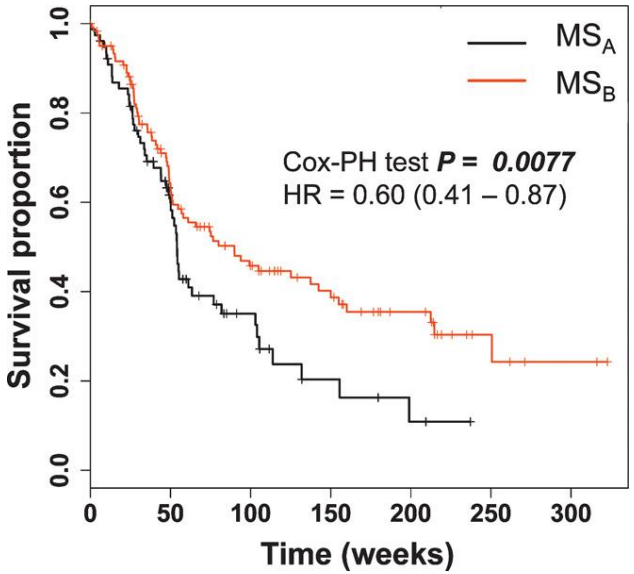
RNA profiling and MS disease activity

- The authors applied an **unbiased, data-driven approach** to divide the patients on the basis of their molecular profile

- A specific gene expression signature enabled the patients to be stratified into two subsets, MS_A and MS_B



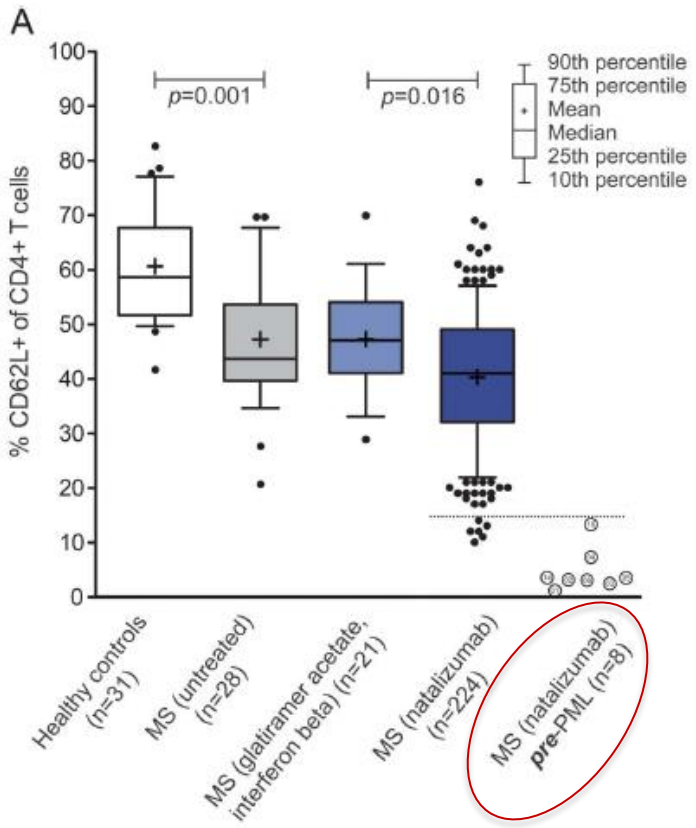
RNA profiles correlate with disease activity in multiple sclerosis. A heat map profile of the 98 probe sets that constitute the RNA signature is presented, with patients classified as MS_A or MS_B on the basis of RNA expression profiles in blood mononuclear cells. The MS_A group was shown to have a greater likelihood of relapse after sampling than the MS_B group. Image courtesy of L. Ottoboni.

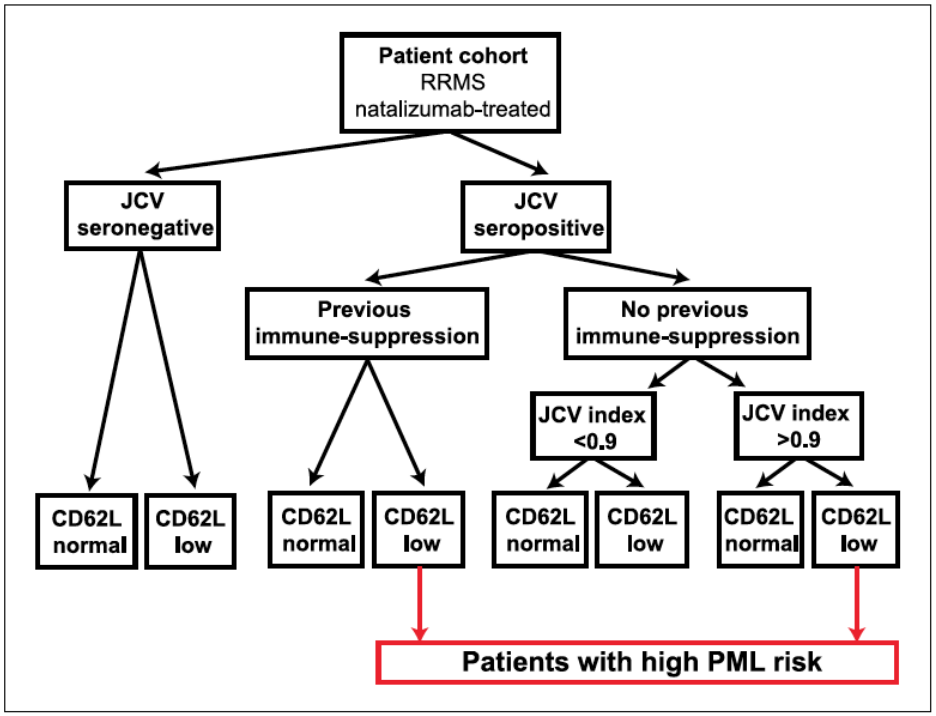


Patients with the MS_A profile were more likely to experience a clinical relapse, and to exhibit new lesions on MRI, than were those with the MS_B profile

Ottoboni et al – *Sci Transl Med 2012*

L-Selectine as a biomarker for PML development in Natalizumab-treated patients





Schwab et al – *Neurology 2013*

Schwab et al – *Mult Scler 2016*

Personalized and Precision Medicine in MS

The BioScreen tool

This new tool attempts to address the challenges of dynamic management of a complex chronic disease and illustrates the extent to which translational digital medicine has the potential to radically transform medical practice

FIGURE 1: Multiple sclerosis (MS) BioScreen prototype application. An image capture from the overview screen illustrates an individual patient's data presented in anonymous mode. The initial layer of the application is a visualization of an individual's overall health information, providing a gateway to the full complement of accessible data. This view introduces the defining characteristics of the patient and the disease course: name, gender, age, disease onset, disease course, duration, and relapses on the top bar. It is otherwise organized by data type, with clinical information (clinical presentation over time, treatments, and attacks) displayed in the panels on the right, and imaging and biomarker data (including MS genetic risk markers) on the left. EDSS = Expanded Disability Status Scale; INF−β = Interferon Beta; IVSM = Intravenous Solumedrol; T2LL = T2 Lesion Load; MSGB = Multiple Sclerosis Genetic Burden. Copyright, Regents of the University of California; all rights reserved.

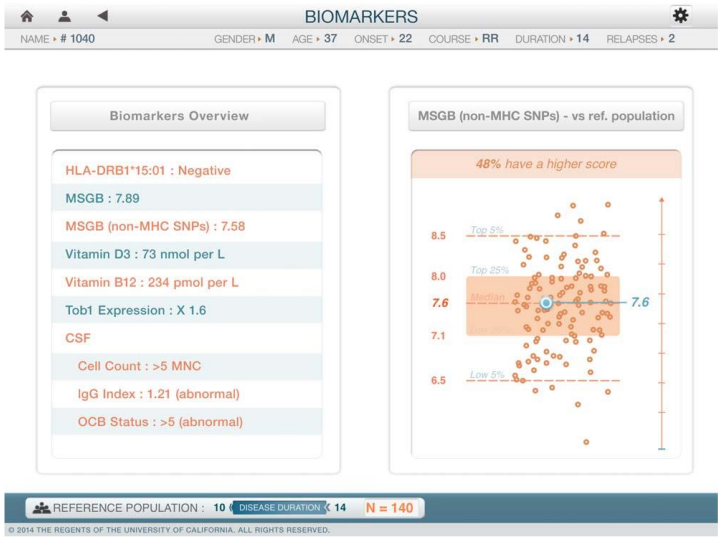


FIGURE 6: Display of biomarker information and contextualized representation of aggregated genetic risk scores. The left panel shows current values for various types of laboratory and biomarker information relevant to multiple sclerosis (MS), including: typing for the disease-associated HLA-DRB1*15:01 allele, value of the Multiple Sclerosis Genetic Burden Score (MSGB) with and without the contribution of the major histocompatibility complex region, vitamin D3, vitamin B12, TOB1 gene expression, and cerebrospinal fluid analyses. In the right panel, the MSGB score was computed30 based on a weighted scoring algorithm using independent 64 single nucleotide polymorphisms associated with MS risk. Similarly to Figures 4 and 5, the orange boxplot displays the 5th, 25th, 50th, 75th, and 95th percentiles of the distribution of the score in the reference population indicated in the bottom blue bar. The application is displayed in anonymous mode. MSGB = Multiple Sclerosis Genetic Burden; MHC = Major Histocompatibility Complex; SNP = Single-Nucleotide Polymorphism; Tob1 = Transducer of ERBB2, 1; CSF = Cerebrospinal Fluid; MNC = Mononuclear Cell; IgG = Immunoglobulin G; OCB = Oligoclonal Bands. Copyright, Regents of the University of California; all rights reserved.

Gourraud et al – *Ann Neurol 2014*

Conclusions

✓Genetic information contribute to better understand the mechanisms underlying treatment response and to move towards a more personalized approach in MS

✓Different tools and huge collaborative efforts are ongoing at the international level, involving also collaboration with pharmaceutical company, to address this unmet medical need

✓A more personalized and individualized approach in on the way in the complex management of MS

Acknowledgements

Thank you all for the attention!

Laboratory of Human Genetics of Neurological Disorders CNS Inflammatory Unit San Raffaele Hospital, Milan - Italy

F Martinelli Boneschi
F Clarelli
F Esposito
S Santoro
E Mascia
A Osiceanu
A Zauli
L Ferre'

PROGRESSO consortium

Department of Neurology San Raffaele Hospital, Milan - Italy

G Comi
V Martinelli
B Colombo
L Moiola
M Radaelli
M Romeo

University of Eastern Piedmont, Novara - Italy

S D'Alfonso
N Barizzone
PROGEMUS consortium

Brigham Women's Hospital & Broad Institute, Boston - USA

PL De Jager
N Patsopoulos

International Multiple Sclerosis Genetic Consortium

SCLEROSI MULTIPLA onlus associazione italiana

fondazione cariplo

BACK-UP