A CANDIDATE-GENE STUDY TESTING THE ROLE OF SPHINGOSINE PATHWAY GENES ON RESPONSE TO FINGOLIMOD IN A COHORT OF ITALIAN RELAPSING-REMITTING MULTIPLE SCLEROSIS PATIENTS

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Introduction and Aim

A substantial heterogeneity in treatment response is observed across MS patients. Given the potential irreversible consequences of partially effective treatments and the presence of several alternative therapeutic options, MS is a typical condition where a more personalized intervention would be highly beneficial, favorably impacting long-term clinical outcomes and optimizing treatment costs.

We performed a study aimed to uncover genetic variants and genes associated to interindividual differences in the response to fingolimod (FTY – Gilenya®) therapy by applying a candidate-gene approach and exploring the sphingolipid signaling pathway. The final aim is to identify patients who would benefit more from the FTY treatment, ideally even before treatment start, based on their clinical and genetic characteristics.

Patients and methods

•We collected relapsing-remitting MS patients, diagnosed according to McDonald Criteria, followed at San Raffaele MS center and treated with FTY. Patients were prospectively followed at San Raffaele MS center for at least 2 years, with clinical visits every 3 months and brain MRI scan on average every year.
Given the known increase in disease activity after natalizumab (NAT) withdrawal, we did not included in the study patients treated with NAT in the year before FTY, in order to limit misclassifications.

•**Treatment response** was assessed using two different approaches:

1. *No evidence of disease activity (NEDA criterion) at 2-year follow-up*
   - No relapses
   - No new T2 or Gd-enhancing lesions at brain MRI scans
   - No EDSS progression confirmed at 6 months.

   Patients with no evidence of any type of disease activity under FTY, but who discontinued the treatment before the 2 year-follow-up (n=5) were not included in this analysis, because we were not able to classify them according to the NEDA criterion.

2. *Time to First Relapse*

   Patients who suspended the treatment within the 2-year follow-up were included in the analysis as long as they received the treatment.

A knowledge-driven candidate-gene approach was adopted by investigating 120 genes belonging to "Sphingolipid signaling pathway" as available in KEGG database and 63 genes manually selected from literature. Gene-level analyses were carried out using VEGAS and SKAT, taking into account pattern of linkage-disequilibrium to compute gene-wise p-values after 10,000 permutations. The selected genes were also tested for evidence of expression modulation by putative SNPs (eSNPs) using different public databases (SNPExpress, SCAN, GTEx and Braineac).

Clinical and demographic features	Entire cohort (n=246)	NEDA (n=132)	EDA (n=109)	p-value
Female:Male ratio	2.3	1.9	2.9	0.15
Age at disease onset, years (sd)	28.3 9.1	29.6 9.1	26.9 8.9	0.02
Age at treatment start, years (sd)	38.6 9.5	39.6 9.5	37.9 9.4	0.15
Disease duration, years (sd)	10.3 7.3	10.0 7.5	10.9 7.0	0.29
Median EDSS at treatment start, (range)	2.0 (1.0 – 6.0)	2.0 (1.0 – 6.0)	2.0 (1.0 – 5.5)	0.24
Annualize relapse rate 2 years before (sd)	0,8 0.8	0.8 0.9	0.9 0.6	0.41
Patients with Gd+ lesions at baseline	31.9 %	25.6%	39.3%	0.03
Number of Gd+ lesions at baseline (sd)	0.7 1.4	0.43 0.9	1.0 1.9	<0.01
Number of New T2 lesions at baseline (sd)	1.7 3.4	1.34 1.9	2.1 4.7	0.11

Table 1 - Clinical and demographic characteristics of the patients enrolled in the study

Statistical analyses

1. *NEDA analysis* → patients were analyzed with a logistic regression model under additive allele coding.
2. *Time to First Relapse analysis* → patients were studied with a Cox regression model adjusted for the ARR in the two years before FTY start.

Results

NEDA analysis (132 NEDA patients *vs* 109 EDA patients)

VEGAS Results					
Gene	Chr	Path	nSNPs	TopSNP p-value	p-value
DEGS1	1	Yes	13	$7.8*10^{-5}$	0.001
SPTLC2	14	Yes	39	0.001	0.001
PLCB4	20	Yes	101	$3.4*10^{-4}$	0.010
MAPK8	10	Yes	19	0.002	0.043
ROCK1	18	Yes	9	0.021	0.050

SKAT Results				
Gene	Chr	Path	nSNPs	p-value
SPTLC2	14	Yes	39	0.001
PLCB4	20	Yes	101	0.002
DEGS1	1	Yes	13	0.009
MAPK8	10	Yes	19	0.010
MAP3K5	6	Yes	38	0.023
ROCK1	18	Yes	9	0.028
CERS2	1	Yes	11	0.030
CD86	3	No	26	0.030
ROCK2	2	Yes	19	0.040
PPP2R2C	4	Yes	111	0.042
CERS5	12	Yes	17	0.045

Table 2 - Top genes selected considering the NEDA outcome, using respectively VEGAS and SKAT softwares

Time to first relapse analysis

VEGAS Results					
Gene	Chr	Path	nSNPs	TopSNP p-value	p-value
DEGS1	1	Yes	13	$8.9*10^{-4}$	0.010
PRKCG	19	Yes	12	0.002	0.018
PPP2R2B	5	Yes	113	0.001	0.018
CD86	3	No	26	0.001	0.029
NSMAF	8	Yes	23	0.020	0.033
RELA	11	Yes	13	0.016	0.035
PPP2R5B	11	Yes	9	0.005	0.036
PPP2R5C	14	Yes	36	$8.5*10^{-4}$	0.038
CLDN5	22	No	7	$7.2*10^{-4}$	0.044
PPP2R2A	8	Yes	25	0.011	0.047
MAPK1	22	Yes	22	0.015	0.048

SKAT Results				
Gene	Chr	Path	nSNPs	p-value
DEGS1	1	Yes	13	0.006
PPP2R5C	14	Yes	36	0.010
IL23A	12	No	8	0.023
PPP2R5B	11	Yes	9	0.030
NSMAF	8	Yes	23	0.030
PPP2R2B	5	Yes	113	0.033
PIK3CG	7	Yes	20	0.045
RELA	11	Yes	13	0.047
CR1	1	No	24	0.050
MAPK1	22	Yes	22	0.050

Table 3 - Top genes selected considering the Time to First Relapse outcome, using respectively VEGAS and SKAT softwares

eSNPs analysis

NEDA/EDA outcome

eSNP	Gene	Path	Database	p-value	A1	OR
rs4965320	*CERS3*	Yes	Braineac/GTEx/SCAN	$7.2*10^{-5}$	A	0.46
rs2361340	*SPTLC3*	Yes	SNP Express	$5.1*10^{-4}$	A	0.36

Time to first relapse outcome

eSNP	Gene	Path	Database	p-value	A1	HR
rs3859170	*GNAI1*	Yes	SCAN	$7.3*10^{-5}$	G	2.2597
rs7640727	*SGMS1*	Yes	SNP Express	$2.7*10^{-4}$	A	0.2361
rs1417371	*GAB2, MAPK11*	Yes	SNP Express	$4.8*10^{-4}$	A	2.5179
rs12238640	*RELA*	Yes	SNP Express	$5.4*10^{-4}$	G	2.3299
rs12423616	*PTEN*	Yes	SNP Express	$7.5*10^{-4}$	G	2.0997
rs4774452	*ADORA1*	Yes	SNP Express	$8.2*10^{-4}$	A	3.4478
rs927418	*CCL4*	No	SNP Express	$9.4*10^{-4}$	G	2.8787
rs4633531	*ASAH1, PTEN, SPTLC2*	Yes	SNP Express	$9.6*10^{-4}$	A	2.0880
rs11115178	*ASAH1, PTEN, SPTLC2*	Yes	SNP Express	$9.6*10^{-4}$	G	2.0871

Table 4 - Top genes with evidence of eQTL modulation considering the NEDA and Time to First Relapse outcomes, according to data reported in public databases (SNPExpress, SCAN, GTEx and Braineac)

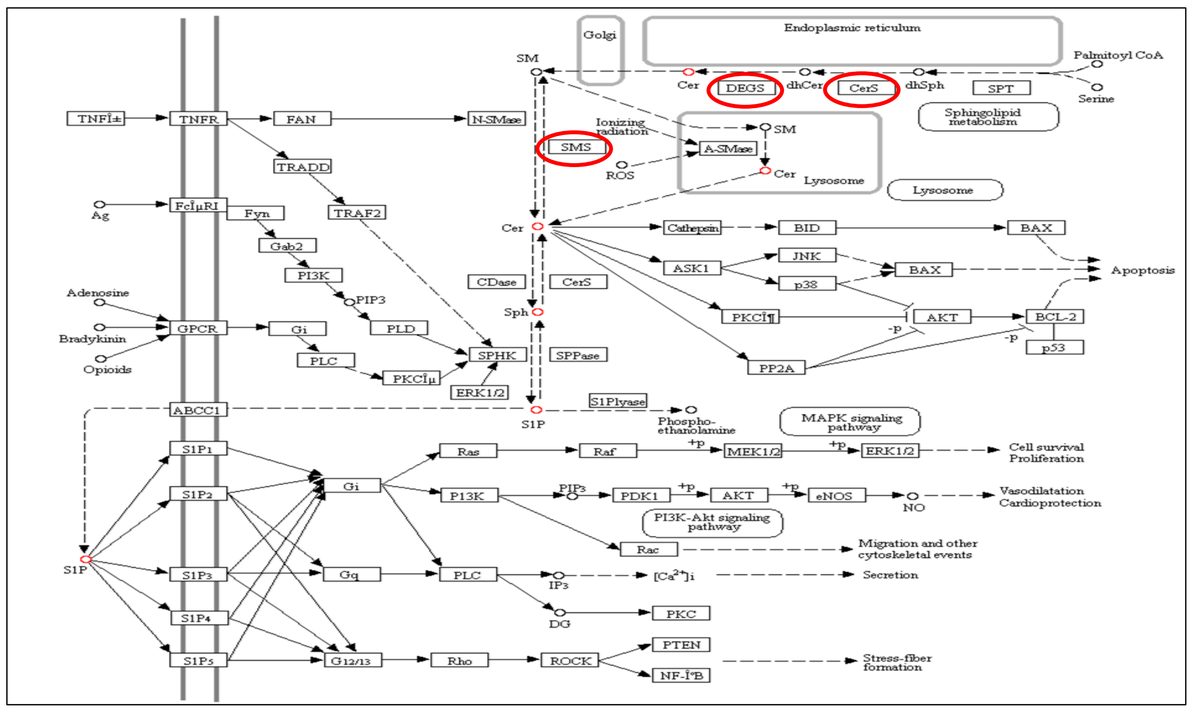

Figure 1 - "Sphingolipid signaling pathway" as available in KEGG database

•The *DEGS1* gene was consistently replicated across outcomes and different tools.
•It encodes a member of the fatty acid desaturase family and catalyzes the last step in the main ceramide biosynthetic pathway.
•It is expressed in both central nervous system and immune system.
•Recent data suggest that within active MS lesions there is an increased production of ceramide (van Doorn et al, 2012).
•Preliminary data suggest that FTY is able to reduce the production of ceramide by astrocytes (van Doorn et al, 2012).

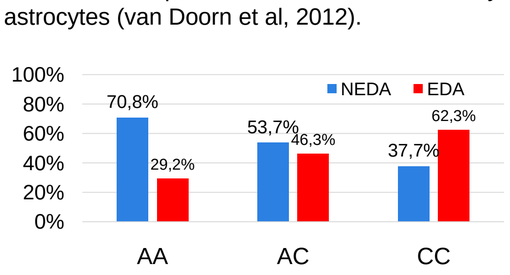

Figure 3 - Proportion of NEDA and EDA patients across the three different genotypes

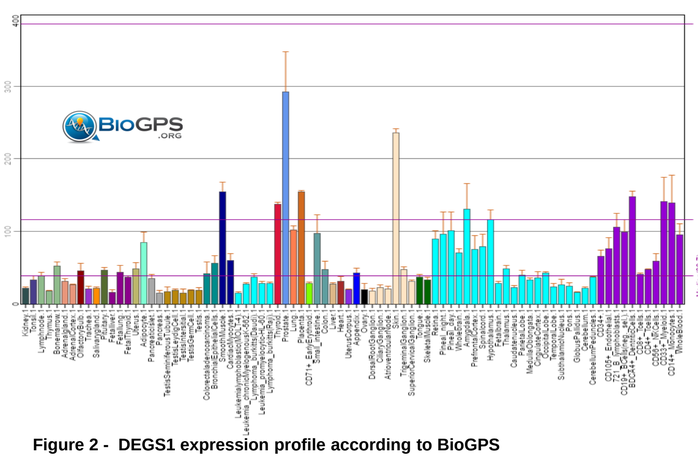

Figure 2 - DEGS1 expression profile according to BioGPS

The top SNP mapping to DEGS1 (p-value $7.8*10^{-5}$) seems to have an additive effect, with the C allele being associated with worse response to FTY.

Discussion

•This candidate gene study suggests a possible involvement of the sphingosine pathway in influencing the response to FTY in MS patients.
•The *DEGS1* (Delta(4)-Desaturase, Sphingolipid 1) gene has been selected across different tools and different treatment response outcomes. The association results obtained with VEGAS and SKAT tools are very close to the stringent Bonferroni threshold at both the gene and SNP level.
•According to our data, the best SNP mapping to *DEGS1* (p-value $7.8*10^{-5}$), seems to have an additive effect, with the C allele being associated to a worse response to FTY.
•*DEGS1* is involved in the ceramide biosynthesis. Recent data suggest that FTY may reduce the production of pro-inflammatory lipids, limiting the subsequent transendothelial leukocyte migration (van Doorn et al, 2012).
•The results from the eSNPs analyses show significant gene-expression modulations of several genes belonging to the sphingosine pathway, with a specific focus to the the genes involved in ceramide production.
•Taken together these data point the attention to an alternative FTY mode of action and suggest that genetic variants within the genes involved in the ceramide synthesis may contribute to explain the interindividual differences in FTY response.
•Additional analyses are planned to replicate these data in bigger and independent cohorts.

Disclosures

F. F. Esposito received honoraria from TEVA and Merck. L. Moiola received honoraria for speaking at meetings or for attending to advisory board from Sanofi-Genzyme, Biogen-Idec, Novartis and TEVA. B. Colombo received travel grant from Biogen-Idec, Merck, Bayer, Genzyme. V. Martinelli has received honoraria for consulting and speaking activities from Biogen-Idec, Merck, Bayer, TEVA, Novartis and Genzyme. M.A. Rocca received speakers honoraria from Biogen Idec, Novartis, Genzyme, Sanofi-Aventis and Excemed and receives research support from the Italian Ministry of Health and Fondazione Italiana Sclerosi Multipla. M. Filippi is Editor-in-Chief of the Journal of Neurology; serves on scientific advisory board for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Biogen Idec, Excemed, Novartis, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Teva Pharmaceutical Industries, Novartis, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, Alzheimer's Drug Discovery Foundation (ADDF), the Jacques and Gloria Gossweiler Foundation (Switzerland), and ARiSLA (Fondazione Italiana di Ricerca per la SLA). G. Comi has received compensation for consulting services with the following companies: Novartis, Teva, Sanofi, Genzyme, Merck, Biogen, Excemed, Roche, Almirall, Chugai, Receptos, Forward Pharma and compensation for speaking activities from Novartis, Teva, Sanofi, Genzyme, Merk, Biogen, Excemed, Roche. F. Martinelli Boneschi has received compensation for activities with Teva Neuroscienze as speaker and/or advisor. F. Clarelli, L. Ferre', E. Mascia, G. Sferruzza, M. Radaelli, F. Sangalli, M. Rodegher have nothing to disclose.

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