GWAS associated Variants, Non-genetic Factors, and Transient Transcriptome in Multiple Sclerosis Etiopathogenesis: a Colocalization Analysis

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Abstract

A clinically actionable understanding of multiple sclerosis (MS) etiology goes through GWAS interpretation, prompting research on new gene regulatory models. We previously suggested a stochastic etiologic model where small-scale random perturbations could reach a threshold for MS development. The recently described mapping of the transient transcriptome (TT), including intergenic and intronic RNAs, seems appropriate to verify this model through a rigorous colocalization analysis. We show that genomic regions coding for the TT were significantly enriched for MS-associated GWAS variants and DNA binding sites for molecular transducers mediating putative, non-genetic, etiopathogenetic factors for MS (e.g., vitamin D deficiency, Epstein Barr virus latent infection, B cell dysfunction). These results suggest a model whereby TT-coding regions are hotspots of convergence between genetic ad non-genetic factors of risk/protection for MS, and plausibly for other complex disorders. Our colocalization analysis also provides a freely available data resource (www.mscoloc.com) for future research on MS transcriptional regulation.

Introduction

A large body of literature agrees that regulatory genomic intervals, especially those encompassing enhancers, are enriched with disease-associated DNA elements. Most of this evidence comes from genome wide association studies (GWAS) based on single polymorphism nucleotides (SNPs) representing common variants (Ernst et al., 2011; Farh et al., 2015; Gusev et al., 2014; Maurano et al., 2012; Vahedi et al., 2015), even though a recent study showed that low-frequency and rare coding variants may somewhat contribute to multifactorial diseases (chris.cotsapas@yale.edu & Consortium, 2020). Several characteristics of regulatory disease-associated genetic variants complicate GWAS interpretation, prompting research on new gene regulatory models: (*i*) SNPs are chosen as haplotypes to spare the genotyping work needed for the large number of samples used in GWAS, therefore fine mapping and epigenetic studies are required to integrate GWAS data (Calderon et al., 2019; Mumbach et al., 2017; Ohkura et al., 2020; van Arensbergen et al., 2019); (*ii*) a fraction of supposedly causal disease-associated variants directly alters recognizable transcription factor binding motifs as it might be expected, according to their regulatory function (Farh et al., 2015); (*iii*) the identified GWAS signals are likely to exert highly contextual (i.e., time- and position-dependent) regulatory effects, that may change according to the tissue and to the time when they receive an input from inside or outside the cell. In summary, current gene regulatory models help only in part to fully detail which disease-associated SNP signals are causal, and by which exact mechanisms they are causal. Recent studies on the biological spectrum of human DNase I hypersensitive sites (DHSs), that are disease-associated markers of regulatory DNA, may help to better rework GWAS data and particularly to contextualize the genomic variants according to tissue/cell states and to gene body colocalization of DHSs (Meuleman et al., 2020). In this context, the latest version of the Genotype-Tissue Expression project may provide further insights into the tissue specificity of genetic effects, supporting the link between regulatory mechanisms and traits or complex diseases (Consortium, 2020).

Another layer of complexity comes from our recent studies suggesting an MS etiologic model where stochastic phenomena (i.e., random events not necessarily resulting in disease in all individuals) may contribute to the disease onset and progression. This model, embedded between physics of stochastic systems and cell biology, suggests how small-scale random perturbations would impact on large-scale phenomena, such as exceeding the threshold for MS development that is set by genetic and non-genetic susceptibility factors (Bordi et al., 2014; Bordi et al., 2013). Such model is consistent with our previous results on the heterogeneity of MS etiology components in twin pairs studies (Fagnani et al., 2015; Ristori et al., 2006) and with prior bioinformatics analyses that determined a significant enrichment of binding motifs for Epstein-Barr virus (EBV) nuclear antigen 2 (EBNA2) and vitamin D receptor (VDR) in genomic regions

77 containing MS-associated GWAS variants (Ricigliano et al., 2015). We also demonstrated that
78 genomic variants of *EBNA2* resulted to be MS-associated (Mechelli et al., 2015), and other
79 groups expanded our findings showing that enrichment of EBNA2-binding regions on GWAS DNA
80 intervals is involved in the pathogenesis of autoimmune disorders, including MS (Harley et al.,
81 2018).
82 A recent sequencing innovation (namely, TT-seq) allowed to map the transient transcriptome that
83 has a typical half-life within minutes, compared to stable RNA elements, such as protein-coding
84 mRNAs, long-noncoding RNAs, and micro-RNAs, that persists at least a few hours (Michel et al.,
85 2017; Schwalb et al., 2016; Villamil et al., 2019). The transient transcriptome (TT) includes mostly
86 enhancer RNAs (eRNA), short intergenic non-coding RNAs (sincRNA) and antisense RNAs
87 (asRNA). Overall, these transient RNAs (trRNA) are relatively short in length, generally lack a
88 secondary structure, and would not present those chemical modifications that characterize
89 unidirectional and polyadenylated stable RNAs (Natoli & Andrau, 2012; Schwalb et al., 2016).
90 Other recent works based on time-resolved analysis, agree on the eRNAs very rapid functional
91 dynamics model while interacting with the transcriptional co-activator acetyltransferase CBP/p300
92 complex (Bose et al., 2017; Weinert et al., 2018). This confirms the highly contextual role of
93 eRNAs through the control of transcription burst frequencies, which are known to influence cell-
94 type-specific gene expression profiles (Larsson et al., 2019). Along these lines, a recent study
95 showed that T cells selectively filter oscillatory signals within the minute timescale (O'Donoghue
96 et al., 2021), further supporting the aforementioned model.
97 In summary, on the basis of our previous research (i.e., the heterogeneity in the MS etiology
98 components; the stochasticity in the interaction between genetic and non-genetic factors
99 contributing to disease development; the enrichment of binding sites for environmental factors in
100 MS-associated DNA intervals) and leveraging the recent sequencing innovations in the mapping
101 of the transient transcriptome (i.e., the erratic time dynamics and the highly contextual
102 expression) (Michel et al., 2017; Schwalb et al., 2016), we hypothesize that MS-associated
103 GWAS signals prevalently fall within regulatory regions of DNA coding for trRNAs. In theory, the
104 genomic intervals coding for this transient transcriptome may be the hotspots where
105 temporospatial occurrences (stochastic in nature, as said) may coalesce and so contribute to
106 physiological (developmental and/or adaptive) outcomes, or possibly give rise to disease onset or
107 progression. This study is aimed at verifying this working hypothesis through a colocalization
108 analysis and its further dissection in the context of MS.
109

Results

MS-associated GWAS signals colocalize with regulatory regions of DNA plausibly coding for trRNAs

We set up our region-of-interest (ROI) inside GWAS catalogue (Buniello et al., 2019) by considering all MS GWAS that were published, extracting all SNP positions, and creating a single set of genomic coordinates that therefore encompass all GWAS-derived or GWAS-verified signal for MS. We then refined the SNP list by pruning out about 1.5% of the SNPs as they did not contain intelligible genomic annotations or were duplicates. The final ROI list is reported in Supplementary Table S1 and consists of 603 unique single-nucleotide regions; to provide a "threshold" against which the match ROI<>Database would be benchmarked, we used 107,423 regions as Universe, that corresponded to the signals coming from the entire GWAS Catalog.

Next, we matched through colocalization analyses our ROI with lists of regions resulting from the work by Michel et al., which mapped the transient and stable transcriptome captured by TT-seq after T cell stimulation (Schwalb et al., 2016). We found a significant enrichment of MS-associated genetic variants in the transient transcriptome (p-value=2.80×10^{-9}; Table 1). Of note, when we split the transcriptome list in two subsets for long (≥ 60 minutes) and short (< 60 minutes) half-life, we found that only the short half-life subset significantly colocalized with the ROI (p-value 2.06×10^{-8} vs 0.09). This finding was indicative of the relationship between MS-associated GWAS signals and the regulatory regions of DNA coding for trRNA.

When we further dissected the mapping of the ROI colocalization signals, we found a significant excess of intergenic and intron regions (as anticipated), as well as their prevalent distribution away from the transcription start site (TSS; Figure 1A). Notably, when we extended this analysis to GWAS data coming from other multifactorial diseases or traits, dividing immune-mediated and other complex conditions, we found highly comparable profiles (Figure 1B, 1C, Supplementary Table S2), suggesting that the colocalization between MS-associated DNA intervals and intergenic or intronic sequences, plausibly referring to trRNA coding regions, is shared by the genetic architecture of most multifactorial disorders.

To consolidate this result and gain a deeper biological insight, we extended the colocalization analysis matching the ROI with a vast set of databases of regulatory DNA regions, including enhancers and super-enhancers, derived from experiments on diverse tissue types (a total of 4,697,782 DNA regions, plausibly coding for trRNA, were extracted from a wide variety of raw data sources; referenced in Supplementary Table S3). To improve interpretability of the results through ranking, we implemented a harmonic score (HS), based on the Odd Ratio, the -log (p-value), and the support of each match. Statistically significant results came from sets included in SEA, seDB, dbSuper and other single lists of enhancers and non-coding RNAs (Figure 2).

146 Interestingly, we found a strong enrichment of MS-associated genetic variants in cell lines of
147 hematopoietic lineage, including CD19+ and CD20+ B lymphocytes, CD4+ T helper cells, and
148 CD14+ monocytes. Moreover, among the top-scoring hits, we found microglial-specific
149 enhancers, which is in line with recent reports on brain cell type-specific enhancer-promoter
150 interactome activities and the latest GWAS on MS genomic mapping (Consortium, 2019; Nott et
151 al., 2019). On the other hand, non-relevant tissues serving as controls (such as kidney, muscle,
152 glands, etc.) scored low in the ranking, crowding the bottom-left corner of Figure 2.
153
154 **Genetic and non-genetic factors for MS etiology converge in genomic regions plausibly**
155 **coding for the transient transcriptome**
156 Independent studies support the fact that MS GWAS intervals are enriched with DNA binding
157 regions (DBRs) for protein 'transducers' mediating non-genetic factors of putative etiologic
158 relevance in MS, such as vitamin D deficiency or EBV latent infection (Harley et al., 2018;
159 Ricigliano et al., 2015). Therefore, we further inquired whether DNA regions plausibly coding for
160 trRNA would share these features (i.e., they colocalize with such DRBs). We set up 4 new ROIs
161 corresponding to the DBRs for VDR, activation-induced cytidine deaminase (AID), EBNA2, and
162 Epstein Barr nuclear antigen 3 (EBNA3C), chosen among viral or host's nuclear factors
163 potentially associated to MS etiopathogenesis (Bäcker-Koduah et al., 2020; Marcucci & Obeidat,
164 2020; Sun et al., 2013). The DBRs for each nuclear factor were derived from recent literature
165 (Supplementary Table S4) and matched with the GWAS-derived MS signals to confirm and
166 expand previous results. We found statistically significant results for VDR, EBNA2, and AID for all
167 the SNP position extensions (±50, 100, 200 kb up- and down-stream), while for EBNA3C
168 significant results came out at extension of ±100 and 200 kilobases. This finding suggests that
169 several DBRs can impact on the MS-associated DNA intervals through colocalization (Table 2).
170 Building once again on the work by Michel et al. (Michel et al., 2017), we inquired whether there
171 was a colocalization between genomic regions containing MS-associated variants, DBRs for
172 VDR, EBNA2, EBNA3C, AID, and DNA intervals plausibly coding for trRNA. To this end, we
173 considered the transient transcriptome that proved to be enriched with MS-associated variants
174 (Table 1), and we then matched the corresponding coding regions with the DBRs for the four
175 molecular transducers. For this analysis DBRs for EBNA2 (6,880 regions), EBNA3C (3,835
176 regions), AID (4,823 regions), and VDR (23,409 regions), represented the ROI, while the
177 ENCODE database of Transcription Factors Binding Sites served as Universe (13,202,334
178 regions; Figure 3a). We report the results of this analysis in Table 3, which shows the significant
179 colocalization of DNA regions plausibly coding for trRNA with both MS-relevant GWAS signals,
180 and DBR of 3 out of 4 factors active at nuclear level, and potentially associated with MS. The

181 DBR for EBNA3C did not reach statistical significance, though it showed higher values of support
182 for short half-life transcripts.
183 To review and confirm previous colocalizations, we considered the genomic regions resulting
184 from the above reported match between the MS-associated GWAS intervals and the databases
185 of regulatory DNA regions, containing enhancers and super-enhancers, plausibly enriched in
186 trRNA-coding sequences (see results in Figure 2 and the online data resource). We therefore
187 matched these DNA regions with the DBR for VDR, EBNA2, EBNA3C and AID, finding significant
188 enrichments that allow to contextualize and prioritize genomic positions, cell/tissue identity or cell
189 status associated to MS. Considering the harmonic score obtained from these colocalization
190 analyses, the top hits in EBNA2, EBNA3C, and AID involved lymphoid (CD19+ B cell lines and
191 lymphomas; T regulatory cells; tonsils) and monocyte-macrophage lineages (peripheral
192 macrophages; dendritic cells) from experiments included in the ENCODE, dbsuper,
193 roadmapEpigenomics databases (Figure3B-E, see also Supplementary Table S5). Even though
194 immune cells prevailed also in VDR top hits, a less stringent polarization was seen, somehow
195 reflecting the wide-spreading actions of this transducer in human biology. However, with a more
196 stringent cutoff of Harmonic Score>40 that selects the most significant hits (Figure Supplement
197 1), a core subset of MS-relevant cell lineages, shared across all four examined transducers,
198 became evident (Supplementary Table S6 and the online data resource).
199
200 **A data resource for future research on transcriptional regulation in MS**
201 A public web interface for browsing the results of our colocalization analysis is freely available at
202 www.mscoloc.com. This is a comprehensive genomic atlas disentangling specific aspects of MS
203 gene-environment interactions to support further research on transcriptional regulation in MS. It
204 includes the whole list of results derived from ROI, DBRs and database matches (Figure 4a)
205 across all performed experiments that yielded significant results. The user can navigate across
206 the results and perform tailored queries searching and filtering for a variety of parameters,
207 including MS-associated variant, DBR, experimental cell type, other match details (see Figure 4b
208 for all available search and filter modalities). Moreover, personalized HS, p-value, support and
209 Odd Ratio threshold can easily be set to screen results, that are readily displayed in tabular
210 format. To provide an example, we select *"AID, EBNA2, EBNA3C, VDR"* in the '*Matched DBR*
211 *region (s)*' panel and obtain the list of MS-associated SNPs targeted by all four transducers
212 (Figure 4b-c). Through this approach we searched for MS-associated regions shared by the
213 DBRs analyzed, and we were able to prioritize 275 genomic regions (almost half of the MS-
214 associated GWAS SNPs) capable of binding at least 2 molecular transducers. These regions are
215 'hotspots' of interactions between genetic and nongenetic modifier of MS risk/protection: all four
216 proteins (VDR, AID, EBNA2, EBNA3C) proved to target 24 regions, 3 of them 115 regions, and 2

217 of them 136 regions. A detailed legend and more example queries may be found on the online
218 data resource website.

219 **Discussion**

220 Our study supports the hypothesis that investigations on the transient transcriptome may
221 contribute to clarify how the GWAS signals affect the etiopathogenesis of MS and possibly of
222 other complex disorders. Specifically, we show that genomic regions coding for the transient
223 transcriptome recently described in T cells (Michel et al., 2017), are significantly enriched for both
224 MS-associated GWAS variants, as well as for DNA binding sites for protein 'transducers' of non-
225 genetic signals, chosen among those plausibly associated to MS. The colocalization of GWAS
226 intervals and some DNA-binding factors involved in MS etiology has already been reported
227 (Harley et al., 2018; Mechelli et al., 2015; Ricigliano et al., 2015), and here we reinforce this
228 premise further suggesting a model in which trRNA-coding regions are hotspots of convergence
229 between genetic ad non-genetic factors of risk/protection for MS. Our analysis showed that these
230 hotspots are shared by two or more of the chosen transducers, indicating possible additive
231 pathogenic effects or a multi-hits model to reach the threshold for MS development (see Figure 4
232 and Supplementary Table S6).

233 In homeostatic conditions, it can be hypothesized that DNA sequences coding for trRNA are
234 composed of regulatory regions where genetic variability and non-genetic signals interact to finely
235 regulate the gene expression according to cell identity, developmental or adaptive states, and
236 time-dependent stimuli. As a matter of fact, the sequence variability of these regions and the strict
237 time-dependence of their transcription could be instrumental to adaptive features; however, these
238 same features make these regions susceptible to become dysfunctional or to be the targets of
239 pathogenic interaction. In some instances, these detrimental interactions come from outside the
240 cell, such as in the case of EBV interference with host transcription (Mechelli R., 2021, Accepted;
241 Park et al., 2020), and the pathogenic consequences of vitamin D deficiency; in other cases, the
242 dysfunction develops within the cell, such as the tumorigenic activity of AID in B cells (Meng et
243 al., 2014; Qian et al., 2014).

244 The mapping of transient transcripts by TT-seq approach fits very well with our results obtained
245 from GWAS data for MS and other multifactorial conditions, showing a significant excess of
246 intergenic and intronic regions (coding for eRNA, sincRNA, and asRNA), and having a distribution
247 in DNA intervals mostly far off from transcription start sites (TSS; see Figure 1). This is in
248 agreement with recent evidence of regulatory DNA region markers which contain genetic variants
249 for complex disease or traits; indeed, a systematic framework of common coordinates for these
250 markers showed that about half of them lie within introns and most are located away from the
251 TSS (Meuleman et al., 2020).

252 To further support the relationship between trRNA and transcription of regulatory DNA regions,
253 we matched a large dataset of enhancers and super-enhancers with MS-GWAS signals and DBR
254 for VDR, EBNA2, EBNA3C and AID. The significant enrichment in cell lines and cell status

255 coming from the hematopoietic lineages and the CNS-specific cell subsets corroborates data
256 coming from recent reports showing the relevance of contextualizing and prioritizing the role of
257 MS-associated GWAS signals (Consortium, 2019; Factor et al., 2020; Nott et al., 2019; Orrù et
258 al., 2020). Our analysis supports the pivotal regulatory role of enhancer transcription (i.e., a main
259 component of transient transcriptome) that was recently reported as not dispensable for gene
260 expression at the immunoglobulin locus and for antibody class switch recombination (Fitz et al.,
261 2020), though more research is needed to unravel such topic at a finer grain.
262 Reports on the dynamics of time-course data are a recent area of focus within the analysis of
263 gene expression, specifically in immune cells. Although current studies use methods that
264 investigate time points related to the stable transcriptome (RNA-seq performed with time spans of
265 hours), they clearly show that gene expression dynamics may influence allele specificity,
266 regulatory programs that seem to depend on autoimmune disease-associated loci, and different
267 transcriptional profiles based on cell status after stimulation (Gutierrez-Arcelus et al., 2020). A
268 recent work showed that an *IL2ra* enhancer, which harbors autoimmunity risk variants and was
269 one of the first MS-associated loci from GWAS, has no impact on the gene level expression, but
270 rather affects gene activation by delaying transcription in response to extracellular stimuli
271 (Simeonov et al., 2017). The importance of the timing in the gene expression control emerges
272 also from several studies implicating enhancers and super-enhancers in the process of phase
273 separation and formation of condensates. In this context, the transcriptional apparatus steps-up
274 to drive robust genic responses (. The overall process seems to be highly dynamic, with time
275 spans of seconds or minutes, and hence compatible with the temporal features of the transient
276 transcriptome, which could somehow act upstream for the formation of these phase-separated
277 condensates.
278 We suggest that studies on transient transcriptomes may integrate previous RNA-seq data in
279 accounting for the interplay between genetic variability and non-genetic etiologic factors leading
280 to MS development. Components of a more-complex-than-anticipated regulation of gene
281 expression could include transcriptional noise, transitory time-courses, erratic dynamics, and
282 highly flexibility of some DNA regions, possibly oscillating between bistable states of enhancer
283 and silencer (Halfon, 2020). The availability of tools to map trRNA could further contribute to the
284 development of studies on immune cells isolated from patients and matched controls, aimed at
285 dissecting key aspects of the complex transcriptional response in MS. Our analysis provides a
286 platform for future studies on transient transcriptome, which we support by making our data
287 resource available at www.mscoloc.com. Finally, new gene regulatory models may emerge from
288 this approach in order to better evaluate the meaning of GWAS in complex traits and the impact
289 of the enhancer transcription (Fitz et al., 2020), which was recently reported as an ancient and
290 conserved, yet flexible, genomic regulatory syntax (Wong et al., 2020).

Materials and Methods

Data pipeline

Analyses were performed in Python and R. A data freeze was applied on 3/1/2020. All GWAS data was gathered from the GWAS Catalog through its REST API (Buniello et al., 2019); about 1.5% of this data was filtered out as part of a QC process aimed at homogenizing legacy and more recent data. The MS GWAS regions were extracted from the overall GWAS Catalog data filtering by trait EFO_0003885. All Transcription Factor Binding Site regions (TFBS) were obtained from the ENCODE portal (Sloan et al., 2016). All data was organized in various databases and data pipelines as detailed below. A modular and parallel data pipeline was created to: (i) readily generate and evaluate all experiments in the paper, (ii) manage and organize all data coming from various region collections (42,075 ROI regions; 4,697,782 regions plausibly coding for trRNAs; 13,309,757 Universe regions), multiple ROIs (MS GWAS, EBNA2, EBNA3C, VDR, AID, etc.), databases of vast background regions as they were populated with the data obtained from GWAS Catalog, ENCODE, and other raw data sources, (iii) provide overlaps and intersection among various data elements, annotate them with the original MS GWAS loci that generated the signal, and (iv) generate the overarching data resource available at www.mscoloc.com.

Statistical analysis

For SNP overlaps and region colocalization, we used LOLA (Sheffield & Bock, 2016) and Fisher's exact test with False Discovery Rate (Benjamini-Hochberg) to control for multiple testing. Resulting -log (p-value), support, and Odds Ratio (OR) were combined into a single score inspired by the harmonic mean (Wilson, 2019) and multi-objective optimization (Umeton R., 2011) with the formula below, where the spacing parameter k_p was set to 10.0 and we consider all three contributors equally, setting therefore weights w_i to 1.0. Statistical significance was taken at $p<0.05$.

$$Harmonic_{Score} = k_p * \frac{\sum_i w_i}{\frac{w_1}{-logP} + \frac{w_2}{Supp} + \frac{w_3}{OR}}$$

Data availability

All generated data and results are made available at the website www.mscoloc.com.

326 **Author Contributions**

327 RU, GB, RB, RM, MS, and GR conceived and planned the analysis. RR, VR, EM, CR, SR, and
328 MCB guided data engineering and database generation from raw data. RU, RPU and GB
329 developed the data resource. RU, GB, RPU, GR and MS wrote the manuscript. RU, GB, RB, and
330 RM created all table and figures. RB, RM, MS and GR supervised the project. All the authors,
331 including SR, MCB, RR, VR, EM and CR, contributed to fortnight discussion for data
332 interpretation and new analysis planning.

333 All the authors revised and approved the manuscript.

334 **Competing Interest Statement**

335 The authors have no conflict of interest related to this analysis.

336

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565

Tables

Table 1. Enrichment of MS-associated genetic variants in lists of T-cell transient transcripts extracted from Michel et al. (21). The whole transcriptome list was split in two sub-lists depending on the transcripts' half-life: short (<60') and long (≥60'), respectively. Results are considered significant at $p<0.05$ and are highlighted in bold.

List	-log (p-value)	p-value	Odds Ratio	Support	List Size
Whole transient transcriptome	8.55	**2.80×10^{-9}**	1.65	241	22126
Short half-life transcripts	7.68	**2.06×10^{-8}**	1.63	209	20143
Long half-life transcripts	1.05	0.09	1.29	35	1993

Table 2. Enrichment of MS-GWAS regions (at ±50,100,200 kb range of extension) in lists (number in brackets in the right-most column) of DNA binding sites of human and viral molecular transducers; significant results (p<0.05, corresponding to a -log (p)>1.301) in bold.

	± 50 KB				± 100 KB				± 200 KB			
	-log (pValue)	Odds Ratio	Support	Harmonic Score	-log (pValue)	Odds Ratio	Support	Harmonic Score	-log (pValue)	Odds Ratio	Support	Harmonic Score
EBNA2 *(6880)*	**10.658**	**1.790**	**158**	**45.544**	**8.616**	**1.509**	**239**	**38.327**	**15.444**	**1.542**	**421**	**41.913**
EBNA3C *(3335)*	**0.614**	**1.108**	**55**	**11.765**	**1.647**	**1.227**	**109**	**20.956**	**3.448**	**1.294**	**199**	**28.098**
AID *(4823)*	**4.963**	**1.596**	**99**	**35.793**	**3.890**	**1.374**	**153**	**30.259**	**13.924**	**1.619**	**309**	**43.308**
VDR *(23409)*	**19.348**	**1.575**	**474**	**43.564**	**19.181**	**1.422**	**767**	**39.635**	**32.090**	**1.424**	**1329**	**40.872**

Table 3. Colocalization of human and viral transducer DBRs and MS-GWAS positions (at ±50,100,200 kb range of extension) in DNA regions coding for transient transcripts; significant results (p<0.05, corresponding to a -log (p)>1.301) in bold. The transcript half-life is considered short if <60' and long if ≥60', respectively.

		± 50 KB				± 100 KB				± 200 KB			
		-log (pValue)	Odds Ratio	Support	Harmonic Score	-log (pValue)	Odds Ratio	Support	Harmonic Score	-log (pValue)	Odds Ratio	Support	Harmonic Score
EBNA2	*Long half-life*	0.023	0.478	3	0.644	0.062	0.717	8	1.708	**1.879**	**1.531**	**33**	**24.679**
	Short half-life	**6.163**	**1.920**	**69**	**43.011**	**3.241**	**1.433**	**95**	**29.496**	**8.945**	**1.610**	**189**	**40.642**
EBNA3C	*Long half-life*	0.064	0.572	2	1.669	0.006	0.321	2	0.185	0.182	0.914	11	4.500
	Short half-life	0.070	0.794	16	1.923	0.023	0.752	28	0.661	0.066	0.875	58	1.841
AID	*Long half-life*	0.089	0.682	3	2.303	0.283	1.024	8	6.477	0.051	0.726	11	1.432
	Short half-life	**1.769**	**1.465**	**37**	**23.531**	**1.346**	**1.267**	**59**	**19.367**	**3.954**	**1.442**	**119**	**31.416**
VDR	*Long half-life*	**1.737**	**1.502**	**32**	**23.571**	0.845	1.187	45	14.646	**2.315**	**1.322**	**97**	**25.031**
	Short half-life	**2.221**	**1.239**	**152**	**23.734**	**2.336**	**1.181**	**267**	**23.460**	**11.478**	**1.367**	**548**	**36.561**

Figures and legends

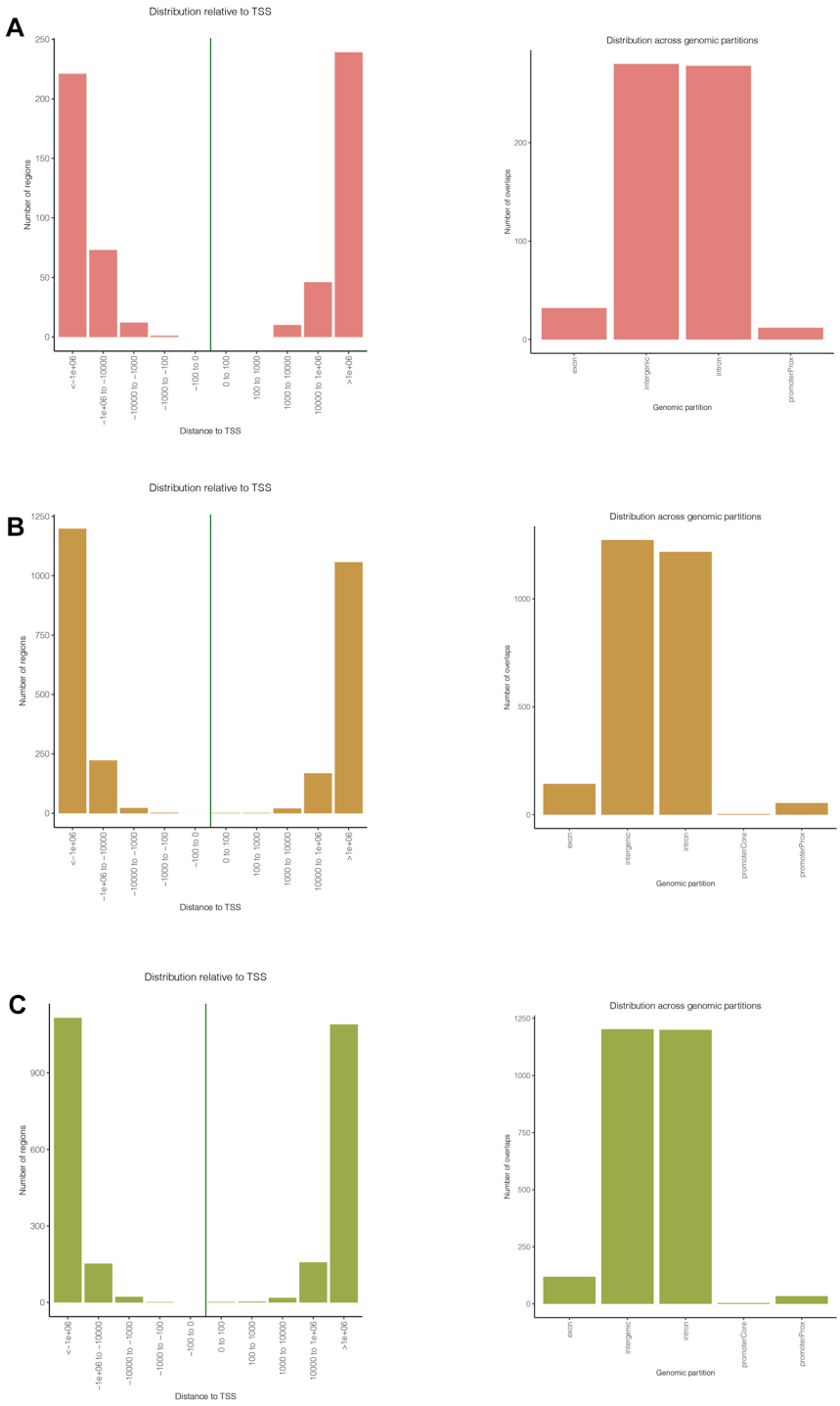

Figure 1. GWAS-associated SNP distribution across genomic partitions and their distance relative to the transcription starting site (TSS). Panel A, Multiple Sclerosis; B, Immune-mediated

conditions: Multiple Sclerosis, Rheumatoid Arthritis, Systemic Lupus Erythematosus, Crohn's Disease, Ulcerative Colitis, Inflammatory Bowel Disease, Celiac Disease, Asthma, Type I Diabetes Mellitus; C, Non-immunological complex conditions: Type II Diabetes Mellitus, Aging, Obesity, Hypertension, Coronary Artery Disease, Bipolar Disorder. Supplementary Table S2 include links to these traits in the GWAS catalog.

Figure 2. Enrichment of MS-associated SNPs in databases of regulatory elements, sorted by experiment/cell lines. X-axis shows the Odd Ratio, y-axis shows the -log (pValue); dot size is proportional to the support of each match, i.e., the number of hits resulting from the colocalization analysis. Color of each point is related to the Harmonic Score (HS), a comprehensive estimation of the relevance of hits, as derived by merging and balancing the OR, pValue and Support of each match. Thus, prioritized hits are represented by the darker dots that occupy the upper-right area of the chart. Labeled points have HS>40. Labels were arbitrarily designated according to the database of origin and the cell lineage where the enrichment occurred.

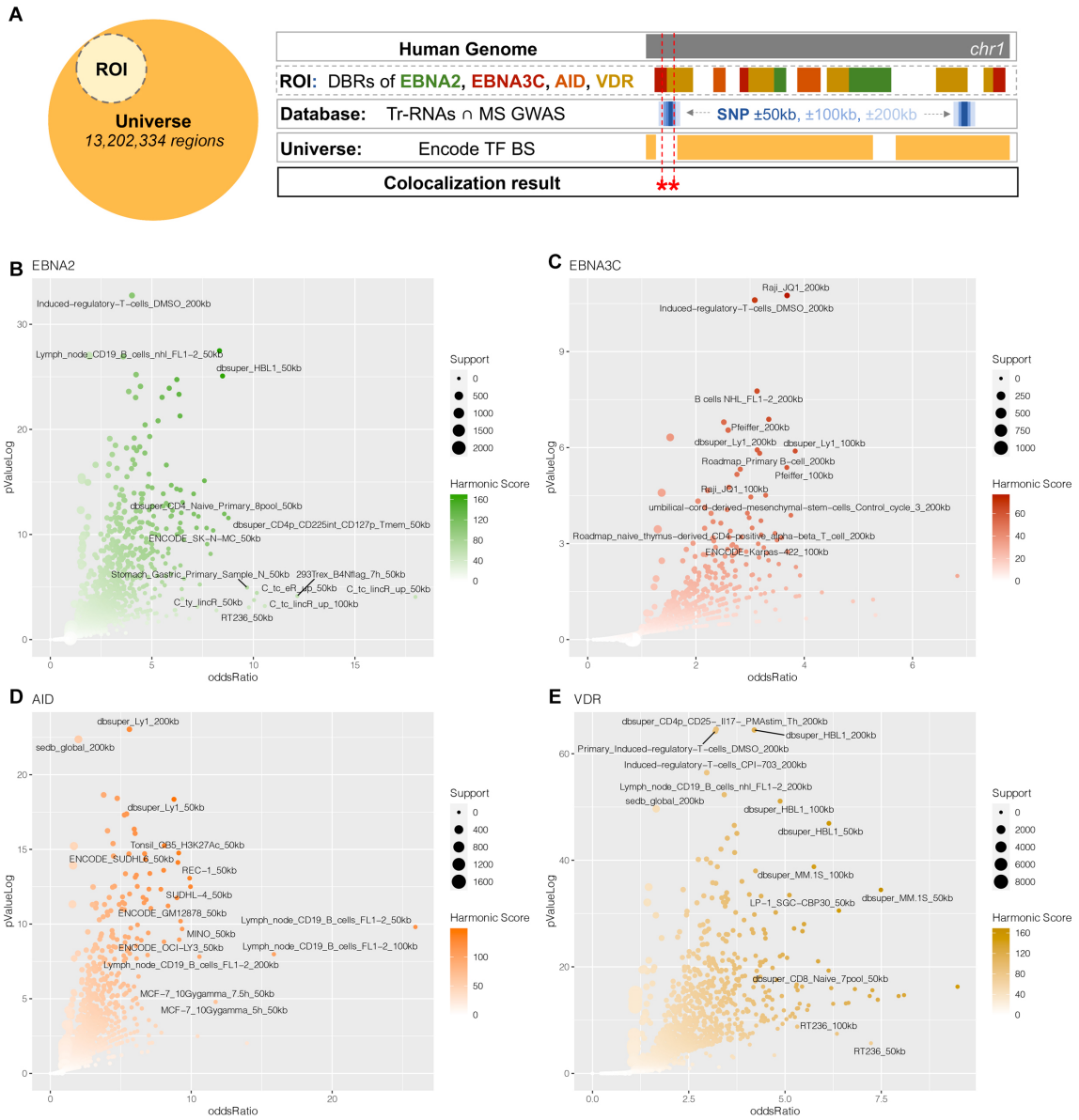

Figure 3. Colocalization analysis of DBRs for human and viral molecular transducers, MS-associated SNPs and DNA regulatory regions derived from databases. (*A),*Schematic representation of the colocalization analysis. (*ROI: Region of interest; DBR: DNA Binding Regions; ENCODE TFBS: Transcription Factor Binding Site).* The figure shows the tracks we considered for the colocalization analyses. In brief, the ROI included the DBRs of MS-related viral and human transducers and was matched with MS-associated SNPs extended by 50, 100, and 200 kilobases that colocalize with regions plausibly coding for trRNAs (Database). As a control (Universe), we took from ENCODE the entire list of transcription factors binding sites. Results were considered significant if a colocalization was found across ROI and a Databases element

without occurring in the Universe as a statistically significant match. *(B-E),* Colocalization results of EBNA2, EBNA3C, AID, VDR. The charts display results of all matches, i.e, with MS-associated SNPs and their extension at ±50, 100, 200 kb. X-axis shows the Odd Ratio, y-axis shows the log (pValue). Dot size is proportional to the support of each match, i.e., the number of hits resulting from each colocalization analysis. The color of each dot is related to the Harmonic Score (HS); labeled points have HS>40. Labels were arbitrarily designated according to the database of origin and the cell lineage where the enrichment occurred.

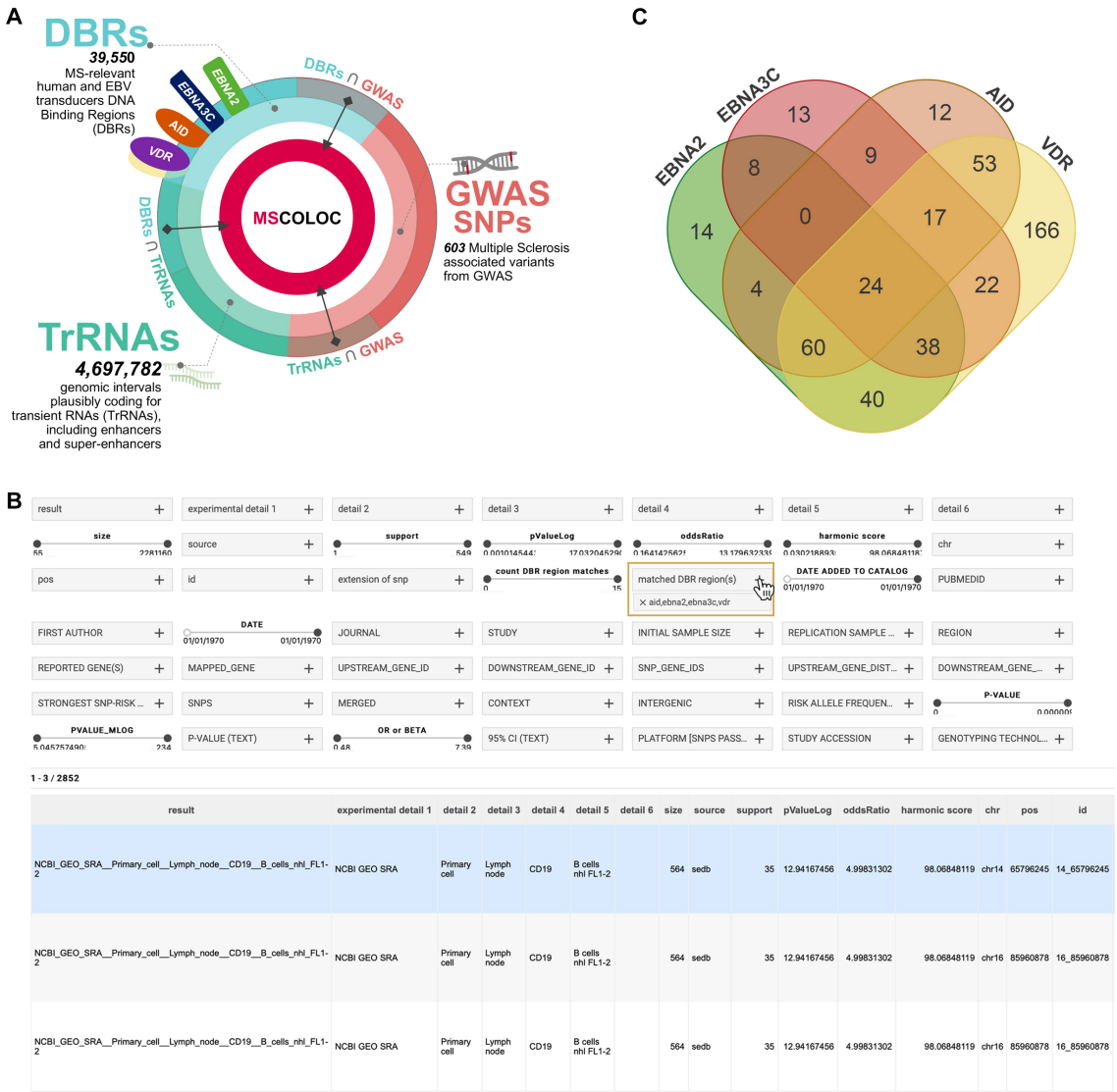

Figure 4. A comprehensive genomic atlas on gene-environment interactions regulating transcription in MS. (*A),* Searchable results at mscoloc.com derive from the matches of GWAS MS regions, DNA binding regions of selected genomic transducers, and more than 4 million of regions annotated as plausible transient RNAs. (*B),* The user interface includes text panels and range sliders allowing extremely personalized queries, that combine statistical significance level (including Odd Ratio, pValue, support, and Harmonic Score), study source, SNP or reported gene, and so on. Filtered results are shown as tables ranked by HS, that can be saved, printed or shared through URL. In the example, the cursor selects '*AID, EBNA2, EBNA3C, VDR'* in the '*matched DBR region (s)'* panel looking for MS-associated SNPs (from the ROI, Supplementary Table S1) and their extensions at ±50, 100, 200 kb that colocalized within DNA binding regions of the molecular transducers. The top hit represents the colocalization of the DBRs, a super-

enhancer region derived from experiments on CD19+B cells included in *sedb,* and the rs8007846 MS-associated SNP on chromosome 14. (*C),* The Venn diagram shows the number of non-redundant MS-associated SNPs derived from the query: for each transducer, SNPs are considered only once if present in more than one match. Intersections show the numbers of regions colocalizing with DBRs of multiple transducers. For instance: 8 regions colocalize with both EBNA2 and EBNA3C DBRs, but not with AID nor VDR DBRs; 24 regions colocalize with all four DBRs, and could be identified as regulatory “hotspots” in MS.

Supplementary material

Supplementary Tables

S1. MS-associated genomic positions from GWAS catalog after QC process filtering, used as Region of Interest (ROI) for the analysis.

S2. GWAS Catalog References for diseases considered in Figure 1.

S3. Sources of DNA regions plausibly coding for trRNAs with references.

S4. Sources of DNA Binding Regions (DBRs) of considered viral and human transducers with references.

S5. Top 10 results of Colocalization analysis.

S6. Cell types for which the colocalization analysis hits reported a harmonic score >40 in all transducers (EBNA2, EBNA3C, AID, VDR).

Figure supplement 1: Harmonic Score threshold defining the top colocalization hits.