A Novel Comprehensive Epigenomic Liquid Biopsy Assay Reveals Disease Biology in Multiple Sclerosis Patients from 1mL of Plasma

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INTRODUCTION

INTRODUCTION: Immunopathological processes in MS include both focal inflammatory infiltrates driven by peripheral immune cell infiltration into the CNS and brain resident innate immune system-driven diffuse inflammation across the CNS. The progression-related pathology in the CNS is variable and is not readily detectable using conventional MRI, driving a need for novel biomarkers.

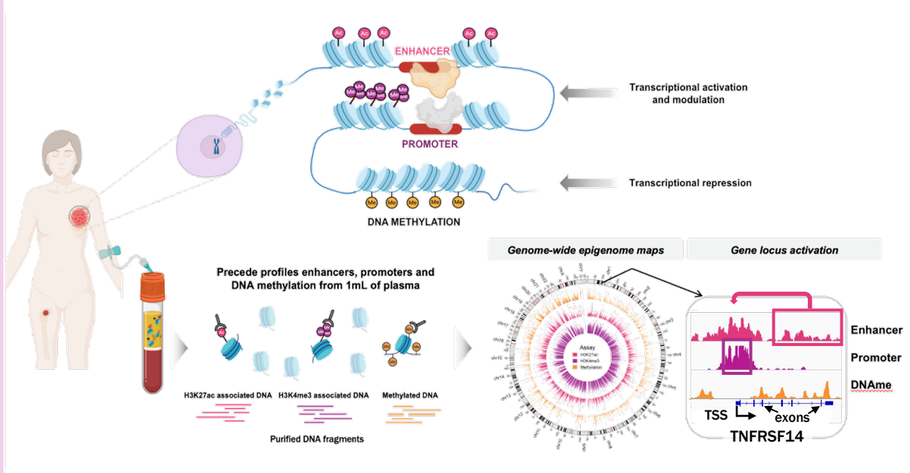
OBJECTIVES/AIMS: Blood-based cell-free DNA (cfDNA) from damaged or dying cells could serve as a powerful biomarker for disease biology and progression. Here we aim to identify MS-associated transcriptional activation and repression utilizing a novel multi-modal liquid biopsy approach, highlighting potential to interrogate ongoing central inflammatory processes in MS by epigenomic analysis of plasma samples.

METHODS

The study consisted of 26 progressive MS [PMS], 4 relapsing remitting MS [RRMS] patients (pts) with clinical evaluation and magnetic resonance imaging, plus 23 healthy controls (HCs). TSPO-PET imaging using [^{11}C]PK11195- ligand and blood sampling were conducted concurrently. One mL of plasma was utilized to capture genome-wide activating enhancers, promoter signals and repressive DNA hypermethylation. Dynamic epigenomic regions were identified between a discovery cohort of 18 MS pts and the HC group; pts from the remaining MS cohort to be added. Plasma from rheumatoid arthritis and systemic lupus erythematosus pts (both N=3) was similarly analyzed.

Figure 1. Comprehensive Epigenomic Platform Offers Dynamic Resolution into Target and Pathway Biology from 1mL of Plasma

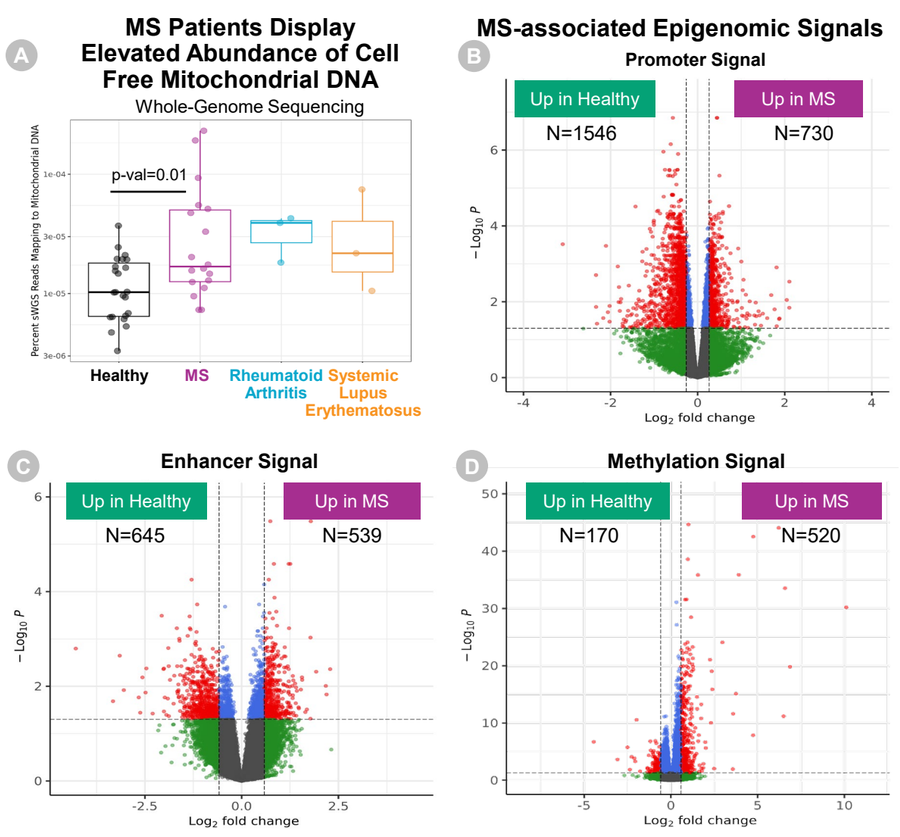
	Total Samples	Samples w/ 3 High Quality Analytes
Disease Type at Sample Collection	PPMS: 3 RRMS: 4 SPMS: 23	PPMS: 1 RRMS: 4 SPMS: 13
Age at Time of Sample Collection	35-65 years old *Median: 50*	35-65 years old *Median: 48*
Age at Disease Onset	17-58 years old *Median: 31*	17-37 years old *Median: 31*
Gender	Female: 19 Male: 11	Female: 9 Male: 9
EDSS	2-7.5 *Median: 4*	2-7.5 *Median: 4*
Number Overall Active Lesions	1-31 *Median: 18*	3-31 *Median: 14*



Cell free DNA derived from tumors exist in circulation as chromatin fragments that faithfully maintain tumor-associated epigenetic modifications on the histones and DNA. Antibodies against H3K27ac marking active enhancers, H3K4me3 marking active promoters and DNA methylation are used to enrich for associated DNA fragments from 1mL plasma and sequenced to define genome-wide epigenomic maps that capture the underlying transcriptional state of the tumor cells.[1]

RESULTS

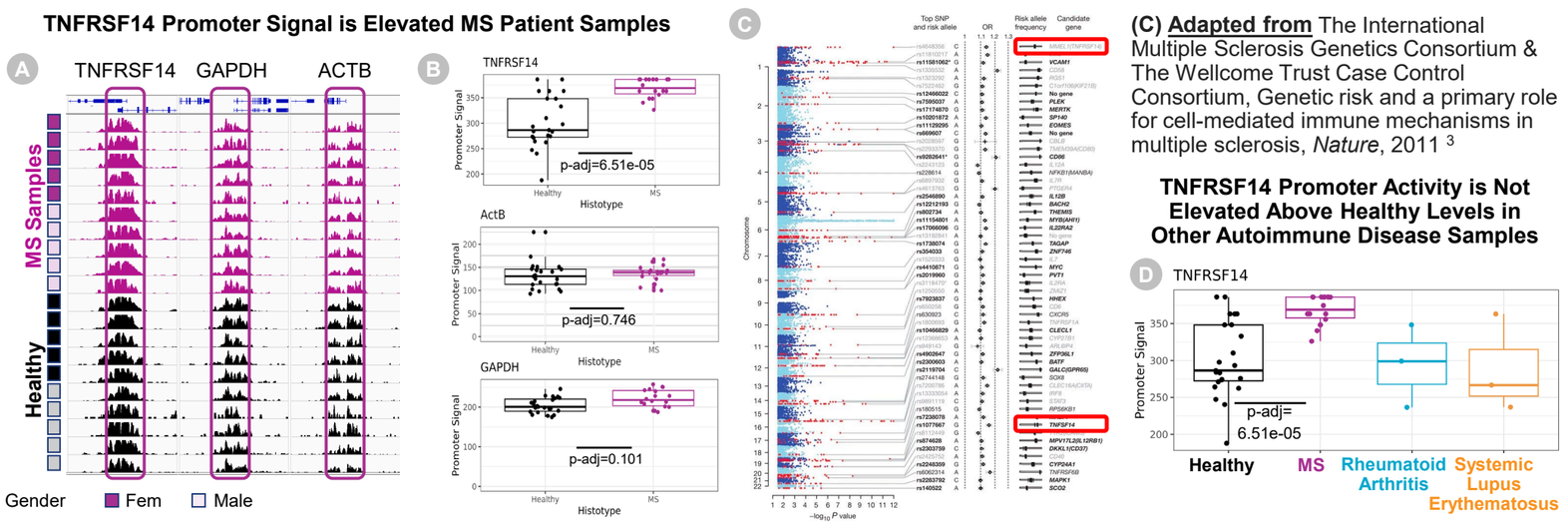
Figure 2. Identification of MS-associated Signals Across Genomic and Epigenomic Analytes



(A) MS patient plasma displays elevated abundance of cell free mitochondrial DNA, quantified by the percent of de-duplicated shallow whole genome reads mapping to the mitochondrial genome, as previously observed[4,5]. Plasma from additional autoimmune patients, suffering rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE) display a similar trend. **(B-D)** Promoter, enhancer, and DNA regions genome-wide were assessed for elevated/depleted signal in MS patients compared to healthy volunteers, revealing hundreds of regions with MS-associated alterations in signal strength.

RESULTS

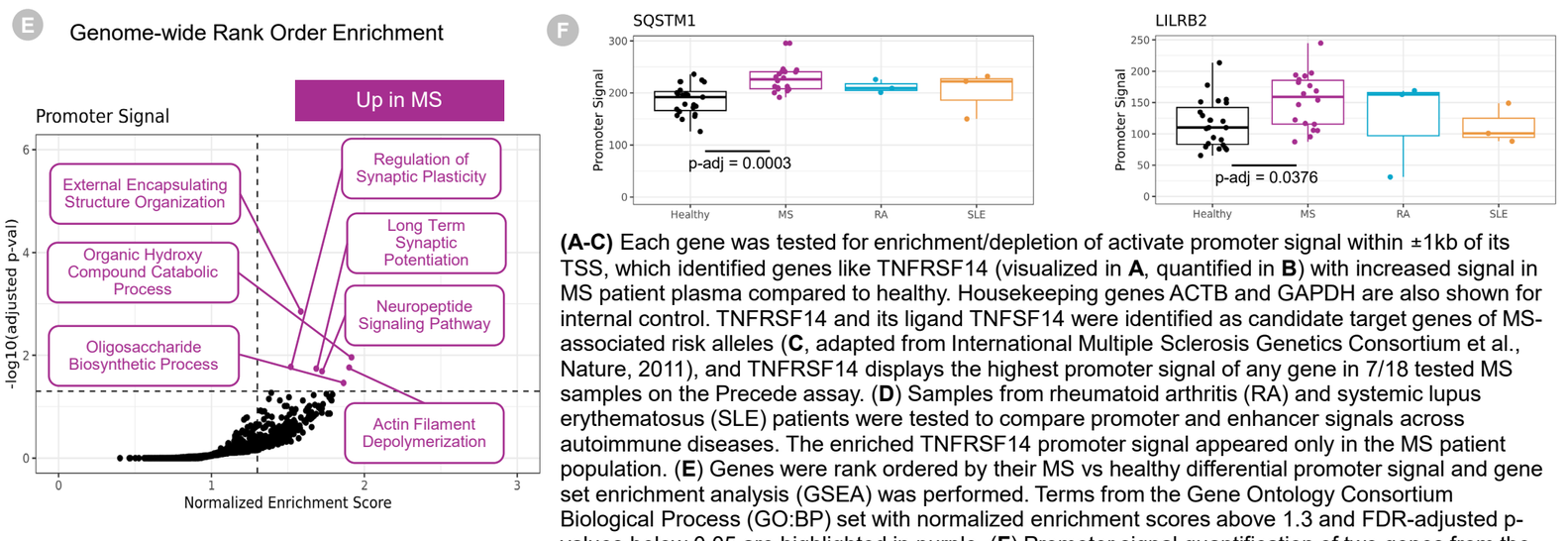
Figure 3. MS-associated Promoter Signal Increase at Synaptic Pathways and Risk Genes



(C) Adapted from The International Multiple Sclerosis Genetics Consortium & The Wellcome Trust Case Control Consortium, Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis, *Nature*, 2011 [3]

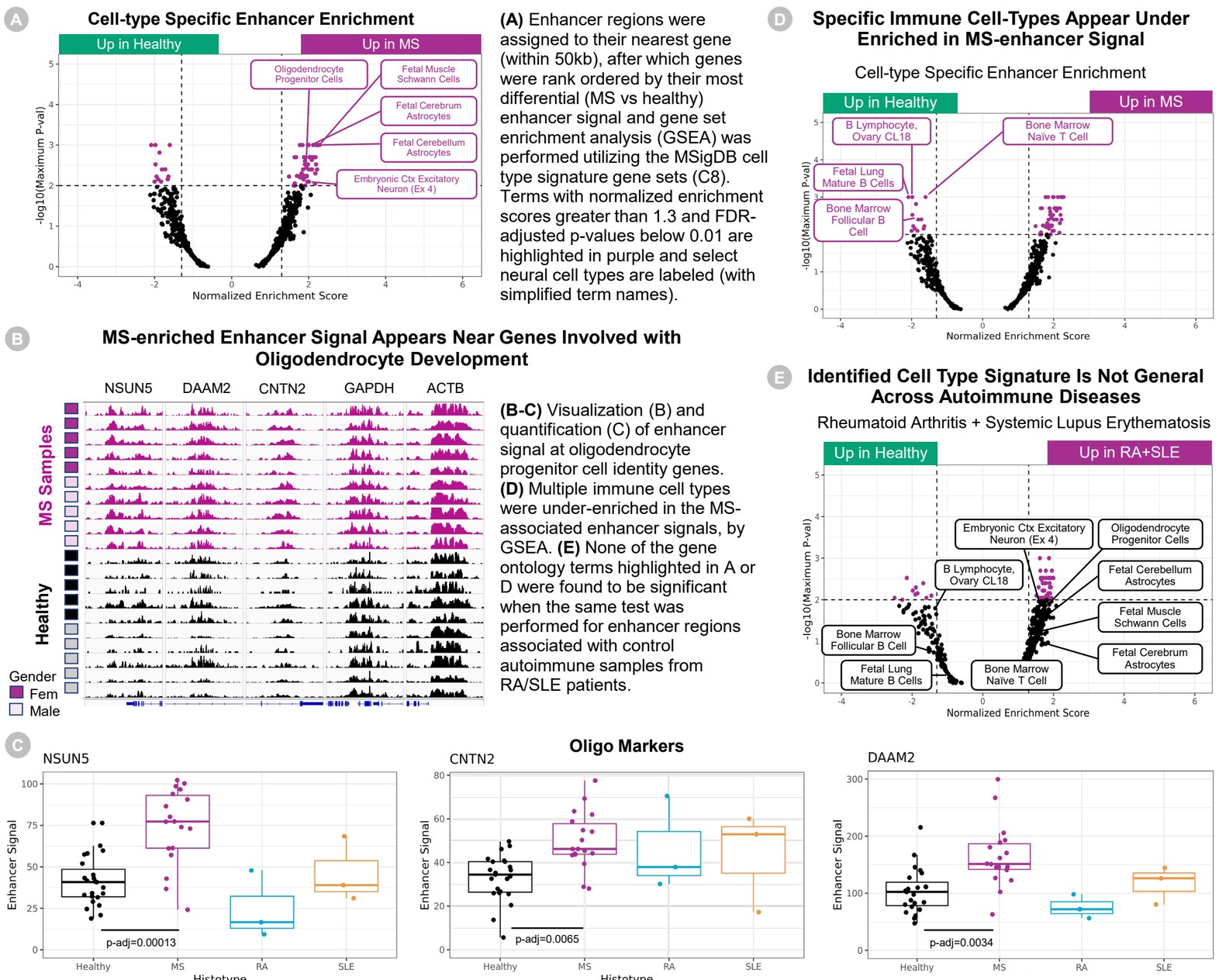
TNFRSF14 Promoter Activity is Not Elevated Above Healthy Levels in Other Autoimmune Disease Samples

MS Patients Display Increased Promoter Signal at Genes Associated with Long-term Synaptic Plasticity



(A-C) Each gene was tested for enrichment/depletion of activate promoter signal within ±1kb of its TSS, which identified genes like TNFRSF14 (visualized in **A**, quantified in **B**) with increased signal in MS patient plasma compared to healthy. Housekeeping genes ACTB and GAPDH are also shown for internal control. TNFRSF14 and its ligand TNFSF14 were identified as candidate target genes of MS-associated risk alleles (**C**, adapted from International Multiple Sclerosis Genetics Consortium et al., Nature, 2011), and TNFRSF14 displays the highest promoter signal of any gene in 7/18 tested MS samples on the Precede assay. (**D**) Samples from rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients were tested to compare promoter and enhancer signals across autoimmune diseases. The enriched TNFRSF14 promoter signal appeared only in the MS patient population. (**E**) Genes were rank ordered by their MS vs healthy differential promoter signal and gene set enrichment analysis (GSEA) was performed. Terms from the Gene Ontology Consortium Biological Process (GO:BP) set with normalized enrichment scores above 1.3 and FDR-adjusted p-values below 0.05 are highlighted in purple. (**F**) Promoter signal quantification of two genes from the synaptic plasticity gene ontology terms highlighted in (E).

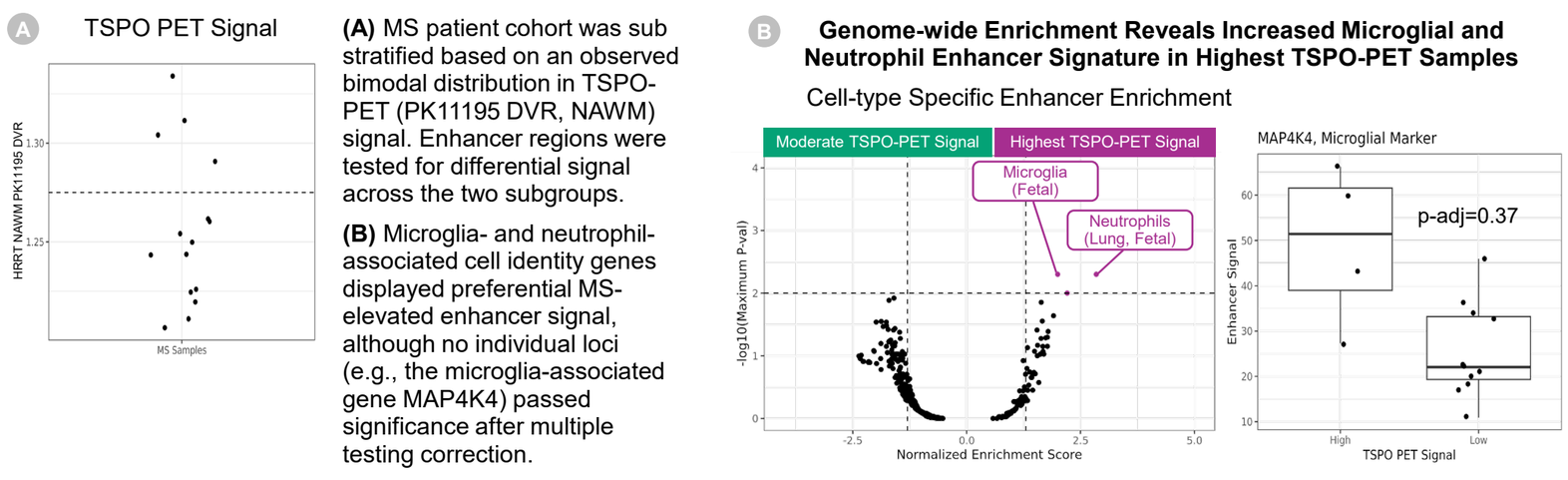
Figure 4. MS-associated Enhancers Appear Preferentially Near Neural Cell Type Genes



(A) Enhancer regions were assigned to their nearest gene (within 50kb), after which genes were rank ordered by their most differential (MS vs healthy) enhancer signal and gene set enrichment analysis (GSEA) was performed utilizing the MSigDB cell type signature gene sets (C8). Terms with normalized enrichment scores greater than 1.3 and FDR-adjusted p-values below 0.01 are highlighted in purple and select neural cell types are labeled (with simplified term names).

(B-C) Visualization (B) and quantification (C) of enhancer signal at oligodendrocyte progenitor cell identity genes. **(D)** Multiple immune cell types were under-enriched in the MS-associated enhancer signals, by GSEA. **(E)** None of the gene ontology terms highlighted in A or D were found to be significant when the same test was performed for enhancer regions associated with control autoimmune samples from RA/SLE patients.

Figure 5. Elevated Microglia and Neurotrophil Enhancers Detected in High TSPO-PET Patients



(A) MS patient cohort was sub stratified based on an observed bimodal distribution in TSPO-PET (PK11195 DVR, NAWM) signal. Enhancer regions were tested for differential signal across the two subgroups.

(B) Microglia- and neutrophil-associated cell identity genes displayed preferential MS-elevated enhancer signal, although no individual loci (e.g., the microglia-associated gene MAP4K4) passed significance after multiple testing correction.

RESULTS & CONCLUSIONS

RESULTS: 1184 enhancers, 2276 promoters, and 690 hypermethylation sites had differential signal in the MS group compared to HCs. Gene set enrichment analysis of genes associated with MS-elevated enhancers revealed enrichment for markers of oligodendrocyte progenitor cells, astrocytes, and excitatory neurons among others. Similarly, MS-elevated promoters were found linked to (1) synaptic plasticity genes, and (2) genes previously linked to MS-risk alleles. These enrichments did not emerge in other autoimmune diseases. Finally, enhancers whose signal across pts trended with increasing TSPO-PET normal-appearing white matter distribution volume ratios were found preferentially near microglial cell identity genes.

CONCLUSIONS: We introduce new methodology with which we identified plasma-based circulating chromatin enriched in MS pts from enhancer and promoter regions linked to genes associated with cell types, risk alleles, and biological pathways underlying MS disease presentation. This assay may represent a valuable, minimally invasive tool to identify and track disease biology.

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