Genome-wide mapping of patient autoantibody targets to understand and predict multiple sclerosis pathogenesis and patient responses to interferon beta-1a therapy

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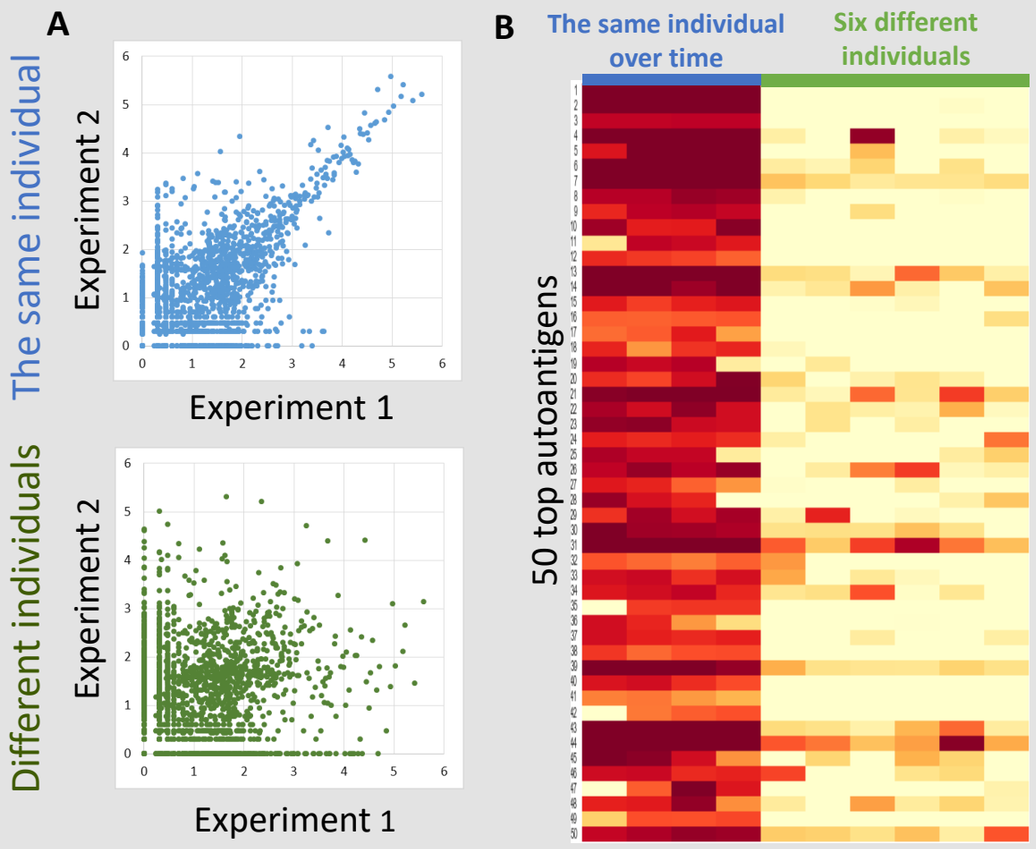
Abstract

The Antigenome Platform is a high-throughput assay that identifies serum autoantibodies reactive with protein domains encoded across the human genome. The Human Antigenome is comprised of all antigens from the genome that can elicit an immune response and the Antigenome technology aims to characterize autoantibody targets to any of the ~20,000 human gene-encoded proteins that could serve as antigens for the quadrillions of blood antibodies in the human body. Linear and conformational epitopes of human protein domains are probed in solution using 1 µL of patient serum IgG to enrich autoantigen identification. This technology identifies autoantibodies that may contribute to multiple sclerosis (MS) pathology, chronicles disease onset and heterogeneity, and predicts therapeutic responses and disease prognosis.

Results: Figure 1

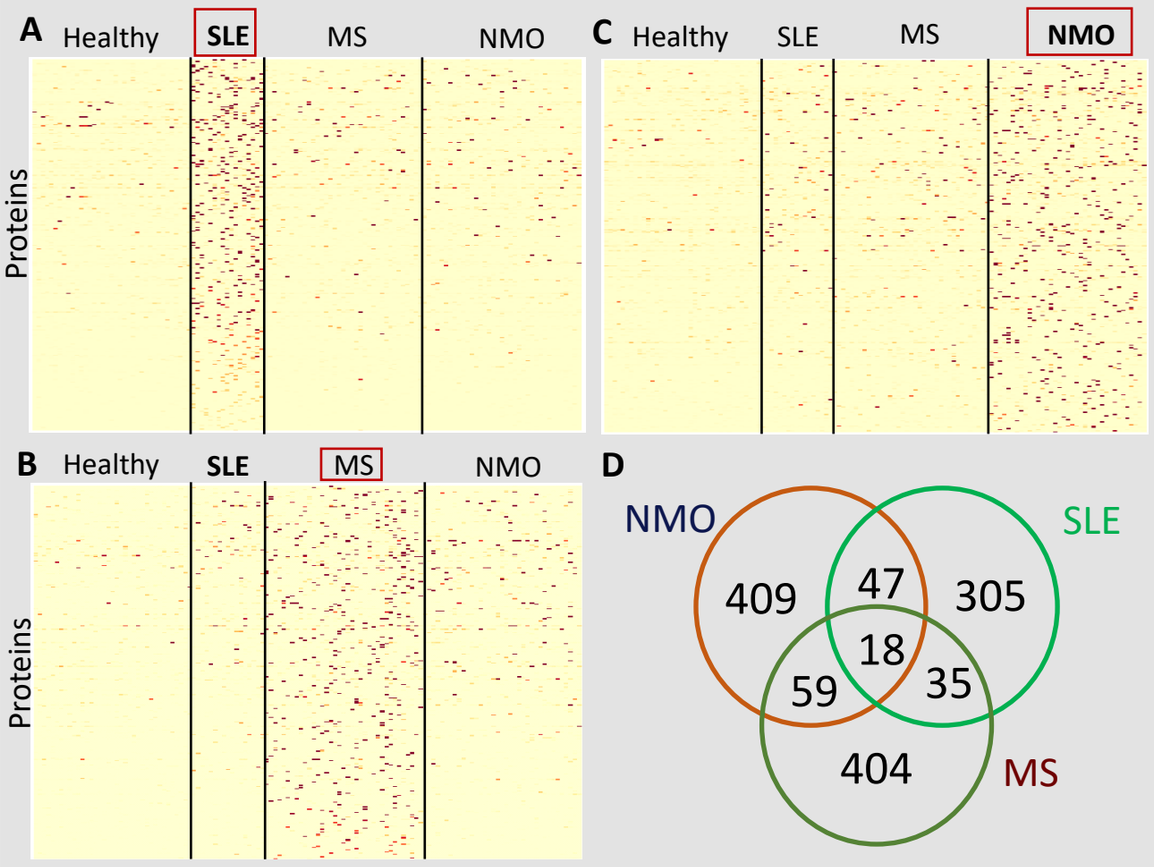
Individual-specific autoantigen signatures are durable over time and reproducible between experiments



A) Autoantigen selection is reproducible. Autoantigens (dots) selected by sera in two independent experiments were only correlated when selected using serum from the same individual. Axes indicate sequencing counts (log_{10} scale). **B)** Top autoantigens selected by sera from the same individual over time versus the same autoantigens selected by different individuals (color scale, 0 to >10^6).

Results: Figure 2

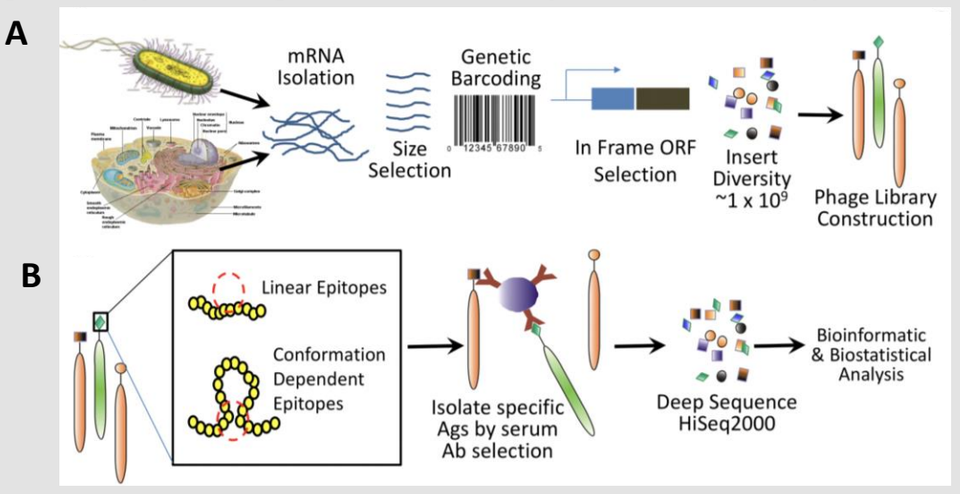
Sera from MS patients reveal autoantigen targets that are distinct from healthy controls and other patients with autoimmune diseases.

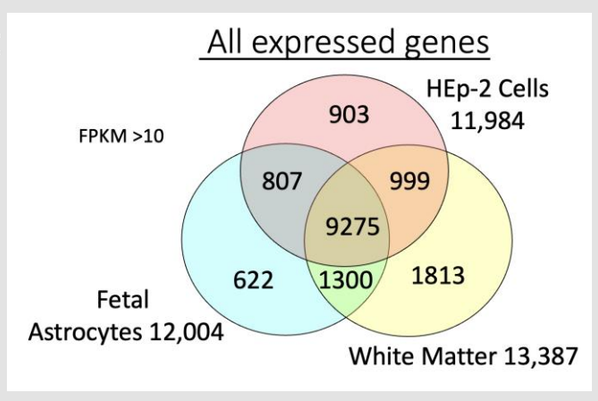


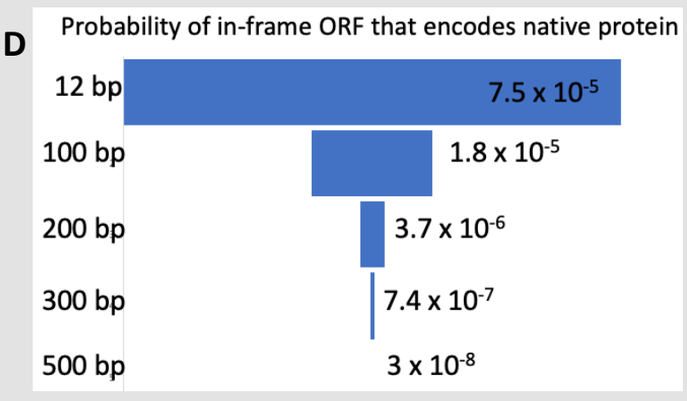
Representative autoantigen signatures for **A**) Systemic Lupus Erythematosus (SLE), **B**) MS, and **C**) Neuromyelitis Optica (NMO) cohorts relative to each other and to healthy control (color scale, 0 to >10^6). MS patients selected for this study represent the spectrum of disease. **D**) There are 404 autoantigens selected exclusively by MS sera and not by other autoimmune diseases.

Methodology

The Antigenome technology utilizes high diversity phage libraries of in-frame, protein domain-sized fragments representing ~5x10^6 domain sequences, which are probed with serum Abs



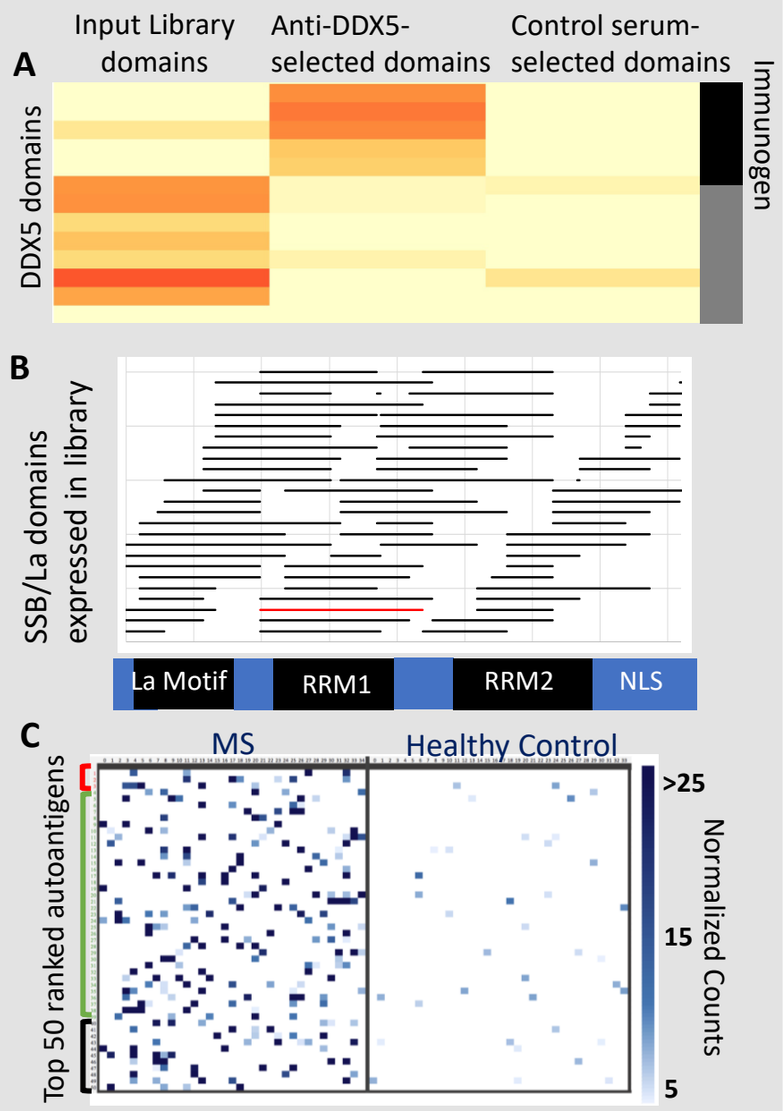




A) Expression library construction. mRNA purified from four types of human cells was fragmented to generate domain-sized open reading frame transcripts encoding polypeptides. Distinct in-frame transcripts (10^9) were genetically barcoded for library construction. **B)** Phage displaying domains with linear and conformational epitopes were immunoselected by serum IgG-bound protein G-paramagnetic beads. Samples were multiplexed, identified by deep sequencing, quantified, and statistically analyzed. **C)** The number of RNA transcripts contributed by each library. FPKM: fragments per kilobase of exon model per million reads mapped. **D)** Since the average domain size for a protein is 100 amino acids, the target library transcript size is 300 bp. Because the frequency of 300 bp transcript inserts are 1 in a million (as shown in chart), rigorous selective pressures are applied throughout library construction and phage selection to reach this target.

Results: Figure 3

Autoantigen Signatures are mapped at domain level

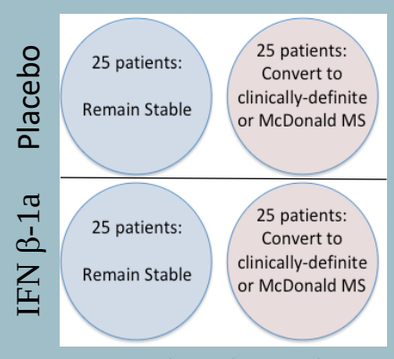


A) Rabbit antisera generated against DDX5 peptide selects appropriate domains with high specificity, despite inappropriate domains having higher library representation. **B)** Expression libraries are complex mixtures of protein domains. Black lines represent expressed library protein domains of the SLE SSB/La autoantigen. **C)** Top 50 antigens selected by MS sera in comparison with healthy control. All top 50 antigens are expressed in the brain (Red = preferentially in brain, Green = highly in brain , black = broadly expressed). MS patients represent the spectrum of disease.

Current Studies

Identify autoantigen signatures that:

1) serve as prognostic biomarkers for MS response to IFNb-1a treatment
2) distinguish patients who will progress to McDonald and clinically-definite MS



- REFLEX study showed IFN β-1a reduces risk of definite and McDonald MS in patients with a clinically isolated demyelinating event[1].
- Autoantigen signatures are being obtained and analyzed for 100 patients at three timepoints each (baseline, 6 months, and 24 months)

Conclusions

While complex patterns of unique autoantibody targets are present in all individuals, regardless of health or disease, shared autoantibody specificities are found among some MS cohorts that may provide new diagnostic, prognostic, or treatment-related markers for different phases of disease progression. Results of these baseline studies are providing a framework for interrogation of the REFLEX trial samples.

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References: 1) Lancet Neurol. 11: 33-41. 2012