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A novel autoantigen discovery platform for the identification of diagnostic and theragnostic autoantibody biomarkers in multiple sclerosis

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Abstract

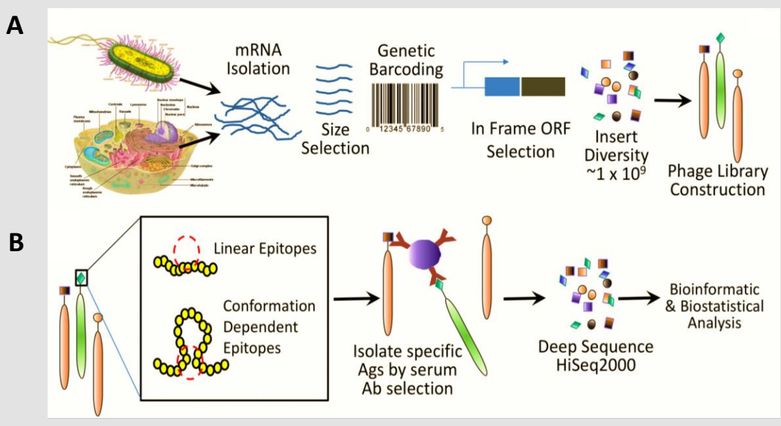
Multiple sclerosis (MS) is a debilitating autoimmune disease characterized by axonal loss and demyelination of the central nervous system. Early diagnosis and treatment of MS are critical for limiting disability and preventing disease progression; however, diagnostic and prognostic biomarkers are currently lacking. Autoantibody profiles offer a unique avenue for detecting and classifying MS patients. This study describes the development and use of a novel autoantigen discovery system, the Antigenome Platform, for the discovery of autoantibody profiles associated with MS diagnosis and treatment.

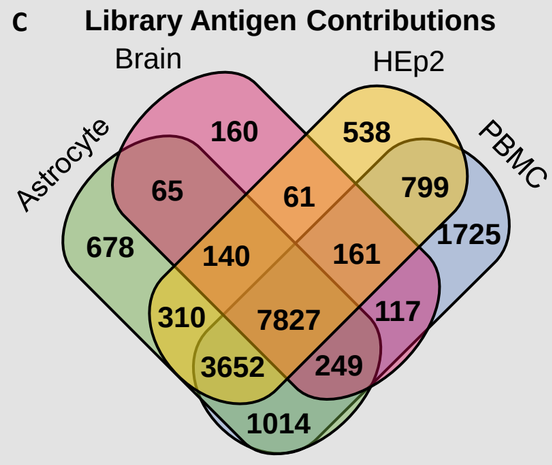
The Antigenome platform agnostically and reproducibly surveys autoantibody binding to large protein fragments (up to 250 amino acids) derived from multiple cell sources, thereby representing 90% of the human genome. The Antigenome Platform was used to screen serum samples from 102 MS patients from the REFLEX (Rebif FLEXible dosing in early MS) trial[1]. This study aimed to identify novel autoantigen targets associated with clinically isolated syndrome (CIS), a stage of disease that often precedes MS, conversion from CIS to MS by McDonald criteria (McDonald MS), and patient response to IFN-beta1a (Rebif®) therapy.

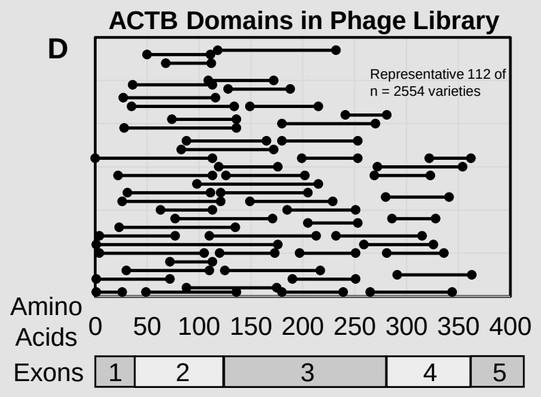
This study identifies expression of autoantibodies to 166 autoantigen targets selected by >10% of MS patients' sera. Of these autoantibodies, 10 serve as biomarkers suggestive of conversion from CIS to MS and 17 associate with interferon beta-1a therapy response. This study suggests widespread autoantibody production occurs in MS and provides novel biomarkers for continued study and prediction of disease progression.

Methodology

The Antigenome technology utilizes high-diversity phage libraries of in-frame, protein domain-sized fragments, which are probed with serum antibodies



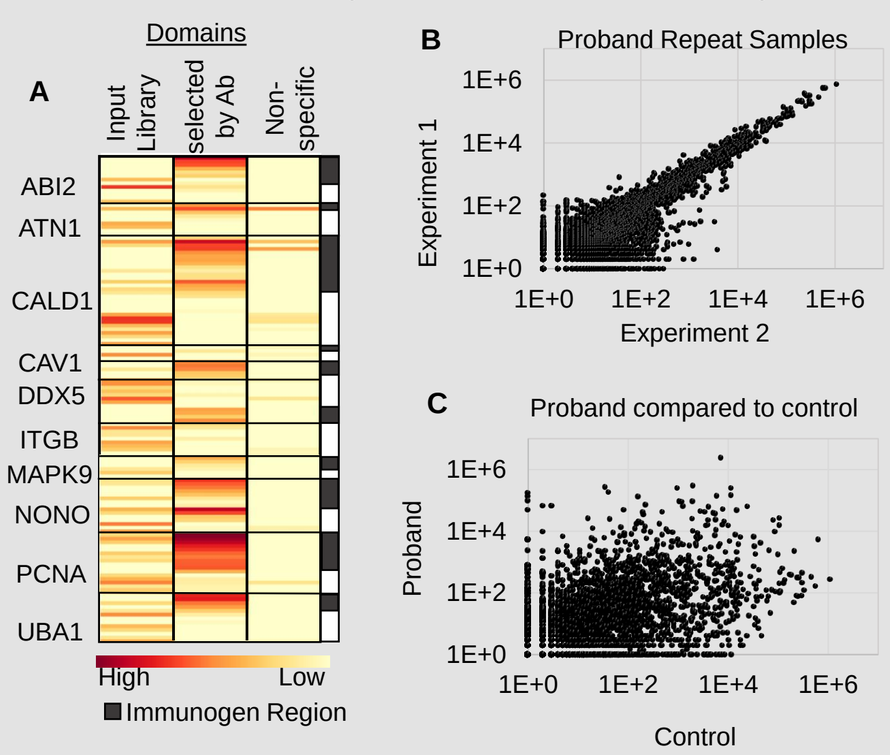




(A) Expression library construction. mRNA purified from four types of human cells was fragmented to generate domain-sized open reading frame transcripts encoding polypeptides. Distinct in-frame transcripts (>10^6) were genetically barcoded for library construction. **(B)** Schema for autoantigen selection by sera and data generation. 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)** Venn Diagram shows the overlap of genes included in the library from each cell source. **(D)** Representative protein domains of the ACTB gene expressed within the pooled cDNA library after selection. Black lines represent expressed library protein fragments of the ACTB protein. The 112 fragments shown represent 2554 total ACTB fragments in the library.

Results: Figure 1

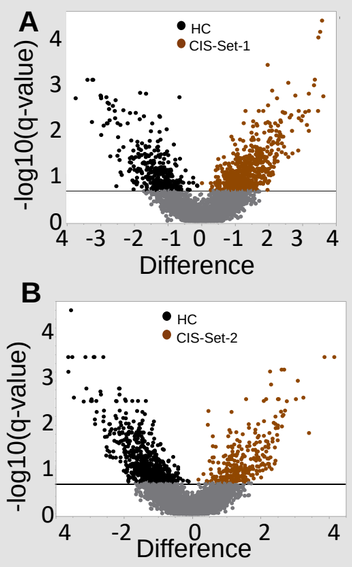
The Antigenome Platform allows antigen-specific selection that is reproducible and individual-specific

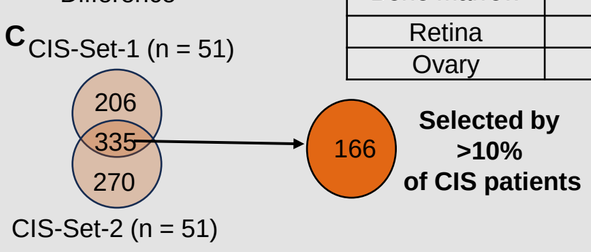


(A) Immunoselection results for nine rabbit antisera generated against domains of the indicated human target antigens. Rabbit antisera generated against specific domains of the following proteins were included: Abl Interactor 2 (ABI2), Atrophin 1 (ATN1), Caldesmon 1 (CALD1), DEAD-Box Helicase 5 (DDX5), Integrin Beta 1 (ITGB1), Mitogen-Activated Protein Kinase 9 (MAPK9), Non-POU Domain Containing Octamer Binding (NONO), Proliferating Cell Nuclear Antigen (PCNA), Ubiquitin Like Modifier Activating Enzyme 1 (UBA1). Protein domains selected by the rabbit antisera antibody (Ab), domains selected by a control serum, and the relative density of domains available for selection in the input library are all shown. **(B-C)** Scatter plot showing autoantigens (dots) selected in two independent experiments using (B) the same serum sample (from Donor #1) or (C) two different serum samples (one from Donor #1 and one from an age-matched control serum). Axes indicate sequencing counts (log10 scale).

Results: Figure 2

Serum autoantibodies from CIS patients consistently select autoantigen targets that are distinct from healthy controls.





D

Tissue	MS-enriched antigens with enriched or enhanced expression
Low tisue specificity	69% (115/166)
Skeletal Muscle	10% (10/166)
Brain	6% (10/166)
Lymphoid tissue	2% (4/166)
Skin	2% (3/166)
Liver	2% (3/166)
Tongue	2% (3/166)
Heart muscle	2% (3/166)
Adipose Tissue	1% (2/166)
Choroid and Plexus	1% (2/166)
Bone marrow	1% (2/166)
Retina	0.6% (1/166)
Ovary	0.6% (1/166)

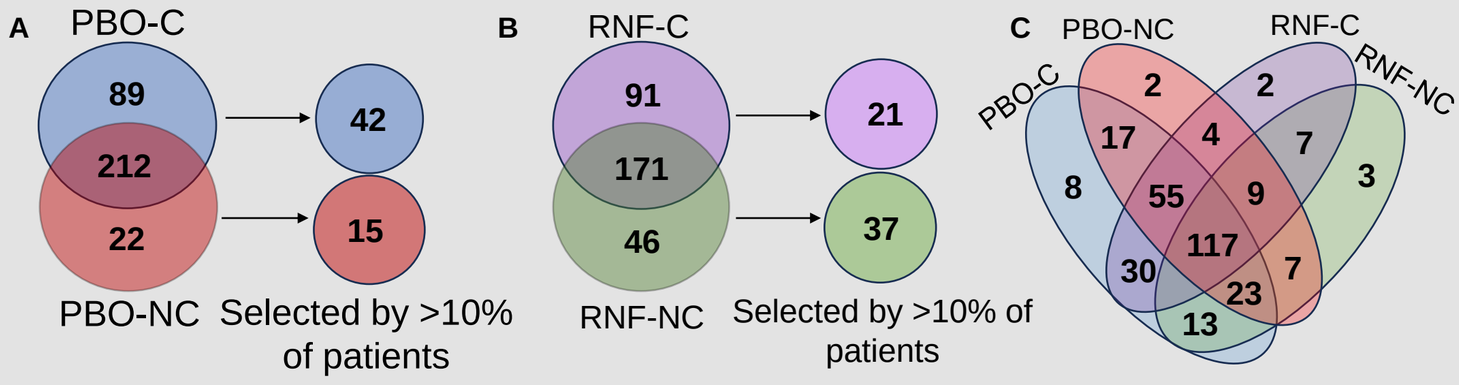
(A-B) The 102 CIS serum samples were evenly divided into two groups, CIS-Set-1 and CIS-Set-2. Individual autoantigens (dots) are statistically compared between the HC group (n=43) and (A) The first group of CIS patients (CIS-Set-1; n=51; month 0), (B) The second group of CIS patients (CIS–Set-2; n=51; month 0). The x-axis represents the difference in sequencing count means between cohorts taken from log2-transformed sequencing counts. The y-axis represents the negative log10 of q-values (higher values indicate greater significance). Autoantigens above the horizontal line (q = .2) are considered significant. Kruskal-Wallis rank test was used for all statistical comparisons. **(C)** Venn diagram shows the overlap of autoantigens selected by CIS-Set-1 and CIS-Set-2. The autoantigens reproducibly selected were extracted and filtered for autoantigens selected by at least 10% of all CIS patients; 166 autoantigens remained. **(D)** Table showing the percent autoantigens (of the 166 CIS-enriched autoantigens from (C)) with enhanced or enriched expression in a human tissue (Human Protein Atlas database).

Conclusions

Patterns of autoantibody expression define MS and MS subgroups, which may be useful as biomarkers and provide insight into the pathogenesis of MS. Patterns of 17 and 10 autoantibodies associate with conversion to MS with and without therapeutic intervention, respectively. Additional studies are needed to validate the proposed autoantibody panels for MS prognosis and therapeutic decision making.

Results: Figure 3

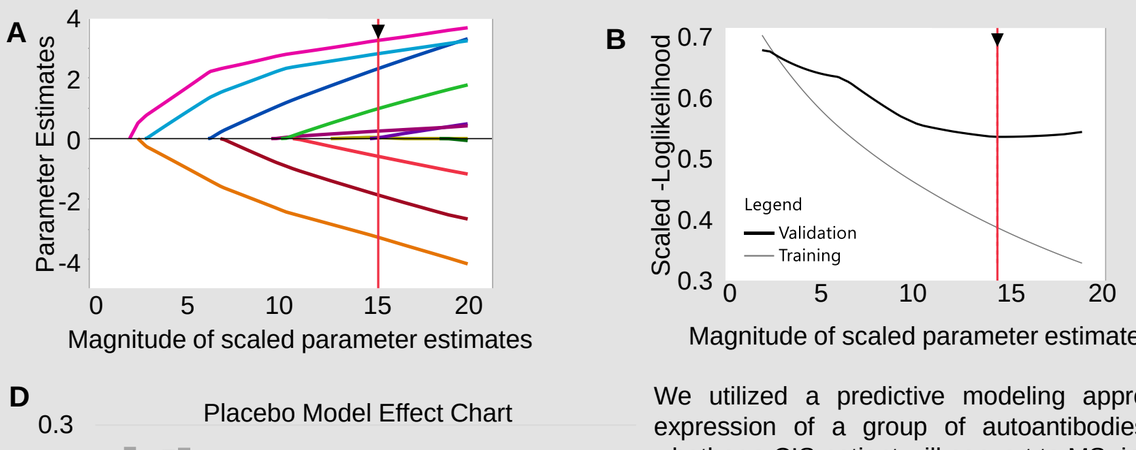
Autoantibody expression associated with conversion from CIS to MS with and without therapeutic intervention



Venn diagram shows the overlap of autoantibody enrichment in CIS patients within the placebo group who converted to MS during the REFLEX trial (PBO-C) and patients who did not convert (PBO-NC) Enrichment is based on statistical comparison (Wilcoxon-Rank test, p-val <.05, q-val <.2) of each CIS group compared to HC. Smaller circles indicate the number of autoantigens selected by >10% of PBO-C (blue) or PBO-NC (red) from the uniquely enriched PBO-C (89) or PBO-NC (22) autoantigens. (B) Venn diagram shows the overlap of autoantibody enrichment in CIS patients within the IFN-beta1a treatment group who converted to MS during the REFLEX trial (RNF-C) and patients who did not convert (RNF-NC). Enrichment is based on statistical comparison (Wilcoxon-Rank test, p-val <.05, q-val <.2) of each CIS group compared to HC. Smaller circles indicate the number of autoantigens selected by >10% of RNF-C (purple) or RNF-NC (green) from uniquely enriched RNF-C (91) or RNF-NC (46) autoantigens. (C) Venn diagram shows the overlap of antigens enriched in each MS subgroup compared to HC.

Results: Figure 4

Expression of 10 autoantibodies predicts conversion or lack of conversion from CIS to MS in placebo patients



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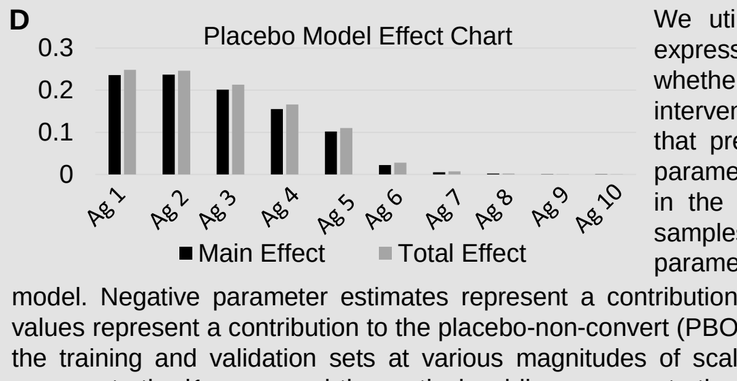
	AUC
Training	0.9921
Validation	0.8750
Test	0.7600



We utilized a predictive modeling approach to evaluate whether expression of a group of autoantibodies at baseline can predict whether a CIS patient will convert to MS in the absence of therapeutic intervention (using placebo samples). We identified 10 autoantibodies that predict conversion or lack of conversion. (A-E) LASSO model parameters for predicting conversion/non-conversion from CIS to MS in the absence of therapeutic intervention using REFLEX placebo samples. (A) The Solution Path Plot displays values of the estimated parameters, where each curve represents a predictive term in the model. Negative parameter estimates represent a contribution to the placebo-convert (PBO-C) outcome, where as positive values represent a contribution to the placebo-non-convert (PBO-NC) outcome. (B) The Validation Plot includes a curve for both the training and validation sets at various magnitudes of scaled parameter estimates. In each plot (A) and (B), the x-axis represents the l1 norm, and the vertical red line represents the value of the l1 norm for the best and chosen solution. (C) The Area Under the Curve (AUC) values for the Training, Validation, and Test samples. (D) Effects chart. "Main Effect" shows the relative contribution of the predictor to the model alone, and "Total Effect" shows the relative contribution of the predictor when other predictors are also taken into account.

Results: Figure 5

Expression of 17 autoantibodies predicts response to IFN-beta1a

C

	AUC
Training	1.000
Validation	0.750
Test	0.694

We utilized a predictive modeling approach to evaluate whether expression of a group of autoantibodies at baseline can predict whether a CIS patient will convert to MS or not convert while using IFN-beta1a therapy. We identified 17 autoantibodies that predict conversion or lack of conversion. A-G) LASSO model parameters for predicting conversion/non-conversion from CIS to MS in the absence of therapeutic intervention using REFLEX placebo samples. (A) The Solution Path Plot displays values of the estimated parameters, where each curve represents a predictive term in the model. (B) The Validation Plot includes a curve for both the training and validation sets at various magnitudes of scaled parameter estimates. In each plot (A) and (B), the x-axis represents the *l*1 norm, and the vertical red line represents the value of the *l*1 norm for the best and chosen solution. (C) The receiver operating characteristic (ROC) curve for the Training, Validation, and Test samples and the associated Area Under the Curve (AUC) values. (D) Effects chart. "Main Effect" shows the relative contribution of the predictor to the model alone, and "Total Effect" shows the relative contribution of the predictor when other predictors are also taken into account.

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