Identifying novel potential cross-reactive targets in Multiple Sclerosis

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Background:

Multiple Sclerosis (MS) is a debilitating demyelinating autoimmune disease affecting mainly young adults. Next to genetics, environmental factors including Epstein-Barr Virus (EBV) infection have been implicated. MS-specific oligoclonal IgG bands in the cerebrospinal fluid have been identified as EBV-specific and autoantibodies against myelin basic protein, oligodendrocyte specific protein and myelin have been identified. Our group has additionally proven anti-EBV nuclear antigen-1 (EBNA-$1_{(398\text{-}413)}$) antibodies as an independent risk factor for MS. This epitope is of particular interest, as it shares high amino acid sequence homology with human crystallin alpha-B which has already been implicated in cross-reactivity.

Objectives:

- Identify cross-reactive targets for anti-EBNA-$1_{(398\text{-}413)}$ IgG antibodies.
- Compare cross-reactive immune responses between MS and healthy controls serum and plasma against human brain-derived proteins.

Methods:

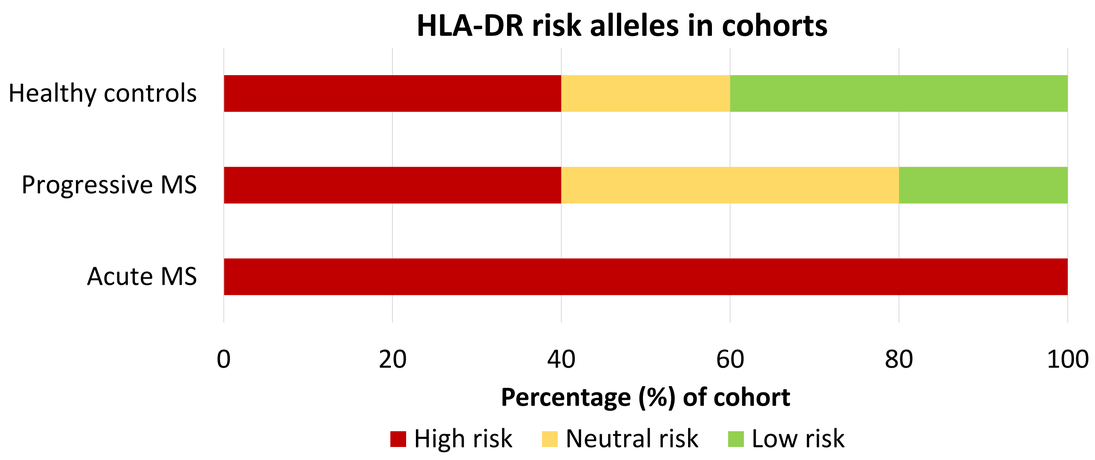
Selecting MS (n=8) and healthy controls (n=10), based on clinical subtype, gender and genetic typing (Human Leukocyte Antigen; HLA; Table 1):

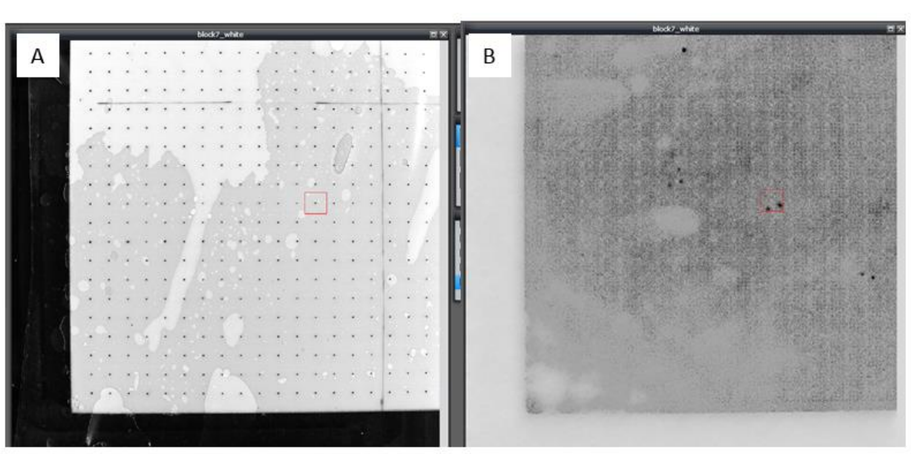
1. Isolate anti-EBNA-$1_{(398\text{-}413)}$ IgG antibodies using pull down columns.
2. Test these selective antibodies and antibodies from whole serum/plasma on a HexSelect macroarray containing recombinant human brain proteins to identify any new potential self-reactive targets of antibodies.

Cohort	Clinical Information	Gender	HLA-DRB1.1	HLA-DRB1.2
MS	Acute relapse	Male	03:01	15:01▲
MS	CIS	Female	15:01▲	15:01▲
MS	Acute relapse	Male	03:01	15:01▲
MS	SPMS, not on treatment	Female	03:01	03:05
MS	SPMS, fingolimod	Female	11:01	13:01
MS	SPMS, not on treatment	Female	04:04▼	13:02
MS	SPMS, interferon	Female	07:01▼	15:01▲
MS	PPMS, not on treatment	Male	03:01	15:01▲
HC		Female	10:01	15:01▲
HC		Female	03:01	15:01▲
HC		Female	04:05▼	15:02▲
HC		Female	04:04▼	08:01▲
HC		Male	01:03	04:04▼
HC		Male	03:01	04:01▼
HC		Female	04:02▼	12:01
HC		Female	04:04▼	07:01▼
HC		Male	14:01	14:01
HC		Female	03:01	13:01

◄Table 1. Multiple Sclerosis (MS) patients and healthy controls (HC) used for anti-EBNA-1 antibody isolation and IgG cross-reactivity. CIS: clinically isolated symptom/s; first presentation of MS. SPMS: Secondary Progressive MS. PPMS: Primary Progressive MS. ▲High Risk HLA ▼Low Risk HLA

▼Figure 1. HLA-DR risk alleles for MS and healthy controls. High risk: contains high risk alleles. Low risk: contains low risk alleles and no high risk alleles. Neutral risk: both outside these groups.





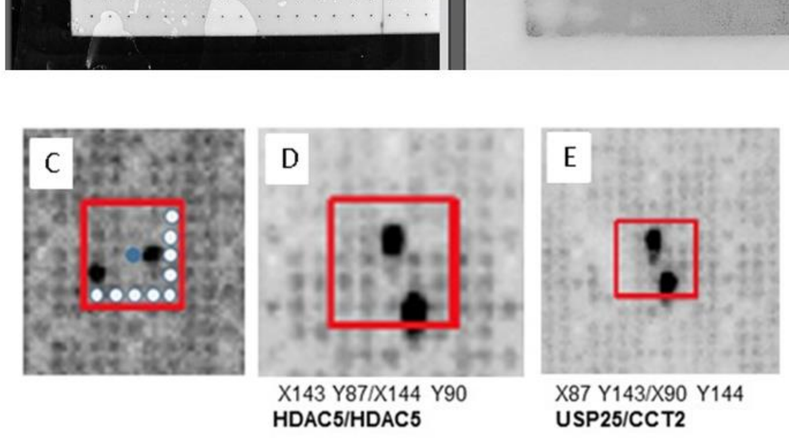


Figure 2. Analysis of protein macroarray. (A) White light "dot blot" image in Pixlr software. (B) Chemiluminescent image in Pixlr software of equal resolution for comparison, with red square on additional layer. (C) Example of 5 x 5 square of dotted proteins (ink dot as a blue circle), with signal as two black dots. (D) X/Y locations of two dots identifying matching duplicate proteins. (E) Two signals identified, but with mismatched protein codes, and thus excluded reactivity.

Results:

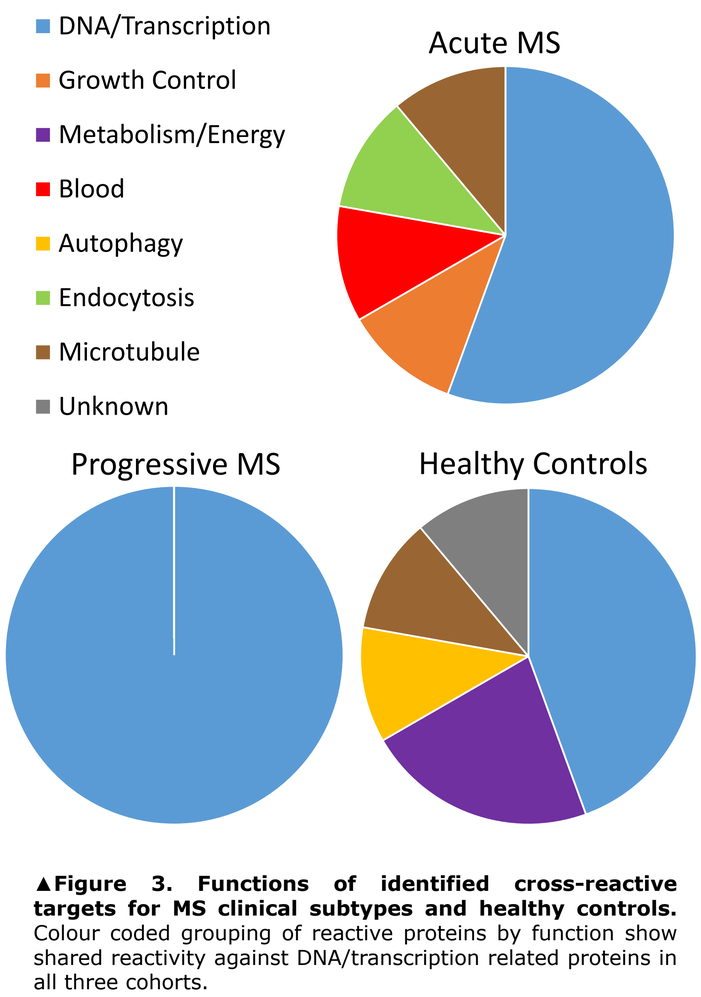
Anti-EBNA-$1_{(398\text{-}413)}$ IgG from MS patients identified two new potentially cross-reactive targets. Both MS patient and healthy control antibodies from serum/plasma reacted against multiple proteins of the array. A total of 18 different protein targets were identified, with some overlap between groups (Table 2). Functionally, proteins were mainly involved in growth control, cell metabolism, autophagy, endocytosis and microtubule destabilization (Figure 3).

Protein Code	Protein Name	Acute MS serum	Acute MS anti-EBNA1$_{(398\text{-}413)}$ antibody	Progressive MS serum	HC plasma	HC plasma (2)
MBD3	Methyl-CpG binding domain protein 3	1				
PIM3	Serine/threonin-protein kinase Pim-3	1				
EPN1	Epsin-1	1				
STMN4	Stathmin-4	4			1	3
HDAC5	Histone deacetylase 5	1			1	2
JMJD8	Jumonji domain-containing protein 8 [transcription/methylation]	1				
SHB	SH2 domain-containing adapter protein B	1				
HBA2	Hemoglobin subunit alpha		1			
EEF1A2	Elongation factor 1-alpha 2 (Statin S1)		1			
TROVE2	60 kDa SS-A/Ro ribonucleoprotein			2		
NFKBIL2	NF-kappa B inhibitor like protein 2			1		
KAT2A	Histone acetyltransferase				1	
CENPB	Major centromere autoantigen B				1	2
MTX1	Metaxin 1				1	
AMPD2	AMP deaminase 2				1	
MAP1LC3A	Microtubule-associated protein 1A/1B light chain 3A precursor					3
UFC1	Ubiquitin-fold modifier-conjugating enzyme 1					1
AZGP1	Zinc-alpha-2-glycoprotein					1

Table 2. Proteins identified as reactive with MS and healthy control serum/plasma IgG and column isolated specific anti-EBNA-$1_{(398\text{-}413)}$ IgG. HC: healthy controls. Numbers indicate the number of duplicates identified across the whole membrane. HC (2) plasma tested a second time after stripping the membrane.

Role	Protein Code	A-MS	P-MS	HC
DNA/ transcription	CENPB			✓
	MBD3	✓		
	JMJD8	✓		✓
	SHB2	✓		
	EEF1A2	✓		
	HDAC5	✓		✓
	TROVE2		✓	
	NFKBIL2		✓	
	KAT2A			✓
Growth control	PIM3	✓		
Metabolism/ Energy	AMPD2			✓
	AZGP1			✓
Blood	HBA2	✓		
Autophagy	MAP1LC3A			✓
Endocytosis	EPN1	✓		
Microtubule	STMN4	✓		✓
Unknown	UFC1			✓

▲Table 3. Grouped functions of target proteins by grouped sources of IgG from three cohorts.
A-MS: Acute MS (antibody or serum).
P-MS: Progressive MS. HC: healthy control plasma.



▲Figure 3. Functions of identified cross-reactive targets for MS clinical subtypes and healthy controls. Colour coded grouping of reactive proteins by function show shared reactivity against DNA/transcription related proteins in all three cohorts.

Discussion, Conclusions, Future Aims:

- Multiple targets were identified for both specific anti-EBNA-$1_{(398\text{-}413)}$ IgG antibodies and whole serum or plasma for both cohorts.
- Different targets were identified for the same cohort (Acute MS) anti-EBNA-$1_{(398\text{-}413)}$ IgG and whole serum, suggesting antibody cross-reactivity could be masked by other stronger binding antibodies.
- Identified targets will be confirmed by western blot, with addition of the protein crystallin alpha-B which was not included on the protein array and previously shown to share homology with the EBNA-$1_{(short)}$ peptide, and myelin based proteins.
- Obtaining a more comprehensive understanding of the potential of cross-reactive antibodies will aid in understanding the underlying MS pathomechanism.

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