Comprehensive Identification of Novel Autoantibody Candidates in OMAS Using Proteome-Wide Screening Platforms

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Introduction

Opsoclonus Myoclonus Ataxia syndrome - OMAS

- ✓ **Rare neuroinflammatory disorder (opsoclonus, myoclonus, ataxia)**
- ✓ **Often paraneoplastic (neuroblastoma) in children → immune-mediated**
- ✓ **Pathogenesis unclear → no reliable biomarkers or specific treatments**

Why OMAS Matters

- ✓ Severe neuroinflammatory disorder(opsoclonus, myoclonus, ataxia)
- ✓ Paraneoplastic (often with NB)
- ✓ Steroid/IVIG effective → Immune-mediated nature
- ✓ No reliable biomarkers → Early Dx difficult
- ✓ Immune insight may inform broader neuroinflammation

Autoimmunity in OMAS

Autoimmunity in OMAS: What We Know - and What's Missing

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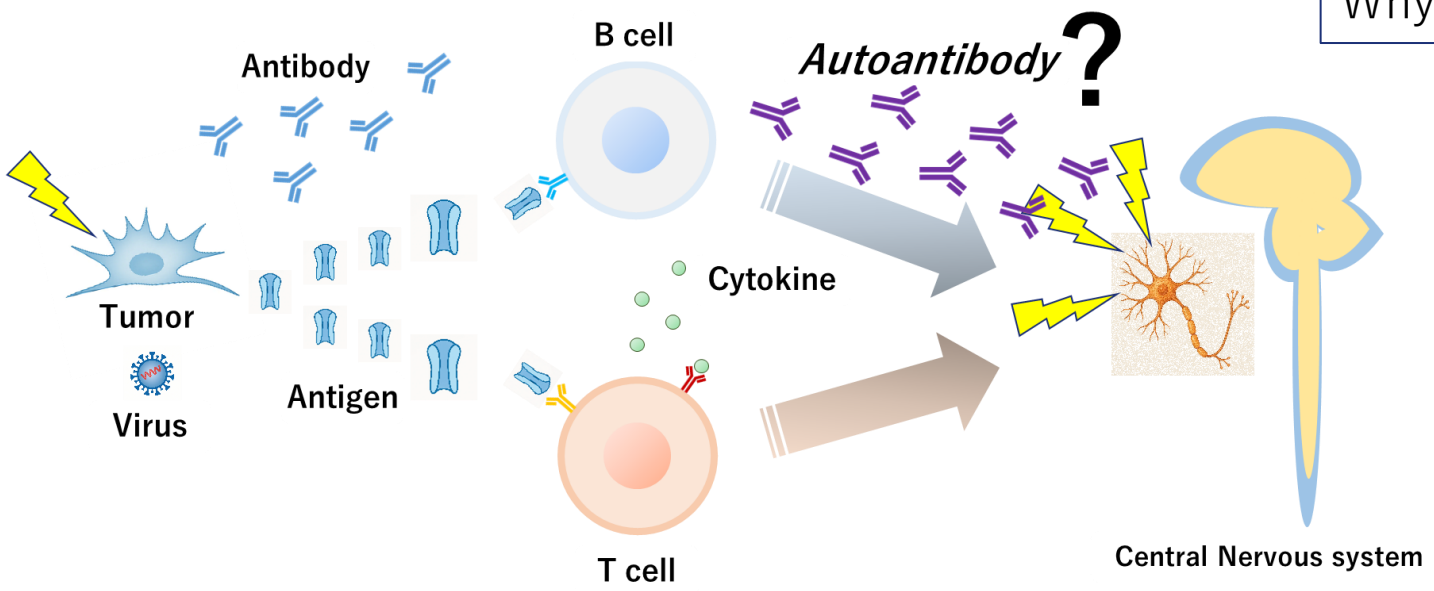
1960s	Kinsbourne first describes OMAS.
1980s-	Recognition of Hu, Yo, Ri, GRID2, KCNC3, etc.
Recent Years	Proteome-wide screening



Why previous autoantibody findings are insufficient:

Limitations of previously reported autoantibodies:

- **Not consistently detected in OMAS patients**
- **Also found in other autoimmune or paraneoplastic conditions**
- **Standard methods (IHC, CBA, cultured neurons) often lack sensitivity for novel or low-abundance targets**

✅ **Key Message:**
Evidence of autoimmunity is strong, but conventional antibody markers are inconsistent, nonspecific, and technically elusive.➤ A comprehensive, proteome-wide screening is needed to uncover novel candidates.

Searching for Autoantibodies - Our Approach

Objectives

- **Comprehensive autoantibody & proteome-wide analysis of CSF from NB-associated OMAS**
- **Identify shared autoantibodies & consistently altered proteins**
- **Correlate findings with clinical data**

→ clarify OMAS pathophysiology & guide future therapies

Materials

- 14 CSF samples from OMAS-NB pediatric patients
  - — Retrospective cohort (JNBSG/JCCG)
- Control samples from ALL (non-CNS disease)

Methods

Screening Antibodies

- **Protein Microarray**
- **Phage ImmunoPrecipitation Sequencing ; PhIP-seq**
- **Liquid Chromatography–Tandem Mass Spectrometry ; LC–MS/MS**

Validation

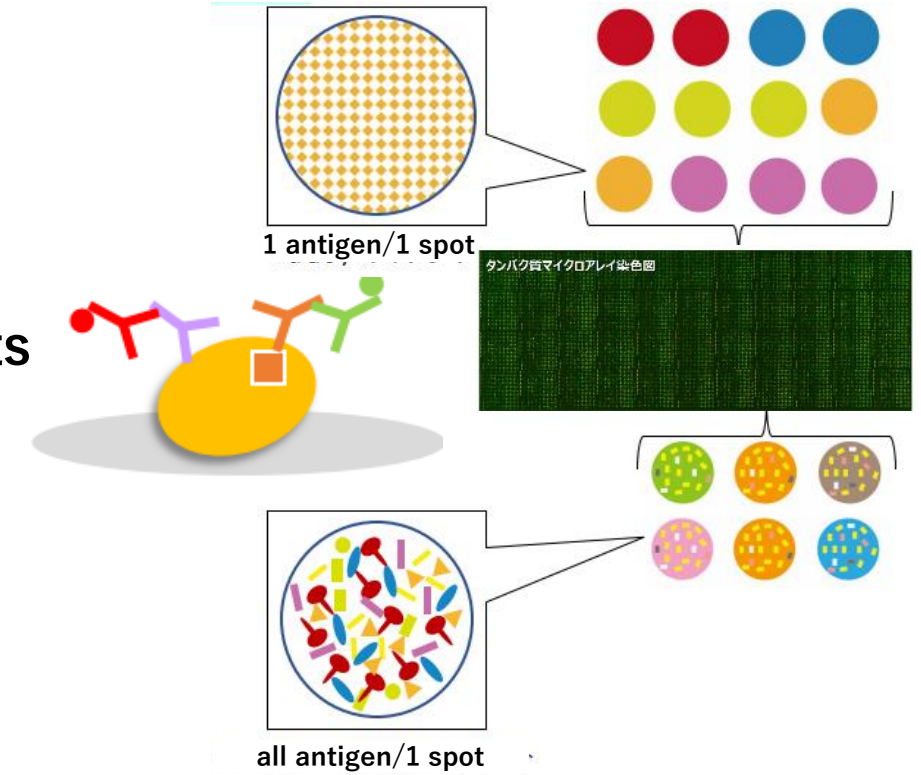
- **Candidate selection across platforms**
- **Confirmatory assays:** ELISA, Western blot, immunocytochemistry
- **Clinical correlation:** neuroblastoma status, immunotherapy response

⚠ Note: Validation methods may not fully capture novel or low-abundance antibodies

→ Functional validation ongoing

Protein Microarray

1. Proteins or peptides densely arrayed on a solid surface
2. Antibodies in the sample bind to their respective targets
3. Fluorescence reveal antibody-antigen interaction



Detectable Protein:
Standard type 14,746 & Variant/Fusion Proteins 1,937

✅ **Key Message:**
High-throughput screening of full-length proteins enables detection of diverse antibody targets, including membrane and intracellular antigens.

Phage ImmunoPrecipitation Sequencing (PhIP-Seq)

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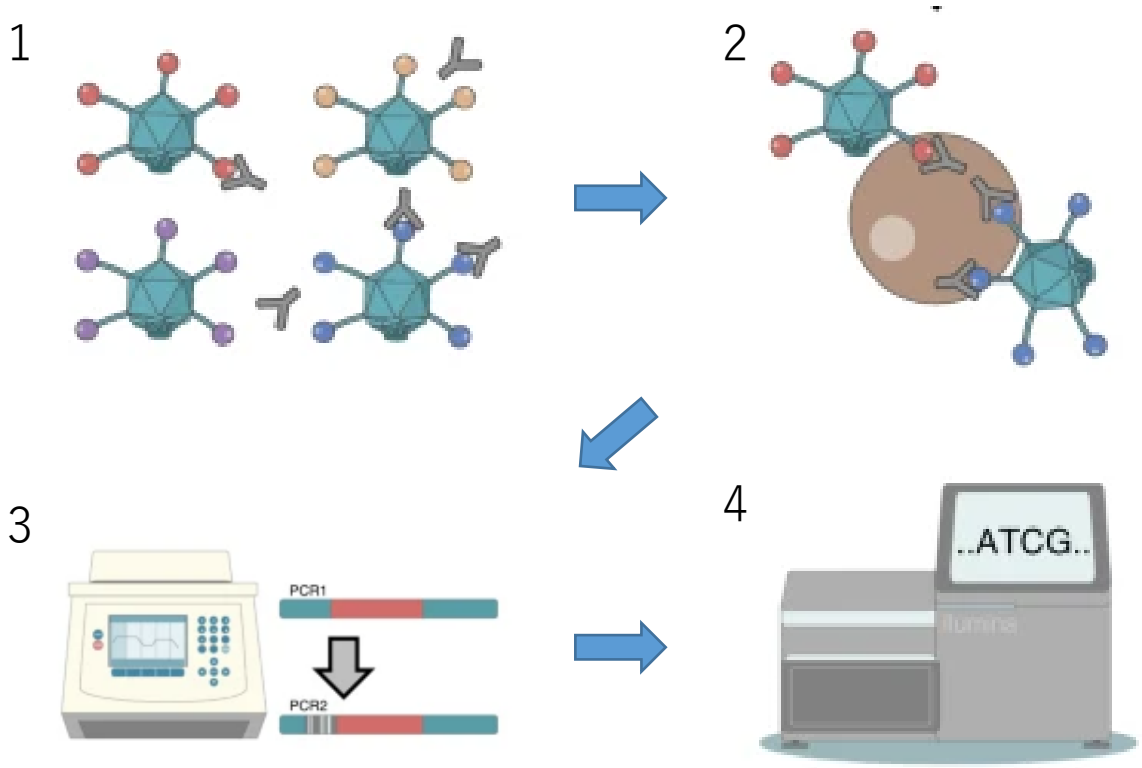
1. Peptide phage display library
2. Incubation with CSF/serum
3. Immunoprecipitation of bound phages
4. NGS-based identification of epitopes

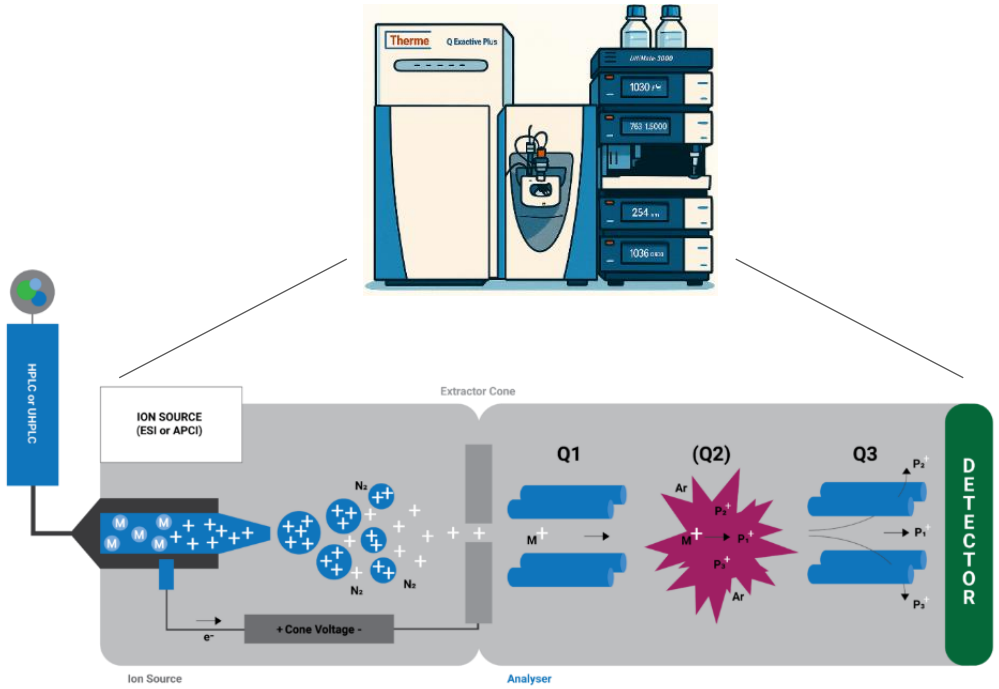


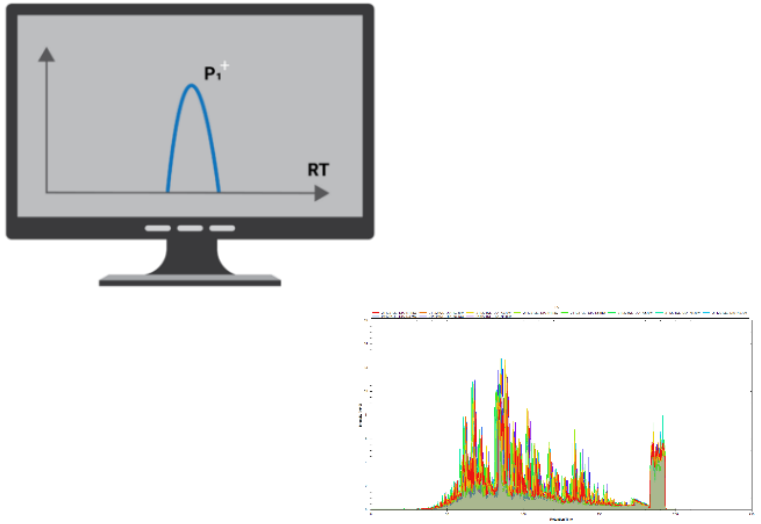
✅ **Key Message:**
PhIP-seq detects linear epitope reactivity, capturing low-abundance or unconventional antibody targets.

LC-MS/MS DIA (data-independent acquisition)

1. LC separates peptides by chemical properties
2. MS/MS fragments and analyzes ions
3. DIA enables reproducible, comprehensive profiling

→ High-sensitivity detection of CSF proteins





✅ Key Message:
Unbiased proteomic profiling reveals differentially expressed proteins, providing context beyond antibody binding.

Workflow

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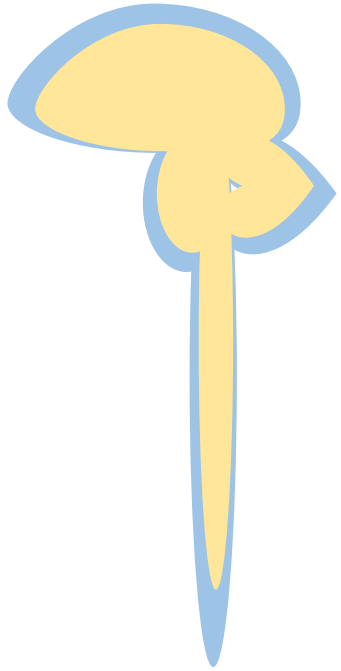
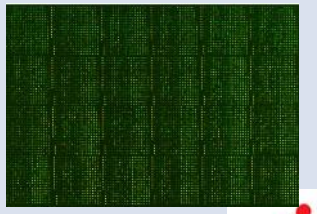
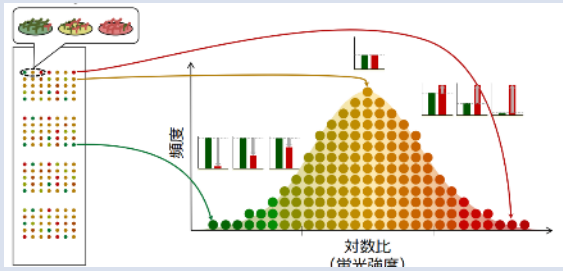
CSF Controll
OMAS

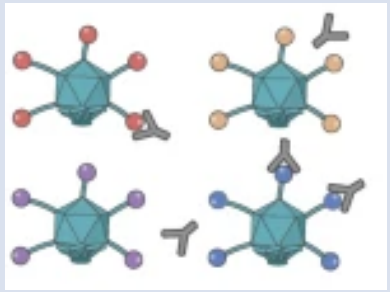


Protein Array

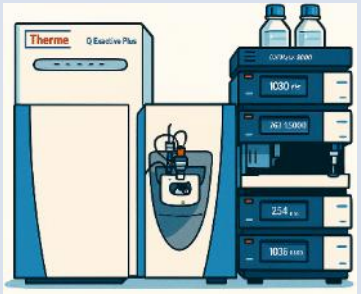


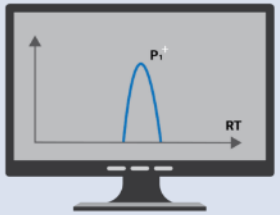


PhiP-seq

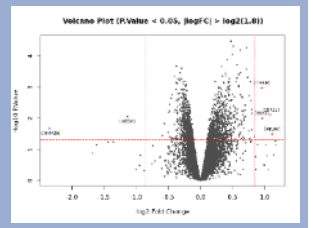


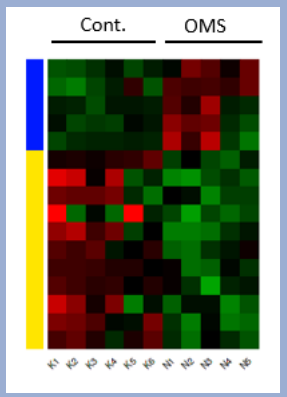
LC-MS/MS



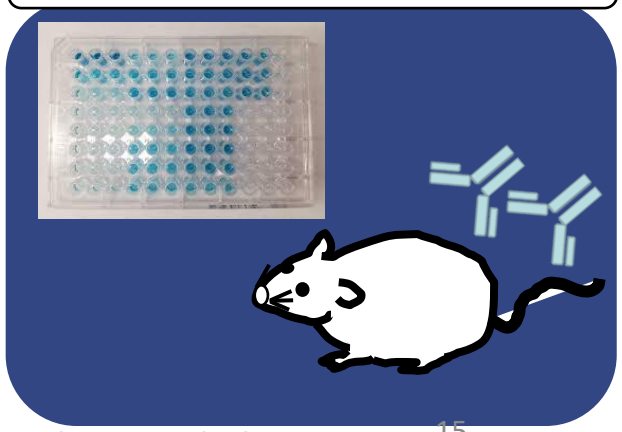


Data Analysis





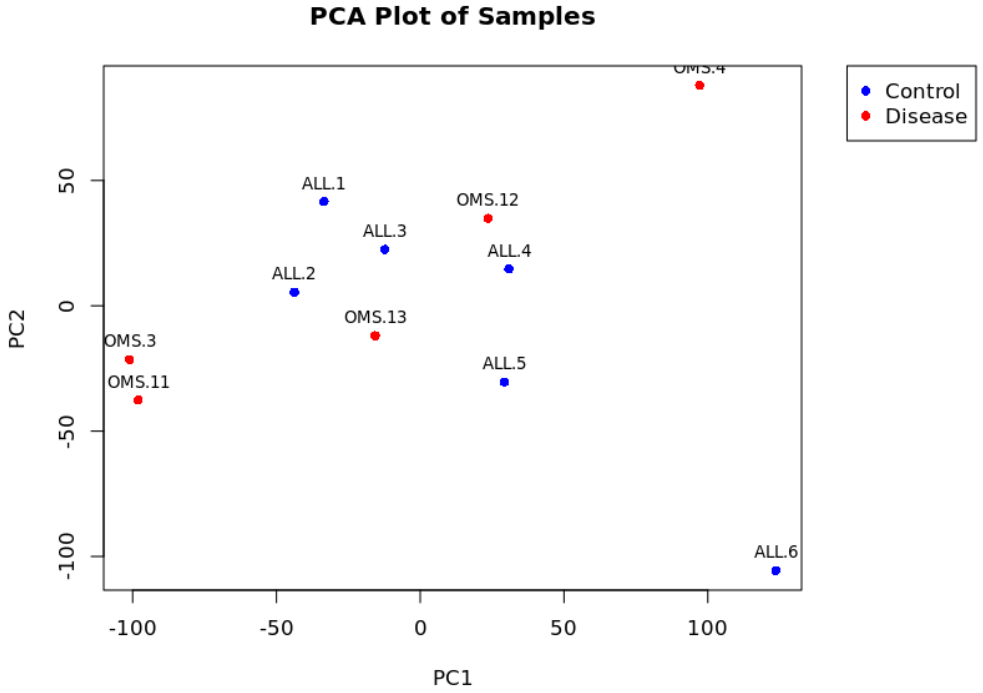
Functional Validation

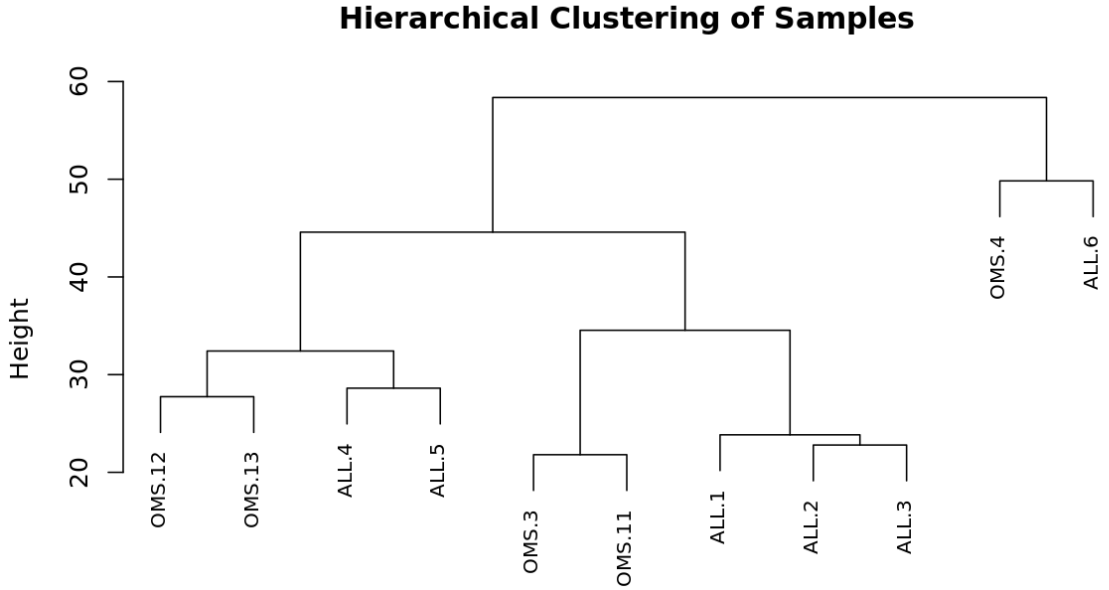


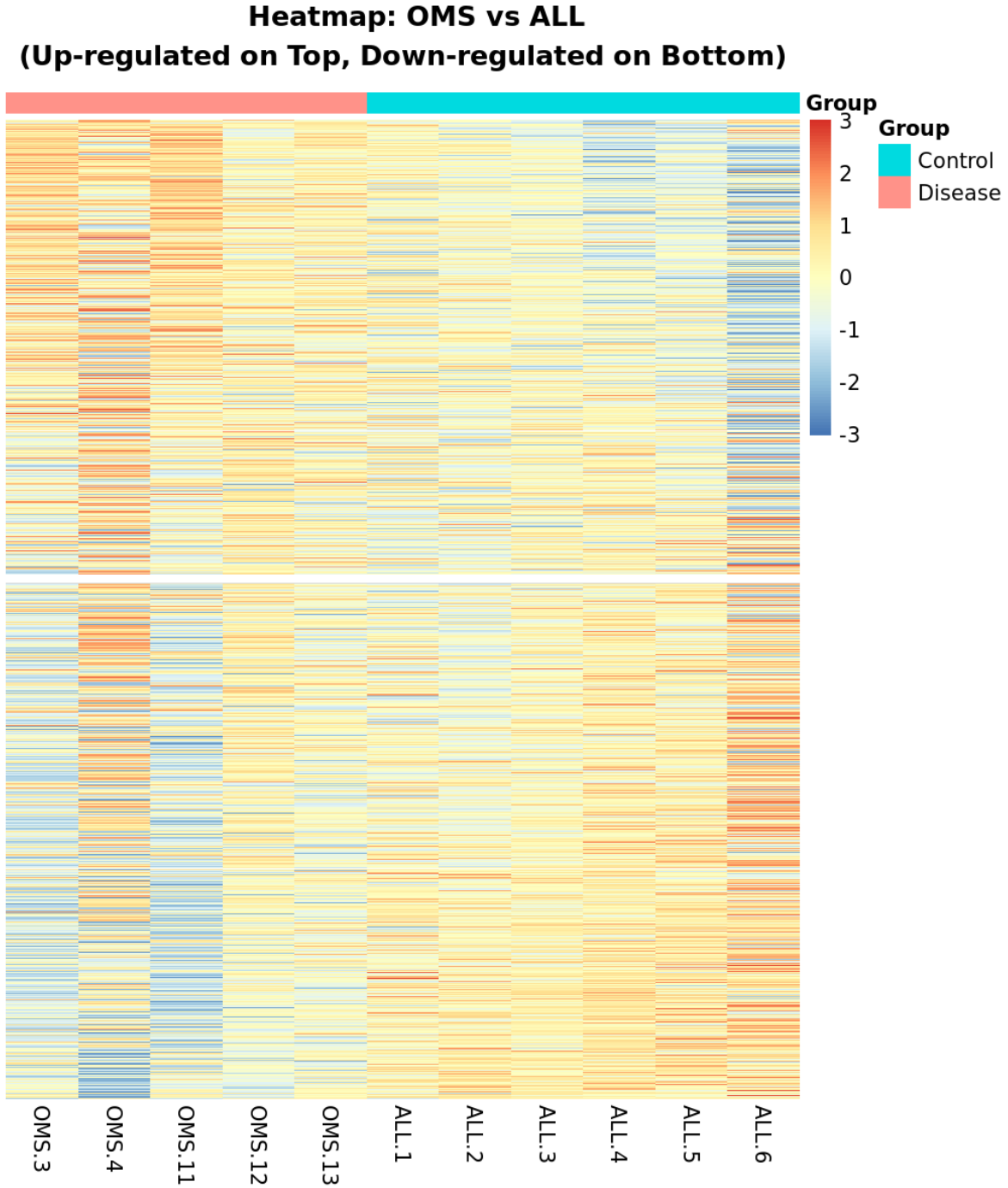
Parallel screening and cross-platform analysis to identify robust autoantibody candidates.

Our Research

Results - Protein Microarray







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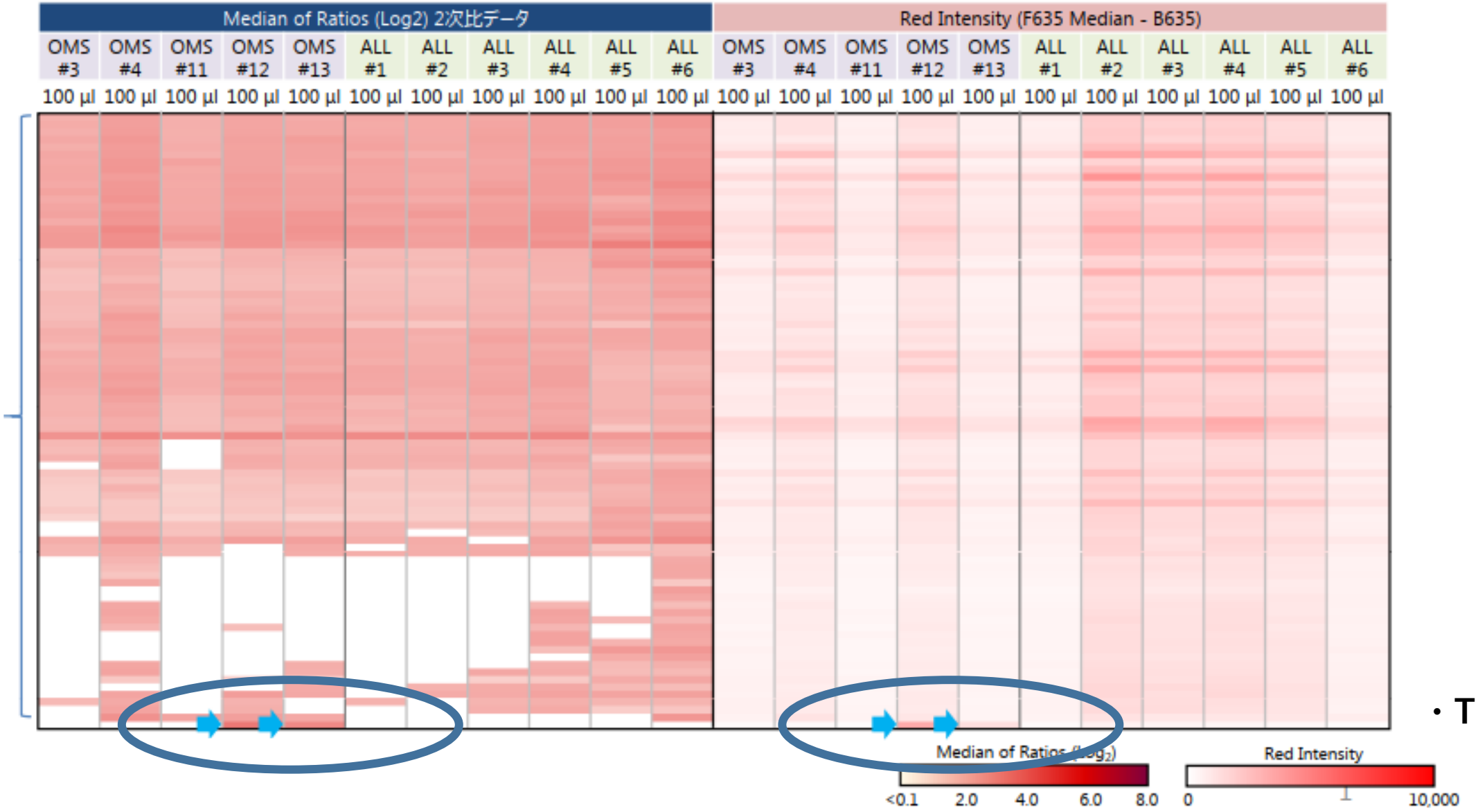
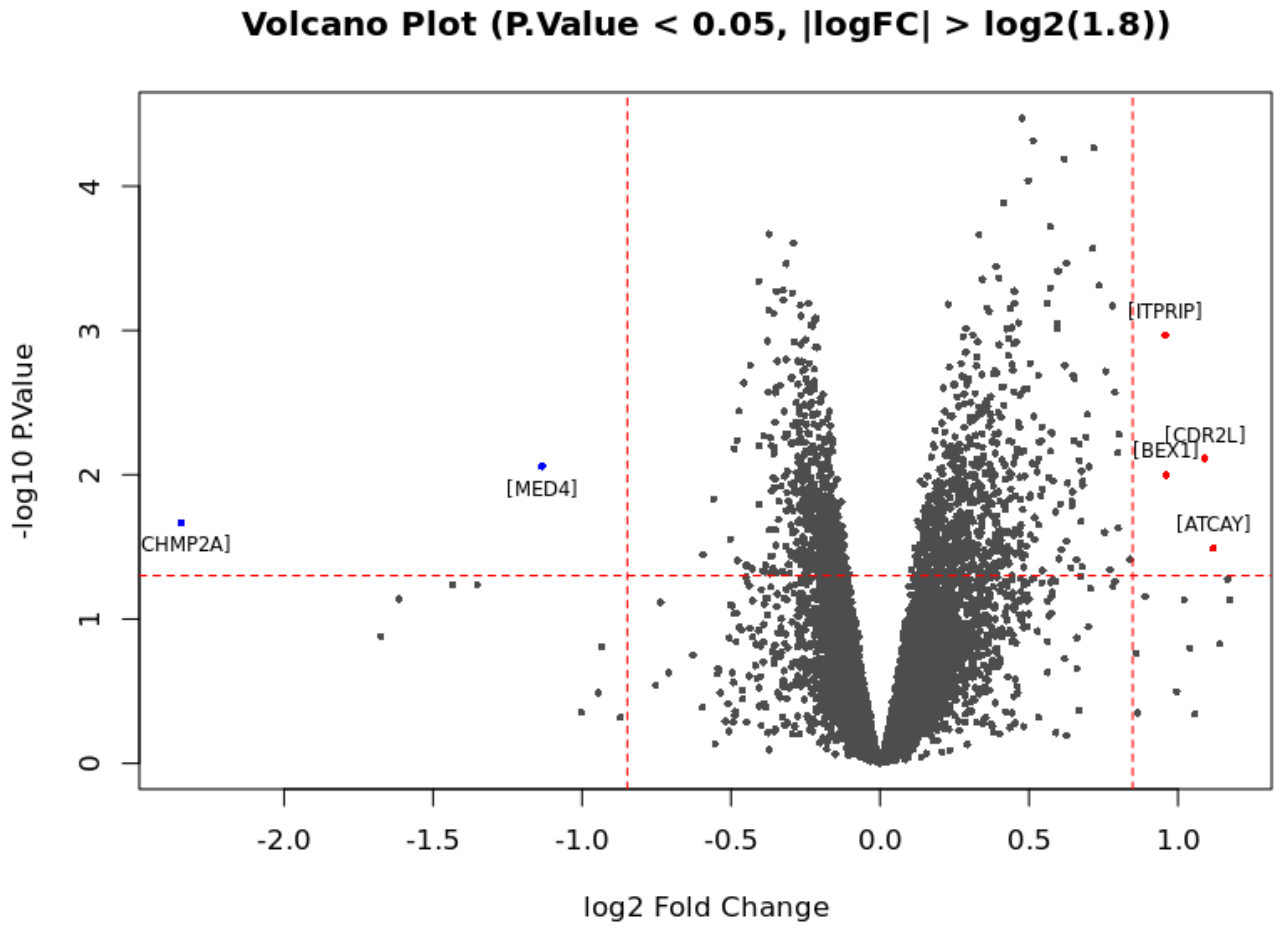

Median of Ratios (Log2) 2次比データ
Red Intensity (F635 Median - B635)
OMS #3
OMS #4
OMS #11
OMS #12
OMS #13
ALL #1
ALL #2
ALL #3
ALL #4
ALL #5
ALL #6
100 µl
Median of Ratios (log2)
<0.1
2.0
4.0
6.0
8.0
Red Intensity
0
10,000

・TMEM240

Differential Expressed Autoantibody Targets

Candidate antigens preferentially recognized in OMAS

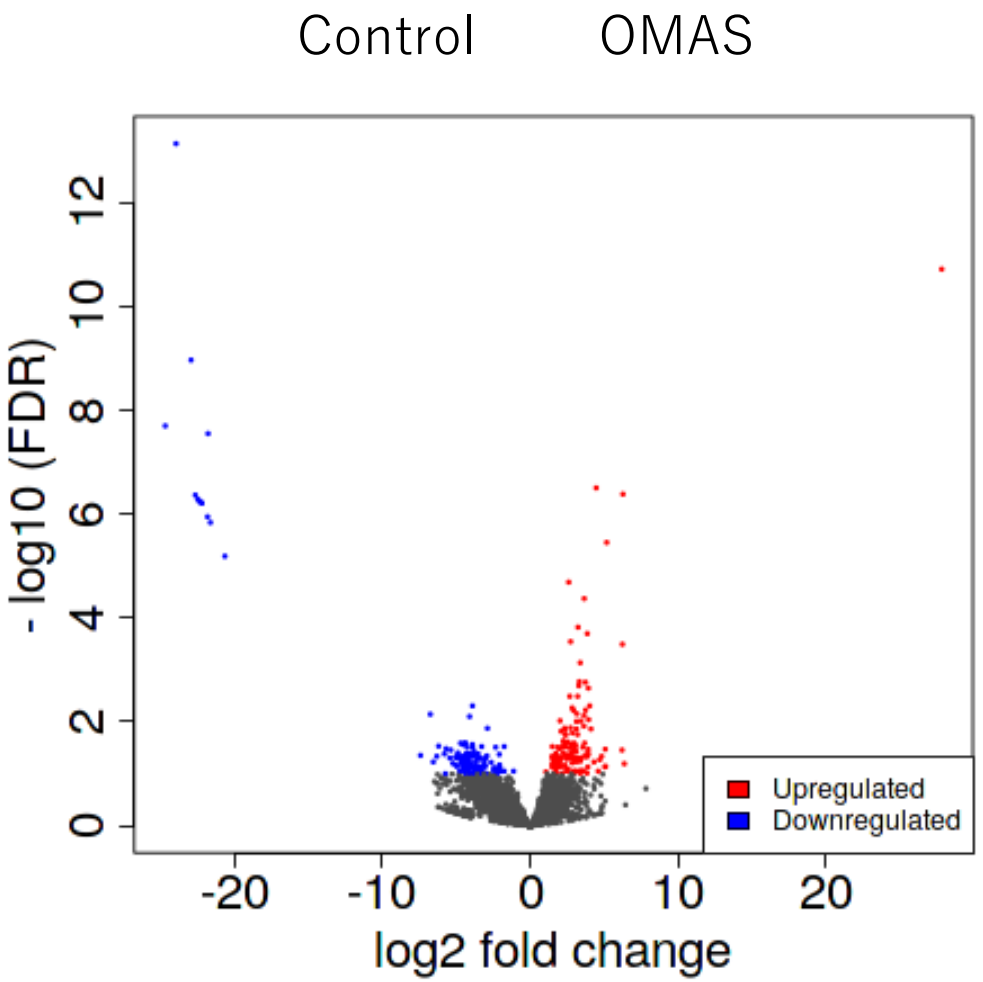


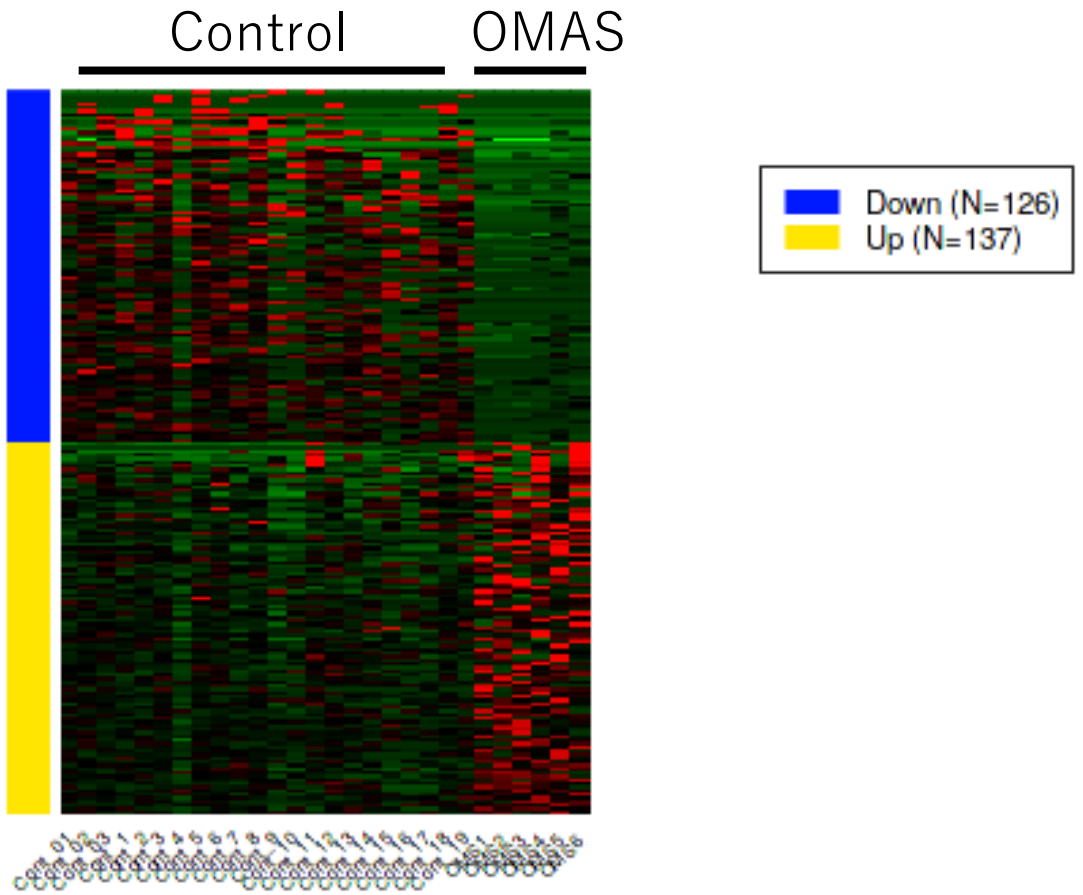
Up
- ATCAY
- CDR2L
- BEX1
- ITPRIP

Down
- MED4
- CHMP2A

✅ **Key Message:**
CDR2L, BEX1, TMEM240 identified as OMAS-enriched autoantigens.
Linked to cerebellar and neurodevelopmental function.

Results - PhiP-seq





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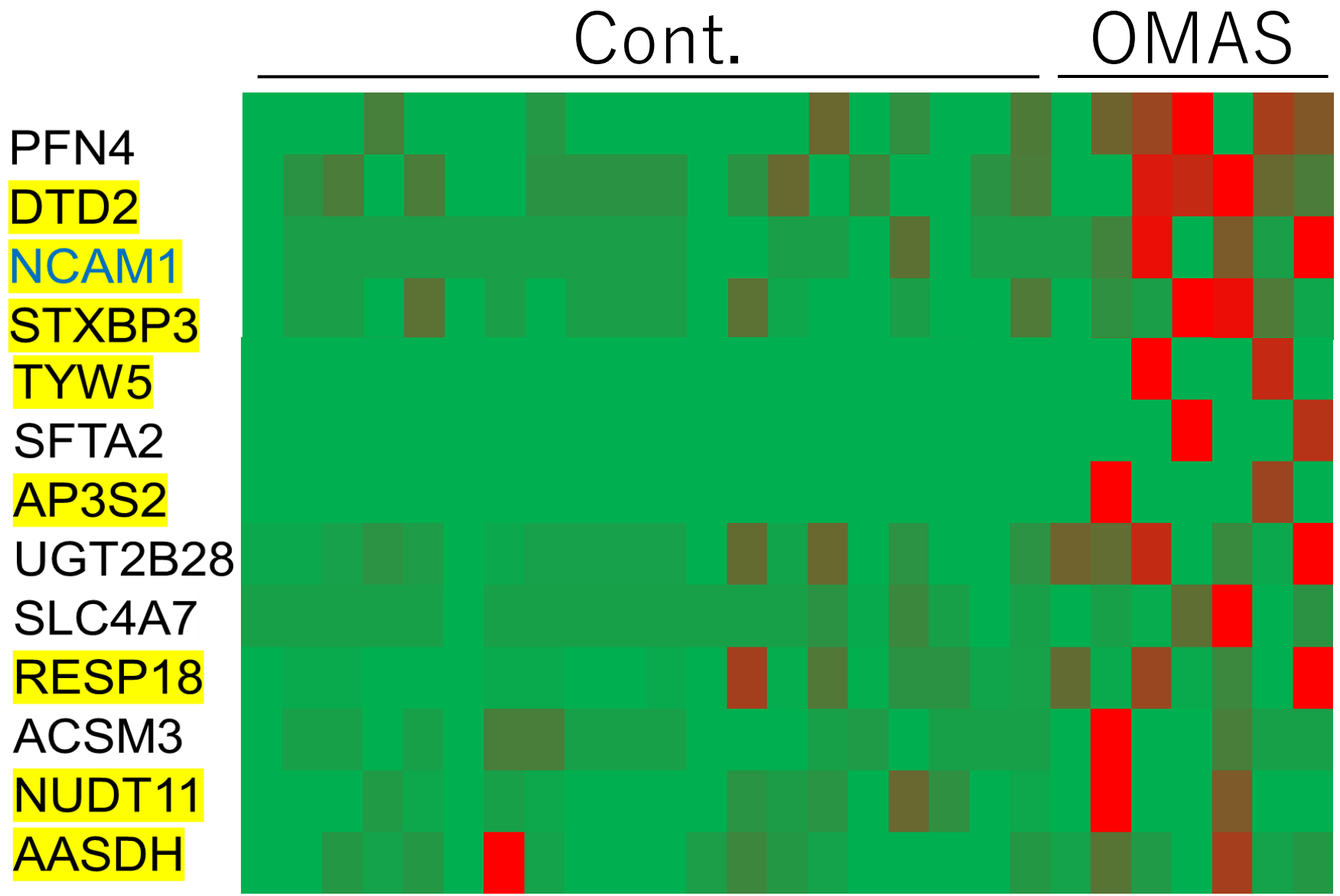
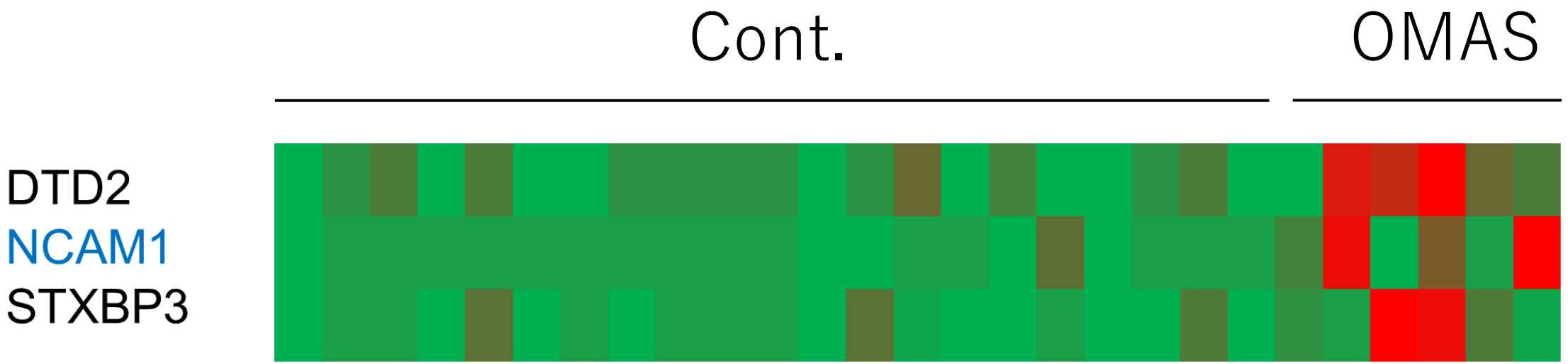
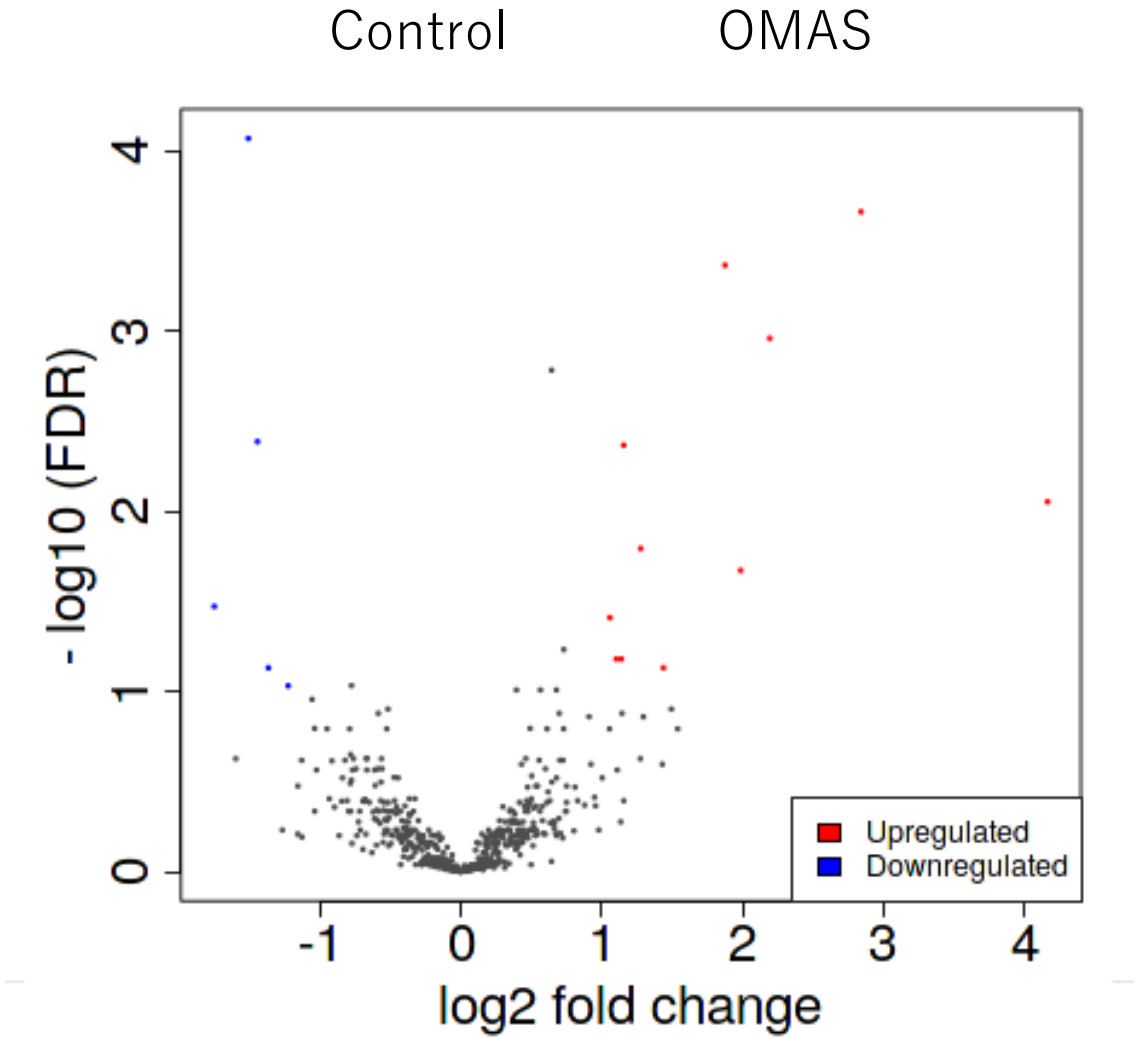


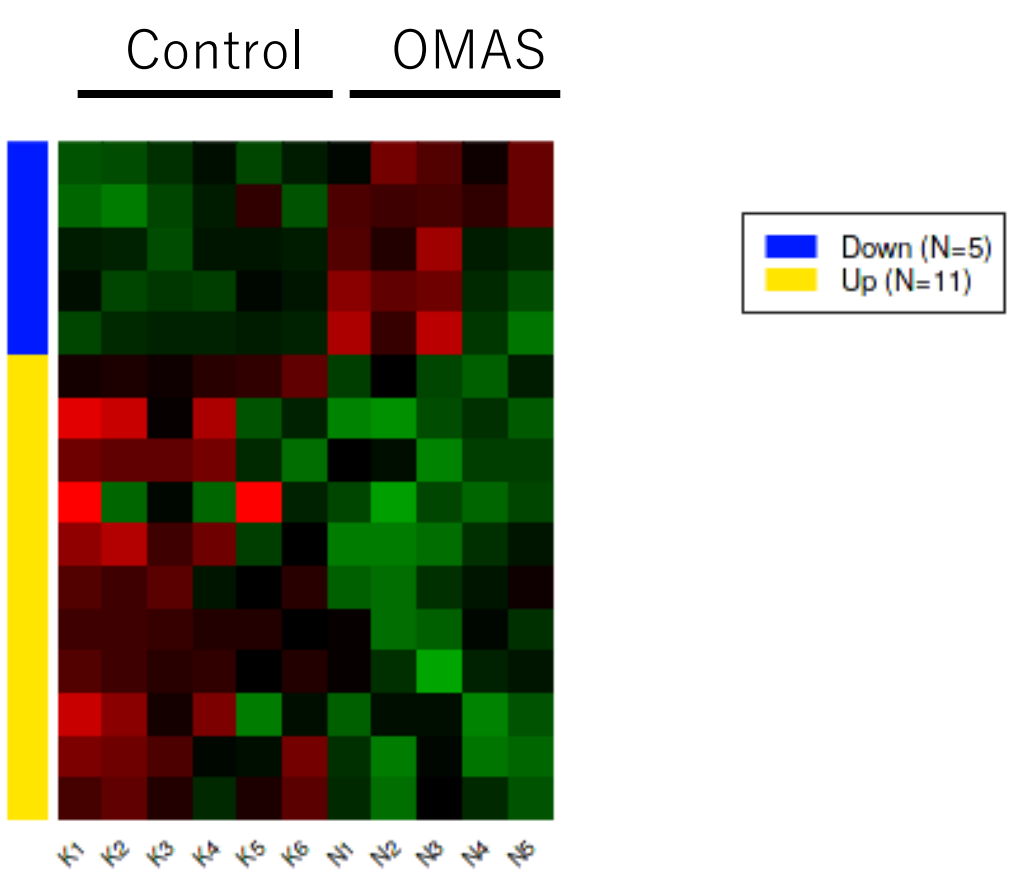
Expressed in the nervous system and observed in the majority of OMAS cases.



Results - LC-MS/MS

Peptides with differential expression identified by LC-MS/MS





Protein-coding genes found to be upregulated in OMAS

Genes
COL3A1
PEBP1
PKM
COL6A3
GDA
COL5A1
FOLR2
C1QC
LYZ
BASP1
GDI1
THBS1

Genes
CDH6
CDH8
CPVL
NUTF2
MIF
CYCS
TXNDC17
WFIKKN2
PFN2
C1QB
IGFBPL1
CHRDL1
CBLN1

The colored genes are those involved in neural function, with CBLN1 being particularly associated with the cerebellum.

Results Summary

Autoantibody Candidates

Gene Symbol	Gene Name	Function	Modalities
ATCAY	Ataxia, Cerebellar, Cayman Type	Involved in neuronal development; mutations linked to cerebellar ataxia.	Protein Microarray
CDR2L	Cerebellar Degeneration-Related Protein 2-Like	Related to neuronal function; potential autoantigen in paraneoplastic syndromes.	Protein Microarray
BEX1	Brain Expressed X-Linked 1	Regulates neurodevelopment and apoptosis; involved in cancer biology.	Protein Microarray
ITPRIP	Inositol 1,4,5-Trisphosphate Receptor Interacting Protein	Modulates IP3 receptor activity and intracellular calcium signaling.	Protein Microarray
TMEM240	Transmembrane Protein 240	Poorly characterized; mutations associated with spinocerebellar ataxia type 21.	Protein Microarray
DTD2	D-Tyr-tRNA Deacylase 2	Hydrolyzes mischarged D-tyrosyl-tRNA; maintains translational fidelity.	PhIP-seq
STXBP3	Syntaxin Binding Protein 3	Involved in vesicle trafficking and synaptic transmission.	PhIP-seq
NCAM1	Neural Cell Adhesion Molecule 1	Mediates cell-cell adhesion; essential for neural development and plasticity.	PhIP-seq
CBLN1	Cerebellin 1 Precursor	Functions in synapse formation and maintenance in the cerebellum.	LC-MS/MS

Conclusions & Future Directions

Overview and Key Finding

- 9 candidate autoantigens enriched in OMAS CSF
  - Cerebellar function: CDR2L, CBLN1, TMEM240, ATCAY
  - Neurodevelopment & synaptic plasticity: BEX1, NCAM1

- Suggests autoimmune targeting may drive ataxia & neuropsychiatric features

Cerebellar-associated Antigens and OMAS Ataxia

- CDR2L: structurally/functionally similar to known PCD antigen CDR2
- CBLN1: essential for cerebellar synapse formation
- TMEM240, ATCAY: linked to cerebellar ataxias

- Findings align with OMAS cerebellar symptoms
- Suggest shared immune mechanisms with cerebellar ataxias

Synaptic and Neurodevelopmental Molecules

- BEX1 & NCAM1: key roles in neuronal differentiation, survival, and synaptic connectivity
- Autoantibodies may disrupt development/plasticity → neuropsychiatric features

- Suggests broader immune impact beyond motor control
- Potential link to attention deficits, behavioral dysregulation in pediatric OMAS

Novel or Understudied Genes with Functional Implications

- **DTD2: Protein quality control (removes mischarged tRNAs)**

  → potential link to neurotoxicity

- **ITPRIP: Modulates IP3 receptor–mediated calcium signaling**

  → ties to neuronal excitability & immune pathways

◆ **Offer new mechanistic clues in OMAS despite limited neuroimmunology data**

Strengths and Limitations

Limitations

- Functional validation is ongoing
- Conventional assays may miss rare or low-affinity antibodies
- Pathogenic role and timing of antibodies still unclear

Strengths

- Multi-platform screening provides cross-validation
- Identified candidates show functional and clinical relevance

Next Steps

- Toward mechanistic studies and biomarker development

Take Home Message

- ✓ **OMAS lacks consistent and specific antibody markers**
- ✓ **We identified 9 novel candidate antigens via proteome-wide screening**
- ✓ **Several are linked to cerebellar or synaptic function**
- ✓ **Highlights the need for improved validation and deeper functional insight**
- ✓ **Toward better biomarkers and understanding of OMAS pathogenesis**