Serum biomarkers of progression by proteomic search in extreme MS phenotypes

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Disclosures

Johanna Oechtering received research support by the Swiss MS Society and served on advisory boards for Roche and Merck.

Eline Willemse, Pascal Benkert, Aleksandra Maceski and Sabine Schaedelin have nothing to disclose.

David Leppert is CMO of GeNeuro; he has received personal compensation for consulting and speaking, and travel reimbursement from Quanterix, Roche, Novartis, Orion, GeNeuro and Sanofi.

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Background and Aims

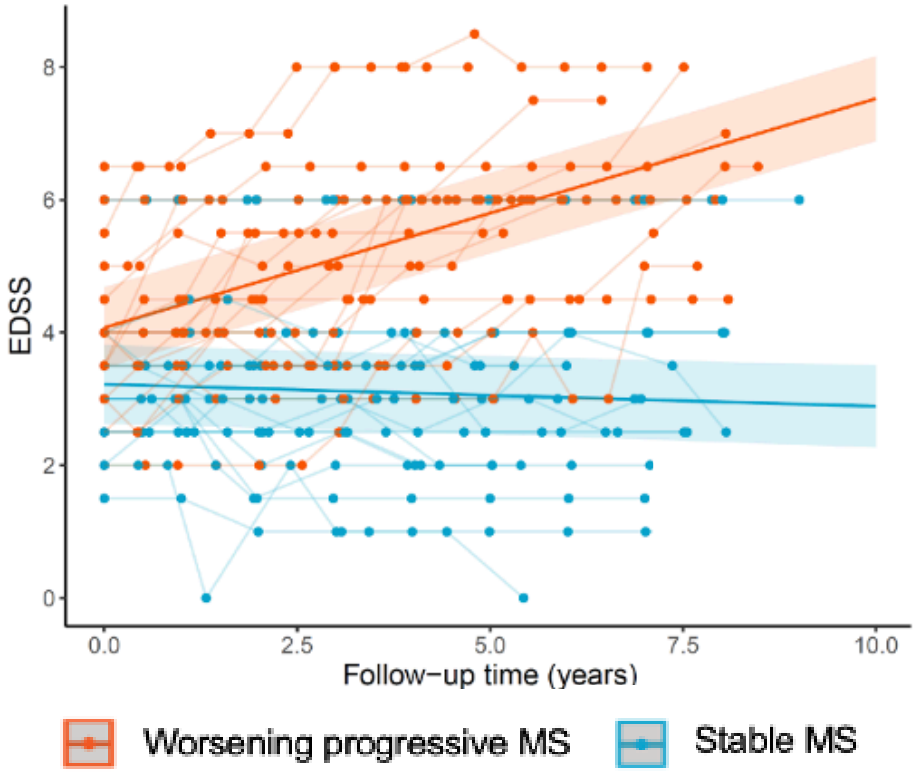
- MS: heterogeneous disease course, with accumulation of disability resulting from:
  1. **Acute** disease activity → **relapse-associated worsening (RAW)**
  2. **Chronic** deterioration of neurologic functions → **progression independent of relapse activity (PIRA)/progression**
- **Aims:**
  - Finding a) serum biomarkers reflecting disease progression and b) to ideally understand underlying pathomechanisms
  - To compare **worsening progressing patients (wpMS)** vs. **stable MS (stMS)** ("extreme phenotypes") prospectively followed by proteomic (Olink) analysis of longitudinal serum samples

Output of the Olink platform is either relative quantitation for large panels or absolute quantitation for focused assay panels with a smaller number of analytes

Patients: Worsening progressive MS vs stable MS

- **relapse-free** during entire follow-up with 6/12M sampling **followed prospectively in the SMSC** (centre Basel)
- wpMS with most pronounced EDSS progression (source pool: n=750); wPMS and stMS matched at baseline
- careful inspection by two neurologists (e.g. worsening ataxia in the upper limbs but stable EDSS excluded; patients with relevant comorbidities excluded)

	Worsening progressive MS	Stable MS	p-value
Samples	**184**	**169**	
Number of patients	18	19	
Baseline			
Age (at BL)	43.8	44.2	0.784
RRMS (at BL)	10	18	0.018
PMS (at BL)	8	1	
EDSS score (at BL)	**4.0**	**3.0**	0.065
Monoclonal antibody DMT (BL)	5 (27.8%)	5 (26.3)	
Follow-up data			
FU time (years)	**6.5**	**7.1**	0.395
EDSS score (last FU)	**6.0**	**2.5**	<0.001
Number of CDP events	**100% (34 events)**	**0%**	<0.001
Monoclonal antibody DMT (last FU)	14 (77.8)	3 (15.8)	<0.001



Proteomic Panels

- **Octave Custom Assay Panel**
  - 19 MS associated proteins including: NfL, GFAP, BAFF, CXCL13, MOG…
  - Assay was developed on the Olink platform:
  - ➢ Extensive analytical validation (Octave)
  - ➢ Clinically validated for MS disease activity assessments in multiple cohorts.[1,2]
  - QC: all samples were within LOQ ranges and inter-assay CV < 10%

- **Olink Target48**
  - Cytokine panel, 37/45 (82%) proteins passed QC
  - QC: more than 50% of sample values > LOD and inter-assay CV < 25%

- **Olink Explore 3072**
  - 2070/2924 (71%) proteins passed QC
  - QC: more than 50% above LOD and inter-assay CV < 35%

- **SIMOA assays for NfL and GFAP** (see presentation Meier et al: Friday at 12.35!)

1. Chitnis et al. Clinical Validation Study Results of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis. Presented at ECTRIMS 2021
2. Kuhle et al. Multivariate Proteomic MS Disease Activity Test Score Performance Evaluated in an Independent Focal Inflammation Cohort. Presented at ACTRIMS 2022

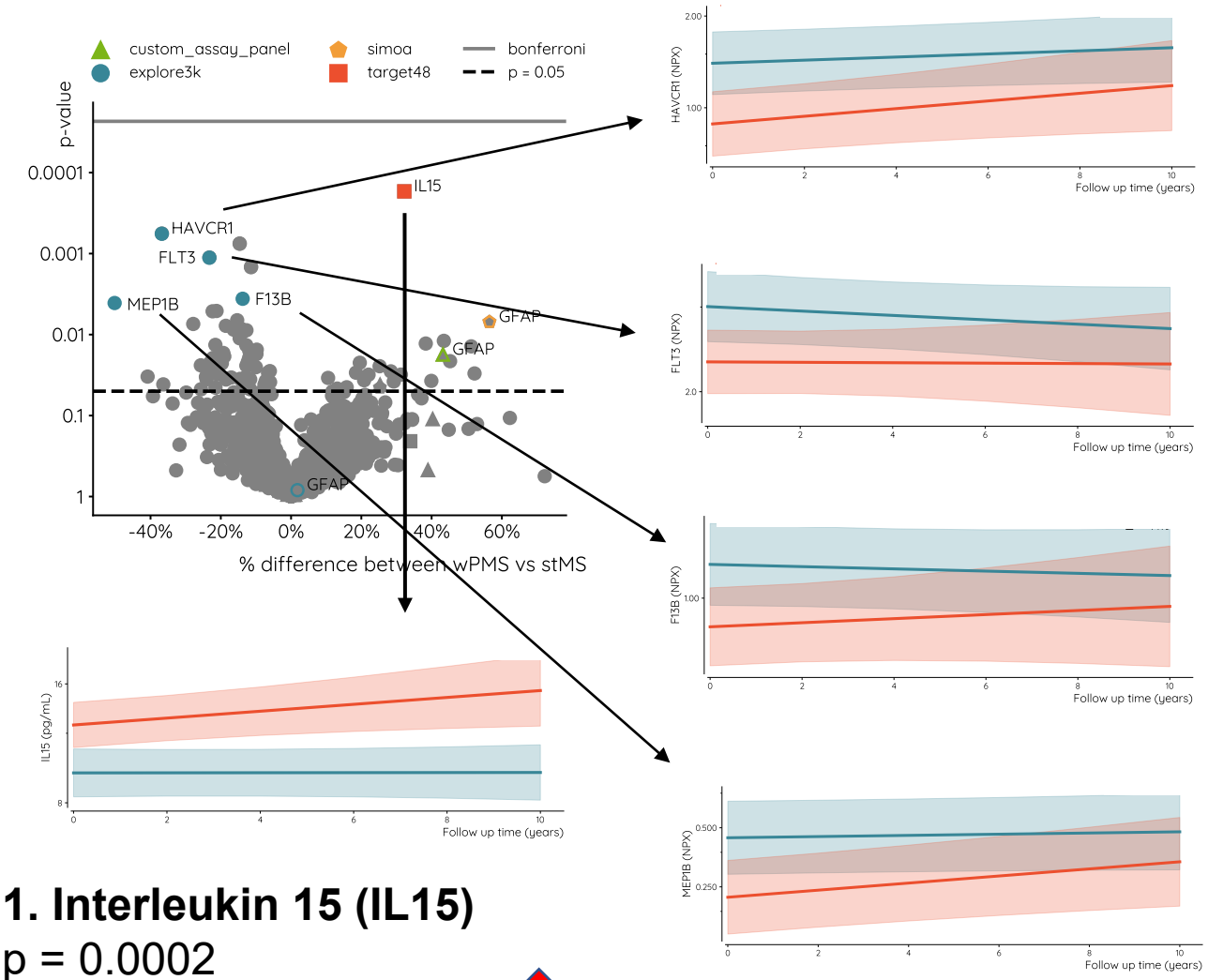
Statistical analysis

- Separate linear mixed effects models per analyte

- Dependent variables: each longitudinal serum (log)biomarker

- Independent covariables:
  - Baseline: age, sex, BMI, disease duration
  - At each sampling: DMT [Orals, Platform, Monoclonal, None], EDSS, follow-up time
  - Target variable 1: wPMS vs stMS
  - Target variable 2: wpMS vs stMS*follow-up time

Different levels between wPMS and stMS



1. Interleukin 15 (IL15)
p = 0.0002
32% higher in wPMS

2. Hep A virus cellular receptor 1 (HAVCR1)
p = 0.0006
37% lower in wPMS

3. Fms related receptor tyrosine kinase 3 (FLT3)
p = 0.0011
23% lower in wPMS

4. Coagulation factor XIII B chain (F13B)
p = 0.0036
14% lower in wPMS

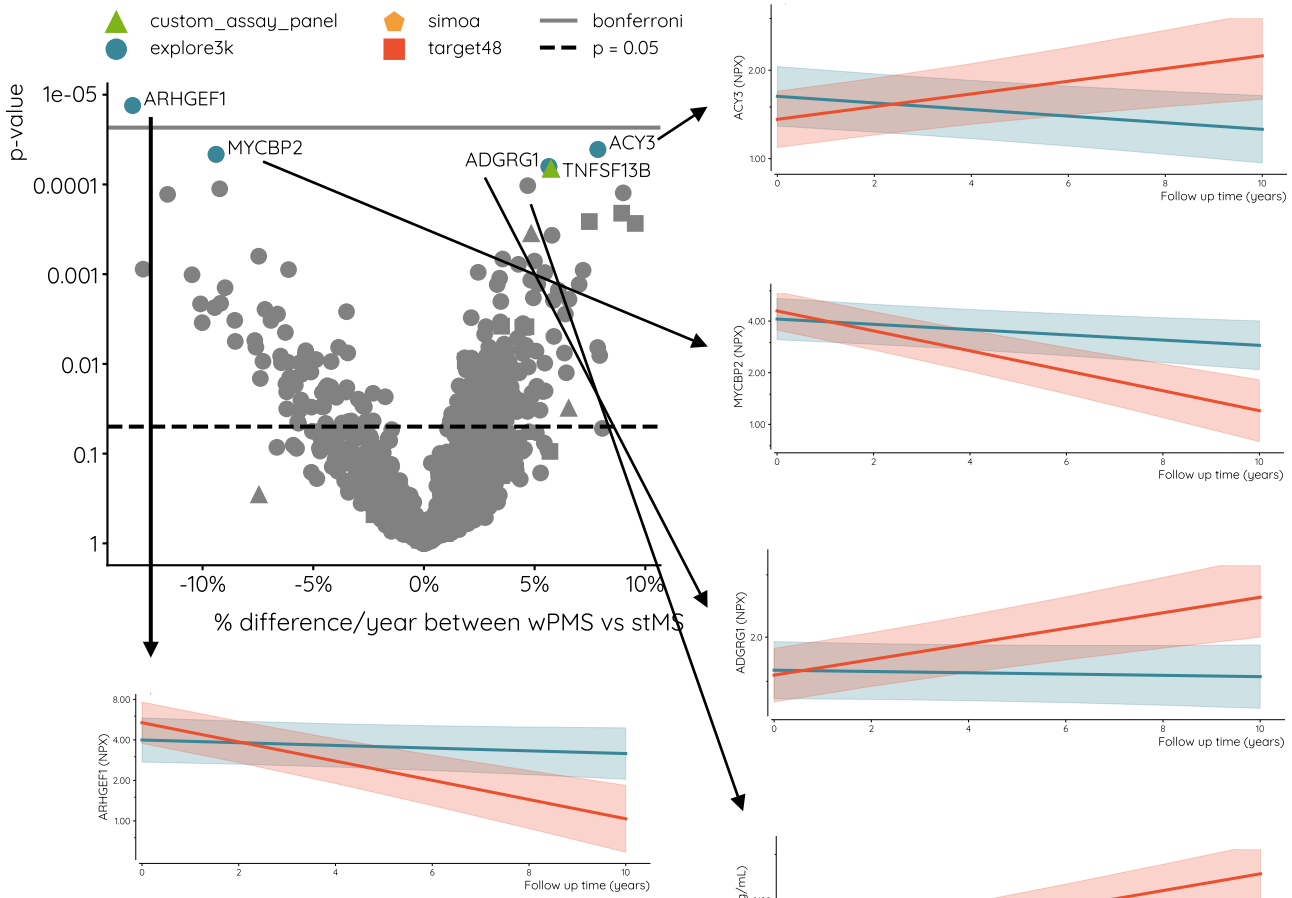
5. Meprin 1B (MEP1B)
p = 0.0041
50% lower in wPMS

Worsening progressive MS

Stable MS

These proteins have no significant time interaction effect

Different slopes between wPMS and stMS (interaction: progressor status*followup time)



1. Rho guanine nucleotide exchange factor 1 (ARHGEF1)
p = 0.00001
wPMS decrease by 13%/year

2. Aminoacylase 3 (ACY3)
p = 0.00004
wPMS increase by 8%/year

3. MYC binding protein 2 (MYCBP2)
p = 0.00005
wPMS decrease by 9%/year

4. Adhesion G protein-coupled receptor G1 (ADGRG1)
p = 0.00006
wPMS increase by 6%/year

5. B-cell Activating Factor (TNFSF13B) "BAFF"
p = 0.00007
wPMS increase by 6%/year

Worsening progressive MS

Stable MS

Summary & Future Steps

- Serum protein differences were found between wpMS and stMS in an exploratory analysis of 2000+ analytes
- Several novel proteins were detected (at higher significance level than serum GFAP)

Group levels			Slope		
Biomarker	**p-value**	**Worsening progressive MS**	**Biomarker**	**p-value**	**Worsening progressive MS**
1. IL15 (Interleukin 15)	0.0002	32% higher	**1. ARHGEF1 (Rho guanine nucleotide exchange factor 1)**	0.00001	13%/year decrease
2. HAVCR1 (Hep A virus cellular receptor 1)	0.0006	37% lower	**2. ACY3 (Aminoacylase 3)**	0.00004	8%/year increase
3. FLT3 (Fms related receptor tyrosine kinase 3)	0.0011	23% lower	**3. MYCBP2 (MYC binding protein 2)**	0.00005	9%/year decrease
4. F13B (Coagulation factor XIII B chain)	0.0036	14% lower	**4. ADGRG1 (Adhesion G protein-coupled receptor G1)**	0.00006	6%/year increase
5. MEP1B (Meprin 1B)	0.0041	50% lower	**5. TNFSF13B (B-cell Activating Factor ("BAFF"))**	0.00007	6%/year increase

Next steps:

- ➢ Understanding the resulting biological relevance of the candidates (ongoing)
- ➢ Investigate prognostic value for brain volume loss in MRI (ongoing)
- ➢ Replicate findings in independent cohort

➢See presentation by Meier et al.; 28.10.22 at 12.35: Serum glial fibrillary acidic protein compared with neurofilament light chain as biomarker for multiple sclerosis disease progression

Thank you for your attention!

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