ADNI Alzheimer's Disease Neuroimaging Initiative

ADNI CSF soluble triggering receptor expressed on myeloid cells 2 (sTREM2) and progranulin (PGRN)

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Contents

Summary

Numerous *TREM2* risk variants have been identified for Alzheimer disease (AD) [1-7]. *TREM2* encodes an innate immune receptor that is expressed exclusively on microglia in the central nervous system [8] and is associated with phagocytosis of cell debris and amyloid-beta [9]. TREM2 is shed by proteases of the ADAM family C-terminal to histidine 157 releasing soluble TREM2 (sTREM2) into the extracellular space [9-11]. *In vivo,* sTREM2 is released into the cerebrospinal fluid (CSF) where it can be quantified by ELISA and mass spectrometry [9,12-17]. Recent studies suggest that CSF sTREM2 levels are elevated early in AD [12-15].

Progranulin (PGRN) has also consistently been associated with AD. Genetic studies have shown that polymorphisms in *GRN* (particularly *rs5848*) increase the risk of AD [18-20]. PGRN is upregulated in microglia surrounding amyloid plaques in AD patients [21] and its deficiency affects amyloid deposition in mouse models of AD [22,23]. Moreover, PGRN deficiency enhance Tau deposition in human tau expressing mice [24] and the presence of the *GRN* polymorphism *rs5848* is associated with CSF tau levels in participants of the ADNI cohort [23].

Together, these studies suggest that CSF levels of sTREM2 and PGRN may be relevant for AD. We aimed to study the levels of CSF sTREM2 and PGRN through the AD *continuum* in the participants of the ADNI study. The following describes the methods of sTREM2 and PGRN measurements in CSF samples from ADNI.

Methods

WU ELISA (Piccio Group) CSF sTREM2 measurement

The soluble TREM2 ELISA assay performed at Washington University in St Louis was developed in-house. Briefly, an anti-human TREM-2 monoclonal antibody (R&D Systems # MAB1828, Clone 263602; 0.5 mg/mL) was used as a capture antibody (dilution 1:250) and coated overnight at 4°C on Maxisorp 96-well plates (Nalge Nunc International, Rochester, NY) in sodium bicarbonate coating buffer (0.015 M Na_2CO_3 + 0.035 M $NaHCO_3$, pH=9.6). After washing, wells were blocked for 4h at 37°C with PBS 10% fetal bovine serum (FBS). Freshly thawed CSF and recombinant human TREM-2 standard (Sino Biological 11084-H08H-50) were incubated in duplicate overnight at 4°C. For detection, a goat anti-human TREM-2 biotinylated polyclonal antibody (R&D Systems # BAF1828; 0.2 mg/mL) was diluted in assay buffer (PBS 10% FBS at 1:3000) and incubated for 1.25h at room temperature (RT) on orbital shaker. After washing, wells were incubated with horseradish-peroxidase labelled streptavidin (BD Biosciences, San Jose, CA; diluted 1:3000) for 1h at RT with orbital shaking. HRP visualization was performed with 3,3',5,5' tetramethylbenzidine (Sigma-Aldrich, St. Louis, MO) added to each well for 10min at RT in the dark. Color development was stopped by adding equal volume of 2.5 N H_2SO_4. Optical density of each well was determined at 450 nm. Washes between the different steps were done four times with PBS 0.05% Tween 20 (Sigma-Aldrich). An internal standard, consisting of a single batch of human CSF positive for sTREM-2 was run in all the assays. Samples were run in duplicate in each assay. Raw values are provided as pg/mL. Interassay variability was calculated from duplicate internal standards across 27 plates (CV = 24.01), intra-assay variability from duplicate measurements was < 9.5%.

MSD ELISA (Haass Group) CSF sTREM2 measurement

CSF sTREM2 measurements were done with the ELISA protocol previously established by the Haass' group with minor changes. The assay is based on the MSD platform and it is comprehensively described in previous publications [9,14,15]. The assay consists of a Streptavidin-coated 96-well plates (MSD Streptavidin Gold Plates, cat. no. L15SA-1); a biotinylated polyclonal goat IgG anti-human TREM2 antibody (R&D Systems, cat. no. BAF1828; 0.25 µg/mL, 25 µL/well) as capture antibody, which is raised against aminoacids 19-174 of human TREM2; a monoclonal mouse IgG anti-human TREM2 antibody (Santa Cruz Biotechnology, B-3, cat. no. sc373828; 1 µg/mL, 50 µL/well) as a detection antibody, which is raised against aminoacids 1-160 of human TREM2; and a SULFO-TAG-labeled goat polyclonal anti-mouse IgG secondary antibody (MSD, cat. no. R32AC; 0.5 µg/mL, 25 µL/well). All antibodies were diluted in 1% BSA and 0.05% Tween 20 in PBS (pH=7.4) buffer. Recombinant human TREM2 protein (Hölzel Diagnostika, cat. no. 11084-H08H), corresponding to the extracellular domain of human TREM2 (aminoacids 19-174) was used as a standard (62.5 to 8000 pg/mL). In brief, Streptavidin-coated 96-well plates were blocked overnight at 4°C in blocking buffer [3% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4); 300 µL/well]. The plates were next incubated with the capture antibody for 1 hour at RT. They were subsequently washed four times with washing buffer (200 µL/well; 0.05% Tween 20 in PBS). Thereafter, the recombinant human TREM2 protein (standard curve), the blanks, and the CSF samples and the internal standards IS (duplicates; dilution factor: 4) were diluted in assay buffer [0.25% BSA and 0.05% Tween 20 in PBS (pH=7.4)] supplemented with protease inhibitors (Sigma; Cat. # P8340) and incubated (50 µL/well) for 2 hours at RT. This dilution was previously selected because it showed the best recovery and linearity performance [9]. Plates were again washed four times with washing buffer before incubation for 1 hour at RT with detection antibody. After four additional washing steps, plates were incubated with SULFO-tag conjugated secondary antibody for 1 hour in the dark at RT. Last, plates were washed four times with wash buffer followed by two washing steps in PBS. The electrochemical signal was developed by adding 150 µL/well MSD Read buffer T (Cat. # R-92TC) and the light emission measured using the MESO QuickPlex SQ 120. Raw values are provided as pg/mL.

MSD ELISA (Haass Group) CSF Progranulin (PGRN) measurement

CSF PGRN was measured by an ELISA protocol previously established by the Haass' group using the MSD Platform [25]. The ELISA consists of a Streptavidin-coated 96-well plates (MSD Streptavidin Gold Plates, cat. no. L15SA-1); a biotinylated polyclonal goat anti-human PGRN capture antibody (BAF2420, R & D Systems; 0.2 µg/mL, 25 µL/well); a monoclonal mouse anti-human PGRN detection antibody (MAB2420, R & D Systems; 0.25 µg/mL, 25 µL/well); and a SULFO-TAG-labeled goat polyclonal anti-mouse IgG secondary antibody (MSD, cat. no. R32AC; 0.5 µg/mL, 25 µL/well). All antibodies were diluted in 0.5% BSA and 0.05% Tween 20 in PBS (pH=7.4) buffer. Recombinant human PGRN protein (His Tag PGRN - Sino Biological cat. no. 10826-H08H) was used as a standard (15.6 to 2000 pg/mL). In brief, Streptavidin-coated 96-well plates were blocked overnight at 4°C in blocking buffer [0.5% bovine serum albumin (BSA) and 0.05% Tween 20 in PBS (pH 7.4)]. The plates were next incubated with the capture antibody for 1 hour at RT. They were subsequently washed four times with washing buffer (0.05% Tween 20 in PBS) and incubated for 2 hours at RT with the CSF and the internal standard (IS) samples diluted 1:2.5 in assay buffer [0.25% BSA and 0.05% Tween 20 in PBS

(pH7.4)] or the recombinant human PGRN protein also diluted in assay sample. Plates were again washed four times with washing buffer before incubation for 1 hour at RT with the detector antibody. After four additional washing steps, plates were incubated with the secondary antibody for 1 hour in the dark. Last, plates were washed four times with wash buffer followed by two washing steps in PBS. The electrochemical signal was developed by adding MSD Read buffer T (cat. no. R-92TC) and the light emission measured using the MESO QuickPlex SQ 120. Raw values are provided as pg/mL.

Common considerations for the MSD ELISA measurements (sTREM2 and PGRN)

All CSF samples were distributed randomly across plates and measured in duplicate. The mean intraplate coefficient of variation (CV) was 3.1% (sTREM2 ELISA) and 2.2% (PGRN ELISA); all duplicate measures had a CV < 15%. All the antibodies and plates are commercially available and, for this experiment, they were from a single lot in order to exclude variability between batches. Four internal standards (IS) were run on each ELISA plate to account for interplate variability. All IS used in this study consisted of pooled CSF samples from diagnostic clinical routine leftovers from the Ludwig-Maximilians-Universität (LMU) München department of Neurology (Munich, Germany). All patients gave their written consent and the study was approved by the local IRB. The mean absolute values, interday and interplate CV for each IS are depicted in **Table 1** and **Table 2** for sTREM2 and PGRN respectively. The experiment was performed on four different days between the 26th Nov 2017 and 6th Dec 2017 by operators experienced in running these assays and blinded to the clinical information.

Table 1. Interday and interplate coefficient of variability (CV) in the MSD sTREM2 assay.
The table shows the mean values of CSF sTREM2 (pg/mL) in each day and the interday and interplate CV.

	IS1	**IS2**	**IS3**	**IS4**	
1st day (mean 15 plates)	2881	3703	2828	11618	
2nd day (mean 15 plates)	2416	3047	2876	11972	
3rd day (mean 15 plates)	2304	2978	2767	11407	
4th day (mean 9 plates)	2164	2719	2160	9950	
*Mean (*mean 54 plates)	*2472*	*3155*	*2713*	*11380*	
Interday CV (%) (between 4 days)	12.7	13.5	12.6	7.9	**Mean interday CV** 11.7
Interplate CV (%) (between 54 plates)	11.4	12.2	10.5	7.1	**Mean interplate CV** 10.3

Table 2. Interday and interplate coefficient of variability (CV) in the PGRN assay.
The table shows the mean values of CSF PGRN (pg/mL) in each day and the interday and interplate CV.

	IS1	**IS2**	**IS3**	**IS4**	
1st day (mean 15 plates)	1546	1676	1595	1938	
2nd day (mean 15 plates)	1454	1613	1556	1784	
3rd day (mean 15 plates)	1583	1690	1610	1940	
4th day (mean 9 plates)	1511	1604	1525	1882	
Mean (mean 54 plates)	*1524*	*1650*	*1576*	*1886*	
Interday CV (%) (between 4 days)	3.59	2.63	2.47	3.89	**Mean interday CV** 3.15
Interplate CV (%) (between 54 plates)	4.26	3.79	3.40	5.40	**Mean interplate CV** 4.21

Statistical methods

Measured CSF levels were corrected by plate-specific correction factors; absolute and corrected values are provided with CVs for each sample.
Both the WU ELISA (Piccio group) and MSD ELISA (Haass group) corrected their raw measurements to take into account the interplate variability, following different methodologies described below.

WU ELISA (Piccio Group)

The WU ELISA CSF sTREM2 values were corrected based on the IS loaded on all plates and a subset of duplicate samples that were run on multiple plates. For each plate (x), a set of three samples (s1, s2, s3) were selected to run in another assay plate (y). The IS and duplicate sample measurements (s1, s2, s3) from plate x and plate y were compared and a line (xy) was fit to the four points. The slope and intercept from the xy line was applied as a correction factor for each sample on plate x. The corrected values are provided as the variable WU_sTREM2corrected. Each plate was run in a three-day assay between the 25th May 2017 and 29th Sep 2017 by the same operator who is experienced with the assay and blinded to the clinical information.

MSD ELISA (Haass Group)

In order to harmonize the different analytical run and account for interplate variation, the MSD ELISA CSF sTREM2 and CSF PGRN concentrations were corrected based on the measurements of the four IS that were loaded on all plates. The concentration of each IS in an individual plate (plate x) was expressed as a percentage of the mean concentration across all plates, as follows:

a1 (%) in plate x = [(concentration of IS1 in plate x) / (mean concentration of IS1 in all plates)] ×100
a2 (%) in plate x = [(concentration of IS2 in plate x) / (mean concentration of IS2 in all plates)] ×100
a3 (%) in plate x = [(concentration of IS3 in plate x) / (mean concentration of IS3 in all plates)] ×100
a4 (%) in plate x = [(concentration of IS4 in plate x) / (mean concentration of IS4 in all plates)] ×100

The mean of the percentages (**Ax**) for all the IS (**a1, a2, a3, a4**) in plate x was calculated and the following correction factor was computed for each individual plate:

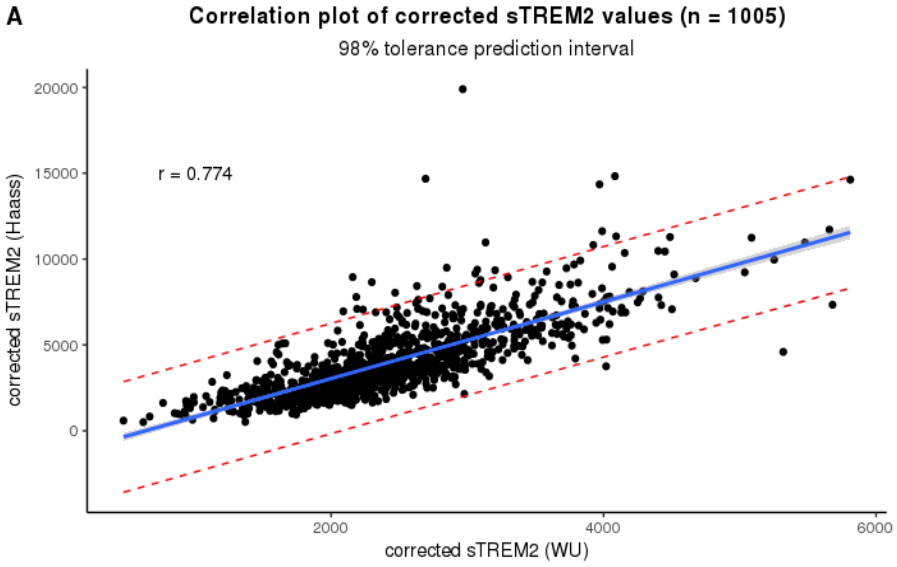
Correction factor for plate x = 100 / **Ax**

The raw values were multiplied by the correction factor of the corresponding plate; the corrected values are provided as variables 'MSD_sTREM2corrected' and 'MSD_PGRNcorrected'.

Comparison of the CSF sTREM2 measurements of the two centers

The strong correlation of corrected sTREM2 values between the two groups is shown in **Figure 1. Table 3** lists the individuals that fell outside the 98% prediction tolerance level.

Figure 1 There was a strong correlation between the corrected values of sTREM2 between the two groups (**A**. Pearson's r = 0.774, 95% CI = 0.747 - 0.797, P = 7.0×10^{-201}). There were 25 individuals that fell outside the prediction intervals (shown as red dotted lines in the correlation plots, based on 98% tolerance) when testing the corrected sTREM2 values. These samples are flagged in the dataset under the variable TREM2outlier, and we recommend excluding them in analyses. We removed these individuals and retested the correlation. After removing the individuals outside the 98% prediction tolerance level the correlation between the corrected sTREM2 values increased (**B**. Pearson's r = 0.834, 95% CI = 0.813 - 0.852, P = 2.2×10^{-254}).



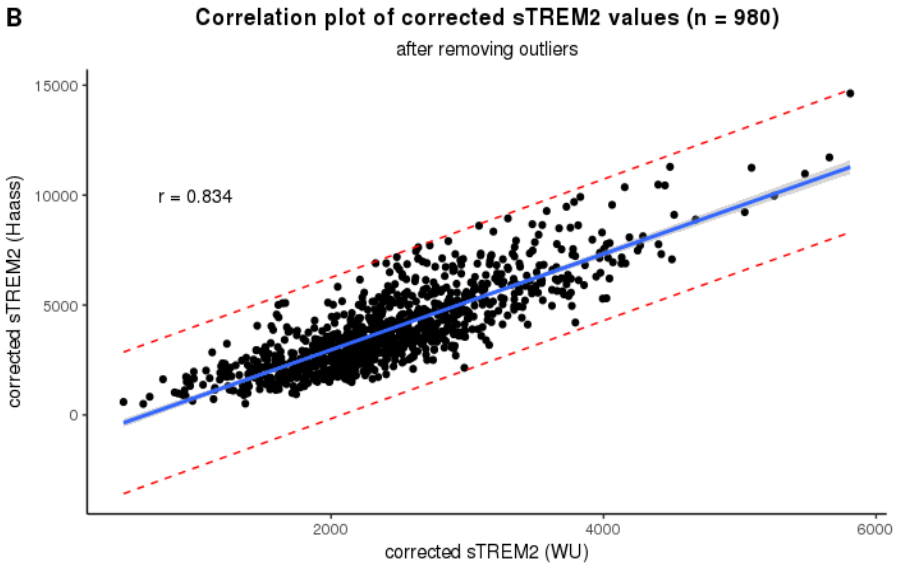


Table 3. Outlying samples based on 98% tolerance prediction interval from correlation between corrected sTREM2 values (N = 25)

ID	MSD sTREM2 corrected	WU sTREM2 corrected
AA80BDD1-05	14351.886	3970.184
BA801CSZ-11	7340.830	5680.439
BA801M2P-12	7098.323	2190.104
CA8017P6-12	6958.254	2089.496
CA801MK4-13	8655.054	2298.833
CA807QDJ-04	3754.182	3097.434
CA8097Z3-06	14628.082	4018.464
DA8016G2-14	8951.230	2158.526
DA807754-05	14831.550	4083.309
EA804J4L-04	9349.995	3203.507
FA801674-17	2145.877	2693.386
FA801R57-14	7183.071	3057.745
FA801STM-14	14679.310	2630.037
FA801W7Z-12	9156.392	2816.230
FA807DKB-06	7713.857	3072.800
GA8015HX-13	4206.288	5318.779
GA801BGY-18	9382.916	3924.427
GA801CVC-13	4596.670	2966.385
GA801F0Q-11	10822.539	2469.922
GA801L75-12	19899.014	2848.632
GA801YHN-14	8041.528	2184.960
GA8023M7-11	9497.511	4091.922
GA807HDC-05	7791.797	3133.730
JA807F5R-05	10971.150	3989.677
KA801K6K-10	11626.103	2234.026

Dataset Information

This methods document applies to the following dataset(s) available from the ADNI repository:

Dataset Name	Date Submitted
ADNI_CSF_sTREM2_PGRN.txt	22 February 2018

References

1 Benitez, B. A. *et al.* TREM2 is associated with the risk of Alzheimer's disease in Spanish population. *Neurobiol Aging* 34, 1711 e1715-1717, doi:10.1016/j.neurobiolaging.2012.12.018 (2013).

2 Guerreiro, R. *et al.* TREM2 Variants in Alzheimer's Disease. *New Engl J Med* 368, 117-127, doi:10.1056/NEJMoa1211851 (2013).

3 Jiang, T. *et al.* TREM2 p.H157Y Variant and the Risk of Alzheimer's Disease: A Meta-Analysis Involving 14,510 Subjects. *Curr Neurovasc Res* 13, 318-320 (2016).

4 Jiang, T. *et al.* A rare coding variant in TREM2 increases risk for Alzheimer's disease in Han Chinese. *Neurobiol Aging* 42, 217 e211-213, doi:10.1016/j.neurobiolaging.2016.02.023 (2016).

5 Jin, S. C. *et al.* Coding variants in TREM2 increase risk for Alzheimer's disease. *Hum Mol Genet* 23, 5838-5846, doi:10.1093/hmg/ddu277 (2014).

6 Jin, S. C. *et al.* TREM2 is associated with increased risk for Alzheimer's disease in African Americans. *Mol Neurodegener* 10, 19, doi:10.1186/s13024-015-0016-9 (2015).

7 Jonsson, T. *et al.* Variant of TREM2 Associated with the Risk of Alzheimer's Disease. *New Engl J Med* 368, 107-116, doi:10.1056/NEJMoa1211103 (2013).

8 Colonna, M. & Wang, Y. TREM2 variants: new keys to decipher Alzheimer disease pathogenesis. *Nat Rev Neurosci* 17, 201-207, doi:10.1038/nrn.2016.7 (2016).

9 Kleinberger, G. *et al.* TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. *Sci Transl Med* 6, 243ra286, doi:10.1126/scitranslmed.3009093 (2014).

10 Schlepckow, K. *et al.* An Alzheimer-associated TREM2 variant occurs at the ADAM cleavage site and affects shedding and phagocytic function. *EMBO Mol Med* 9, 1356-1365, doi:10.15252/emmm.201707672 (2017).

11 Wunderlich, P. *et al.* Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and gamma-secretase-dependent intramembranous cleavage. *J Biol Chem* 288, 33027-33036, doi:10.1074/jbc.M113.517540 (2013).

12 Heslegrave, A. *et al.* Increased cerebrospinal fluid soluble TREM2 concentration in Alzheimer's disease. *Mol Neurodegener* 11, 3, doi:10.1186/s13024-016-0071-x (2016).

13 Piccio, L. *et al.* Cerebrospinal fluid soluble TREM2 is higher in Alzheimer disease and associated with mutation status. *Acta Neuropathol* 131, 925-933, doi:10.1007/s00401-016-1533-5 (2016).

14 Suarez-Calvet, M. *et al.* Early changes in CSF sTREM2 in dominantly inherited Alzheimer's disease occur after amyloid deposition and neuronal injury. *Sci Transl Med* 8, 369ra178, doi:10.1126/scitranslmed.aag1767 (2016).

15 Suarez-Calvet, M. *et al.* sTREM2 cerebrospinal fluid levels are a potential biomarker for microglia activity in early-stage Alzheimer's disease and associate with neuronal injury markers. *EMBO Mol Med* 8, 466-476, doi:10.15252/emmm.201506123 (2016).

16 Piccio, L. *et al.* Identification of soluble TREM-2 in the cerebrospinal fluid and its association with multiple sclerosis and CNS inflammation. *Brain* 131, 3081-3091, doi:10.1093/brain/awn217 (2008).

17 Henjum, K. *et al.* Cerebrospinal fluid soluble TREM2 in aging and Alzheimer's disease. *Alzheimers Res Ther* 8, 17, doi:10.1186/s13195-016-0182-1 (2016).

18 Perry, D. C. *et al.* Progranulin mutations as risk factors for Alzheimer disease. *JAMA Neurol* 70, 774-778, doi:10.1001/2013.jamaneurol.393 (2013).

19 Brouwers, N. *et al.* Genetic variability in progranulin contributes to risk for clinically diagnosed Alzheimer disease. *Neurology* 71, 656-664, doi:10.1212/01.wnl.0000319688.89790.7a (2008).

20 Brouwers, N. *et al.* Alzheimer and Parkinson diagnoses in progranulin null mutation carriers in an extended founder family. *Arch Neurol* 64, 1436-1446, doi:10.1001/archneur.64.10.1436 (2007).

21 Pereson, S. *et al.* Progranulin expression correlates with dense-core amyloid plaque burden in Alzheimer disease mouse models. *J Pathol* 219, 173-181, doi:10.1002/path.2580 (2009).

22 Minami, S. S. *et al.* Progranulin protects against amyloid beta deposition and toxicity in Alzheimer's disease mouse models. *Nat Med* 20, 1157-1164, doi:10.1038/nm.3672 (2014).

23 Takahashi, H. *et al.* Opposing effects of progranulin deficiency on amyloid and tau pathologies via microglial TYROBP network. *Acta Neuropathol* 133, 785-807, doi:10.1007/s00401-017-1668-z (2017).

24 Hosokawa, M. *et al.* Progranulin reduction is associated with increased tau phosphorylation in P301L tau transgenic mice. *J Neuropathol Exp Neurol* 74, 158-165, doi:10.1097/NEN.0000000000000158 (2015).

25 Capell, A. *et al.* Rescue of progranulin deficiency associated with frontotemporal lobar degeneration by alkalizing reagents and inhibition of vacuolar ATPase. *J Neurosci* 31, 1885-1894, doi:10.1523/JNEUROSCI.5757-10.2011 (2011).

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