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Polyglutamine-expanded ataxin-3: a target engagement marker for Spinocerebellar ataxia type 3 in peripheral blood

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Short report

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Abstract

Spinocerebellar ataxia type 3 is a rare neurodegenerative disease, caused by a CAG repeat expansion leading to polyglutamine elongation in the ataxin-3 protein. While no curative therapy is yet available, preclinical gene silencing approaches to reduce polyglutamine-toxicity demonstrate promising results. In view of upcoming clinical trials, quantitative and easily accessible molecular markers are of critical importance as pharmacodynamic and particularly as target engagement markers. We developed a novel ultrasensitive immunoassay to measure specifically polyQ-expanded ataxin-3 in plasma and cerebrospinal fluid. Statistical analyses revealed a correlation with clinical parameters and a stability of polyglutamine-expanded ataxin-3 during conversion from the pre-ataxic to the ataxic phase.

Background

Spinocerebellar ataxias (SCAs) are a heterogeneous group of dominantly inherited, progressive diseases. The worldwide most common among them is Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), a multisystem disorder characterized by degeneration of spinocerebellar tracts, dentate nucleus, brainstem nuclei, and basal ganglia. It is caused by an unstable expansion of a polyglutamine (polyQ) encoding CAG repeat in the *ATXN3* gene resulting in the expression of an abnormally elongated ataxin-3 protein that is considered to be the major cause of neurodegeneration in SCA3. Currently, there is no treatment for SCA3, but new approaches aiming to silence the disease gene are close to clinical trials [1].

To demonstrate target engagement in such trials, availability of an ultrasensitive quantitative immunoassay to measure concentration of polyQ-expanded ataxin-3 in body fluids is mandatory. Recently, an immunoassay that detected polyQ-expanded ataxin-3 in body fluids including cerebrospinal fluid (CSF) and discriminated between SCA3 patients and healthy controls was developed [2]. In addition, an assay based on time-resolved fluorescence energy transfer (TR-FRET) was reported that was capable of measuring ataxin-3 concentrations in peripheral blood mononuclear cells (PBMCs) but failed to detect ataxin-3 in body fluids [3].

Here, we report a novel single molecule counting (SMC™) ataxin-3 immunoassay to specifically measure polyQ-expanded ataxin-3 in plasma and CSF. Using this assay, we found strong correlations between plasma polyQ-expanded ataxin-3 concentrations and clinical parameters. In a longitudinal study, we observe a high stability of polyQ-expanded ataxin-3 in pre-ataxic and ataxic mutation carriers.

Methods

Ethics and Consent to participate

The study was approved by the Local Committees of all participating centers. Informed and written consent was obtained from all study participants at enrolment.

Study participants

Blood and CSF samples were obtained from participants of the European Spinocerebellar ataxia type-3/Machado-Joseph disease Initiative (ESMI) cohort. To test whether the newly developed assay discriminates between mutation carriers and healthy controls, we measured plasma ataxin-3 concentrations in an exploratory cohort of 9 healthy controls and 10 ataxic SCA3 mutations carriers (= exploratory cohort). For validation and more in-depth analysis, we performed plasma measurements in a cohort of 15 healthy controls, 11 pre-ataxic mutation carriers, and 45 ataxic SCA3 mutation carriers (= validation cohort). For a subset of 17 mutation carriers (4 pre-ataxic and 13 ataxic mutation carriers) parallel measurements in CSF were performed. CSF samples from 18 age and sex-matched healthy controls were provided by the biobank of the Donor Institute for Brain, Nijmegen. Longitudinal plasma assessments were available for 37 participants of the validation cohort (4 healthy controls, 5 pre-ataxic, 28 ataxic mutation carriers), with follow-up plasma samples collected one year after the first visit (table 1).

Biosamples were collected under highly standardized protocols at all participating centers. Briefly, blood collection was performed by standard venipuncture. Plasma was separated using cell preparation tubes (CPT) from BD Biosciences. Directly after blood collection, CPT tubes were inverted 8 to 10 times and centrifuged within 2 hours for 30 min at 1,700 RCF. Plasma was collected from the tubes without disturbing the mononuclear cell layer. After processing, all plasma samples were immediately frozen at -80ºC.

CSF collection was performed by lumbar puncture. Samples were processed within 2 hours. First, samples were centrifuged at 1,100 RCF for 10 minutes, afterwards divided in aliquots and immediately frozen at -80ºC.

Age at ataxia onset (AAO) in ataxic mutation carriers was defined as the reported age at onset of gait difficulties. In the pre-ataxic mutation carriers, predicted AAO was calculated on the basis of age at recruitment and CAG repeat length [4]. The scale for the assessment and rating of ataxia (SARA) was used to assess the severity of ataxia [5]. Mutation carriers were classified either as pre-ataxic (SARA ≤ 3 points) or ataxic (score ≥ 3). The inventory of non-ataxia signs (INAS) count was used to assess the degree of non-cerebellar involvement [6]. The CAG repeat length of the expanded allele was determined using PCR-based fragment length analysis (CEQ8000 capillary sequencer, Beckman Coulter).

Anti-ataxin-3 antibodies

For Single Molecule Counting (SMC^{TM}) assay development, two monoclonal antibodies were used: (I) mouse antibody MW1 specifically binding to disease-associated polyQ stretches of HTT, whose generation has been previously described [7] and (II) the mouse anti-spinocerebellar ataxia type 3 antibody, clone 1H9, with the epitope mapped to amino acids E214-L233 of the human ataxin-3 protein, obtained from Millipore (MAB5360). Graphical illustration of epitope binding sites is shown in Figure 1A.

Recombinant purified proteins

Recombinant human ataxin-3 proteins with a polyQ lengths of 15Q and 62Q, respectively, were purified as described elsewhere [8]. Recombinant proteins were pre-diluted to a concentration of 10 µg/mL in artificial CSF (aCSF: 300 mM NaCl; 6 mM KCl; 2.8 mM $CaCl_2$ x $2H_2O$; 1.6 mM $MgCl_2$ x 6 H_2O; 1.6 mM Na_2HPO_4 x $2H_2O$; 0.4 mM NaH_2PO_4 x H_2O), supplemented with 10% Tween-20 and cOmplete protease inhibitor (Roche) and stored at -80 °C. On assay day, recombinant proteins were further diluted to finally 400 pg/mL as a starting concentration for the preparation of standard dilutions. Afterwards, standard was generated by a 1:4 fold serial dilution series for usage as a calibrator standard in the assay.

Sample preparation for SMC™ assay

Plasma and CSF from SCA3 mutation carriers and healthy controls were diluted in aCSF (supplemented with 10% Tween-20 and cOmplete protease inhibitor(PI)) in a ratio of 1:4 (plasma) or 1:5 (CSF) before assay application.

Ataxin-3 SMC™ assay

The assay employs the Single Molecule Counting (SMC™) technology that affords ultra-sensitivity and a wide linear detection [9]. Specific detection of polyQ-expanded ataxin-3 in this assay occurs by bead-based immunoreaction with antibody combination 1H9 and MW1: the 1H9 antibody was labeled and coated to magnetic particles (MP) according to SMC™Capture Labeling Kit manufacturer's instructions by coupling 25 µg of labeled 1H9 antibody per mg of magnetic particles (MP). MP-1H9 suspension was stored at 10 mg/mL in Coated-bead-buffer at 2-8° C. The MW1 antibody was labeled as detection antibody with fluorophore Alexa according to SMC™ Detection Antibody Labeling Kit manufacturer's instructions.

In the assay, capture and detection antibodies were used at final concentrations of 50 ng/well or 5 ng/well, respectively. 50 µL/well of Blocking buffer containing 6% BSA, 0.8% Triton X-100, 750 mM NaCl, and cOmplete protease inhibitor; and 150 µL/well of standard or human sample (CSF prediluted 1:5; plasma prediluted 1:4 in aCSF+1%Tween+PI) were added to a 96-conical assay plate (Axygen). MP-1H9 antibody (100 µL/well) diluted to 1:1000 with Assay/Discovery Buffer (Merck Millipore) was added, and the plate was sealed and incubated under shaking (400 rpm) at room temperature for 1 hour. All washing steps described here were performed with the HydroFlex™ microplate washer (Tecan). The plate was first placed on a magnetic stand and then washed once with 200 µl 1x SMC™ Wash Buffer with ProClin (Merck Millipore). Washing buffer was removed, and 20 µL/well of 5 ng/µl diluted labeled MW1-antibody (1:4000 diluted in Assay/Discovery Buffer and filtered) was added to the plate. The plate was sealed and incubated under shaking (700 rpm) at room temperature for 1 hour. After 4 washes with 200 µl 1x SMC™ Wash Buffer with ProClin and aspiration, 11 µl of elution buffer (acidic glycine solution, 0.1 M, pH 2.7) were added to the plate, and the plate was incubated under shaking (700 rpm) for 6 minutes. The plate was placed on the magnetic stand for 2 min, 11 µl of buffer D (neutralization buffer Tris, 1 M, pH 9)

were added. 20 µl of eluate were transferred to an Aurora plate, 384-well, F-bottom, transparent (Merck Millipore), the plate was shaken for 2 min and then spun down (2 min, 400g) to eliminate foaming and bubble formation. The analysis plate was subsequently measured with the SMCxPro^{TM} platform. All assays were performed by operators blinded to genotype and clinical state of the participant.

The assay´s limit of detection (LOD) was defined as the concentration corresponding to the signal 2.5 SDs above the background (zero calibrator), lower limit of quantification (LLoQ) as the concentration corresponding to the signal 10 SDs above the background, and upper limit of quantification (ULoQ) as the concentration corresponding to the signal 2.5 SDs above the highest calibration standard on the calibration curve.

Statistical analysis

Analyte distribution were tested for normality using Shapiro-Wilk test. Non-parametric group analyses were performed using two-sided Mann-Whitney test with Bonferroni correction for multiple comparison. For linear correlation we used partial Spearman correlation. Data were adjusted for age and CAG-repeat length. CAG repeat adjustment was included as MW1 antibody can bind 16 or more polyQ repeats with increasing intensity for longer repeats.[7] Both, age and expanded CAG repeat length were identified as covariable in our dataset and as independent modifier of SCA3 disease severity [10, 11]. Multivariate analyses revealed that age at onset, disease duration and sex did not correlate with our datasets. Therefore, all statistical analyses were only corrected for age and expanded CAG repeat length. Correlation analyses of plasma and CSF ataxin-3 levels were performed on z-transformed datasets. Effect sizes (r) were calculated as Cohen´s d. Intraclass variation (ICC) was performed to analyze the stability of the analyte ataxin-3 at the longitudinal study design. To test the quality of classification of the cohort into healthy controls and mutation carriers, we calculated receiver operating characteristic (ROC) curves and determined the area under the curve (AUC).

Data are presented as median and interquartile range [IQR]. Statistical significance is demonstrated by *P*-values ($\leq$ 0.05 (*), $\leq$ 0.01 (**), $\leq$ 0.001 (***).

All statistical and graphical evaluations were performed with GraphPad prism 8.0. For linear regression, we used IBM SPSS Statistics version 27.

Results

SMC^{TM} immunoassay quantifies polyQ-expanded ataxin-3 with high specificity and sensitivity

SMC^{TM} immunoassay validation using human recombinant ataxin-3 with normal (15Q) or elongated (62Q) polyQ-length demonstrated a high specificity for polyQ-expanded ataxin-3 over normal ataxin-3 (Fig. 1B). Determination of LOD (0.07 pg/mL), LLoQ (0.252 pg/mL) and ULoQ (427.695 pg/mL) showed a low picomolar detection threshold and broad dynamic range (Fig.1C). Spiking in recombinant polyQ-expanded ataxin-3 protein in human plasma from control subjects revealed a good polyQ-expanded

ataxin-3 signal recovering rate (99.7%, data not shown) and demonstrated the capacity of the assay to measure reference analytes in real human biomaterials. In the exploratory cohort, polyQ-expanded ataxin-3 plasma concentrations in SCA3 mutation carriers ranged from 18 to 87 pg/mL (59.63 pg/mL [48.93-74.37]), whereas polyQ-expanded ataxin-3 was not detectable in healthy controls (1.15 pg/mL [0.27-6.0] (Fig. 1D).

PolyQ-expanded ataxin-3 is quantifiable in mutation carriers

In plasma and CSF samples from the validation cohort, expanded ataxin-3 was quantifiable (plasma: 72.25 pg/mL [52.34-100.3], P<0.0001, r=0.84; CSF: 5.48 pg/mL [4.85-6.77], *P*<0.0001, *r*=0.869) in SCA3 mutation carriers, whereas concentrations were below the detection threshold in healthy controls (plasma: 0.14 pg/mL [0.1-0.4]; CSF: 0.11 pg/mL [0.08-0.15]; Fig. 1E, F). PolyQ-expanded ataxin-3 concentrations were higher in plasma samples of ataxic than of pre-ataxic mutation carriers (83.30 pg/mL [55.38-106.6] vs. 53.80 pg/mL [40.28-63.37]; *P*=0.009, *r*=0.50) (Fig. 1E). CSF concentrations of polyQ-expanded ataxin-3 did not differ between ataxic and pre-ataxic mutation carriers. Correlation analysis failed to reveal an association between CSF and plasma ataxin-3 levels (*R*=0.210*, P=0.45*). There were no sex-specific differences of polyQ-expanded ataxin3 protein levels (Fig. 1D, F). Level of polyQ-expanded ataxin-3 in plasma and CSF perfectly discriminated between mutation carriers and healthy controls with AUC values of 1.00 in the ROC analysis. Plasma polyQ-expanded ataxin-3 showed a good discrimination ability comparing pre-ataxic and ataxic mutation carriers (AUC = 0.78), but failed for CSF (AUC = 0.58) (additional file 1 A-D).

Plasma polyQ-expanded ataxin3 level correlate with clinical parameters and remain stable over a period of one year

Plasma polyQ-expanded ataxin-3 were positively correlated with SARA (*R*=0.5026, *P*=0.020) (Fig. 2B), whereas it was negatively correlated with AAO (*R*=-0.6041, *P*<0.001) (Fig. 2A): these results however were not replicable in CSF samples. No correlation were seen for INAS (*R*=0.3478, *P*=0.295) (Fig. 2C). Cross-sectional analyses of plasma polyQ-expanded ataxin-3 protein level relative to time to predicted/ reported years from ataxia onset revealed a positive linear correlation (*R*=0.3747; *P*=0.005), demonstrating that the polyQ-expanded ataxin-3 protein levels are higher at a later stage of disease (Fig. 2D). Longitudinal measurements of 33mutation carriers over 1 year period revealed a high stability of polyQ-expanded ataxin-3 in SCA3 mutation carriers including three mutation carriers who converted from the pre-ataxic to the ataxic stage (ICC = 0.848 [0.693-0.925]) (Fig. 2E).

Discussion

To demonstrate target engagement in future trials that aim at silencing the SCA3 disease gene, availability of an ultrasensitive, quantitative immunoassay to measure concentration of polyQ-expanded ataxin-3 in body fluids is mandatory [1].

Here we report, on the successful generation and validation of a new ultrasensitive and quantitative immunoassay to specifically measure low concentrations in pg/mL range of polyQ-expanded ataxin-3 in human biofluids like blood plasma and CSF. Our SMC™ immunoassay perfectly discriminated between healthy controls and SCA3 mutation carriers, yielding discrimination values similar to a recent published ataxin-3-specific mesoscale assay [2]. In addition, our assay allowed for a discrimination between pre-ataxic and ataxic mutation carriers in plasma. Moreover, polyQ-expanded ataxin-3 levels correlated with clinical feature of the disease, namely SARA, suggesting that our assay might indeed quantify polyQ-expanded ataxin-3 in a way that reflects severity of ataxia of SCA3. These findings extend our pilot study where we used a TR-FRET-based technique to quantify ataxin-3 in PBMCs [3], by demonstrating that polyQ-expanded ataxin-3 protein serves as a biomarker even in plasma and CSF.

We did not find an association of polyQ-expanded ataxin-3 levels in plasma and CSF. Therefore, the pool of polyQ-expanded ataxin-3 in CSF differs from that in peripheral blood and blood cells, as reported earlier [2]. This notion is further supported by the observation that levels of polyQ-expanded ataxin-3 were > 10 times higher in plasma as in CSF.

So far, neither our SMC™ nor the mesoscale-based immunoassay (comparison of both immunoassays demonstrated in additional Table 1) showed any association between CSF polyQ-expanded ataxin-3 protein levels and clinical features of the disease. This could be explained by the assumption that CSF polyQ-expanded ataxin-3 represents a – rather disease-stage independent – trait biomarker of the disease, as demonstrated for the respective key proteins of other neurodegenerative diseases, e.g. C9orf72 dipeptides in ALS/FTD [12]. Alternatively, it might be due to the sample size and composition of our SCA3 subject group. The total number of CSF samples in general was low. This even more applied to CSF from patients in the later stages of the disease, in whom we found higher plasma concentrations of polyQ-expanded ataxin-3. The lack of correlation of CSF polyQ-expanded ataxin-3 concentrations and disease severity, however, does not call into question the potential usefulness of CSF polyQ-expanded ataxin-3 might serve as a target engagement biomarker in future interventional trial that investigate gene silencing approaches.

Our longitudinal analyses revealed a high stability of plasma derived polyQ-expanded ataxin-3 protein levels over a period of one year. If confirmed in a larger cohort and longer time period, the high degrees of stability of this biomarker would allow to reduce sample sizes in trials that include polyQ-expanded ataxin-3 as one of the endpoints.

In conclusion, our novel SMC™ immunoassay is able to quantify polyQ-expanded ataxin-3 in plasma and CSF, while polyQ-expanded ataxin-3 levels in plasma correlating with disease severity. First longitudinal analyses demonstrated a high stability of polyQ-expanded ataxin-3 over a period of one year. Therefore, this immunoassay has the potential to support clinical development of therapeutic drugs in SCA3, allowing to determine levels of polyQ-expanded ataxin-3 as a target engagement biomarker in human biofluids.

Abbreviations

AAO: Age at onset; CPT: cell preparation tube; CSF: cerebrospinal fluid; ESMI: European Spinocerebellar ataxia type-3/Machado-Joseph disease Initiative; INAS: Inventory of non-ataxia signs; MJD: Machado-Joseph disease; PBMCs: peripheral blood mononuclear cells; polyQ: poly-glutamine; SARA: scale for the assessment and rating of ataxia; SCA: Spinocerebellar ataxia; SMC^{TM}: Single Molecule Counting; TR-FRET: time-resolved fluorescence energy transfer

Declarations

Ethical approval and Consent to participate

The study was approved by the Local Committees of all participating centers. Informed and written consent was obtained from all study participants at enrolment.

Consent for publication:

All authors read and approved the final manuscript.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article and its additional material.

Competing interests:

BvdW is supported by research grants from Radboud university medical centre, ZonMW, Hersenstichting, uniQure, and Gossweiler Foundation; has served on a scientific advisory board with uniQure; and collaborates within a research consortium with Vico Therapeutics.

PG received funding from Reata Pharmacautical, Vico Therapeutic, Triplet Pharmaceutical. She has served on advisory bord of Triplet, Vico and Reata.

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Authors Contribution:

JH-S design and conceptualization of the study, acquisition of data, analysis of data, and drafting and revision of the manuscript. KK and JP development of the assay, acquisition of data, drafting the manuscript, revision of the manuscript. JF subject recruitment, analysis of data, revision of the manuscript. MMS, HH, HJ, KR, HGM, MR, JvG, JI, KMS, JdeV, MMV, PG, LPA, ML, BvdW and TK subject recruitment, acquisition of data, revision of the manuscript. LS and MS subject recruitment, analysis of data, revision of the manuscript. OHR designed and conceptualization of the study, drafting and revision of the manuscript. Additional ESMI study group members are listed in additional table 2 and participated in subject recruitment, acquisition of data and revision of the manuscript.

All authors read and approved the final manuscript.

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Authors information:

KK and JP are members of the company Evotec, which generated and validated the SMC^{TM} ataxin-3 immunoassay described in this study. Both were blinded for genotypes and clinical data of all participants at each time and did not influenced any project hypothesis or outcome measures.

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Tables

Table 1: Demographic and clinical characteristics of participants in the analyzed ESMI cohort

	controls	pre-ataxic SCA3	ataxic SCA3
Exploratory cohort			
Sample size (female)	9 (44%)	ND	10 (50%)
Age (years)	45 (39.5 – 58)	ND	53 (36.7 – 56.5)
Reported Age at onset, AAO (years)	NA	NA	47 (27.5 – 49.5)
SARA score	0.5 (0 - 1)	ND	10.5 (7.5 – 15.5)
INAS count	1 (1 - 2)	ND	3.5 (1.5 - 5)
Repeat count (long allele)	NA	ND	67.5 (64.7 - 69)
Validation cohort			
Sample size (female)	15 (46.6%)	11 (54.5%)	45 (51.1%)
Age (years)	43.5 (21.1 - 68.2)	35 (21 - 42)	49.8 (40.2 - 61)
Predicted/ Reported AAO (years)	NA	44 (36.5 – 49.5)	39.1 (13 - 68)
SARA score	0.2 (0 - 2)	0.7 (0 - 1.5)	15.3 (4 - 34.5)
INAS count	0.2 (0 - 2)	2.4 (1 - 4)	5.7 (2 - 12)
Repeat count (long allele)	NA	68 (62 - 71)	69 (58 - 73)
CSF cohort			
Sample size (female)	18 (55.5%)	5 (80%)	12 (66.6%)
Age (years)	45 (34 – 52)	37 (35.5 – 41)	47.2 (39.2 – 57.7)
Reported AAO (years)	NA	NA	42 (29 -45)
SARA score	0 (0 – 1.2)	1 (1 – 1.5)	10.2 (8 – 17.5)
INAS count	0 (0 – 0.5)	3 (1 – 4)	5 (2.5 – 7.5)
Repeat count (long allele)	NA	69.5 (69 – 70.7)	69.5 (66.5 – 70.7)
Longitudinal cohort			
Sample size (female)	4 (50%)	5 (80%)	28 (53.5%)
Participants which changed disease status	0	3	2

Age (years) at baseline	44.5 (23.5 – 65.9)	36 (26 – 38.5)	51.5 (43.7 – 60.2)
Reported AAO (years): BL and FUP	NA	BL: NA FUP: 31 (26 – 37)	41 (33 – 47.5)
SARA score: BL and FUP	BL: 0.5 (0 – 1.7) FUP: 0 (0 – 1.5)	BL: 1 (0.5 – 1) FUP: 3.5 (1 – 6.2)	BL: 12.5 (6.5 – 17.5) FUP: 11.7 (9 – 19.5)
INAS count: BL and FUP	BL: 0.5 (0 – 1.7) FUP: 0 (0 – 0)	BL: 2 (1.2 – 3.5) FUP: 2 (0.4 – 4)	BL: 5 (2.2 – 7.7) FUP: 5 (4 – 8)
Repeat count (long allele)	NA	69 (65.5 – 70.5)	70 (65.5 – 71.7)
time span between BL and FUP	14.1 (11.5 - 15.2)	13.2 (12.0 - 14.4)	13.0 (11.3 - 17.7)

Data are reported as median and interquartile range.

NA, not applicable. ND, not determined. BL, baseline. FUP, follow up visit.

Figures

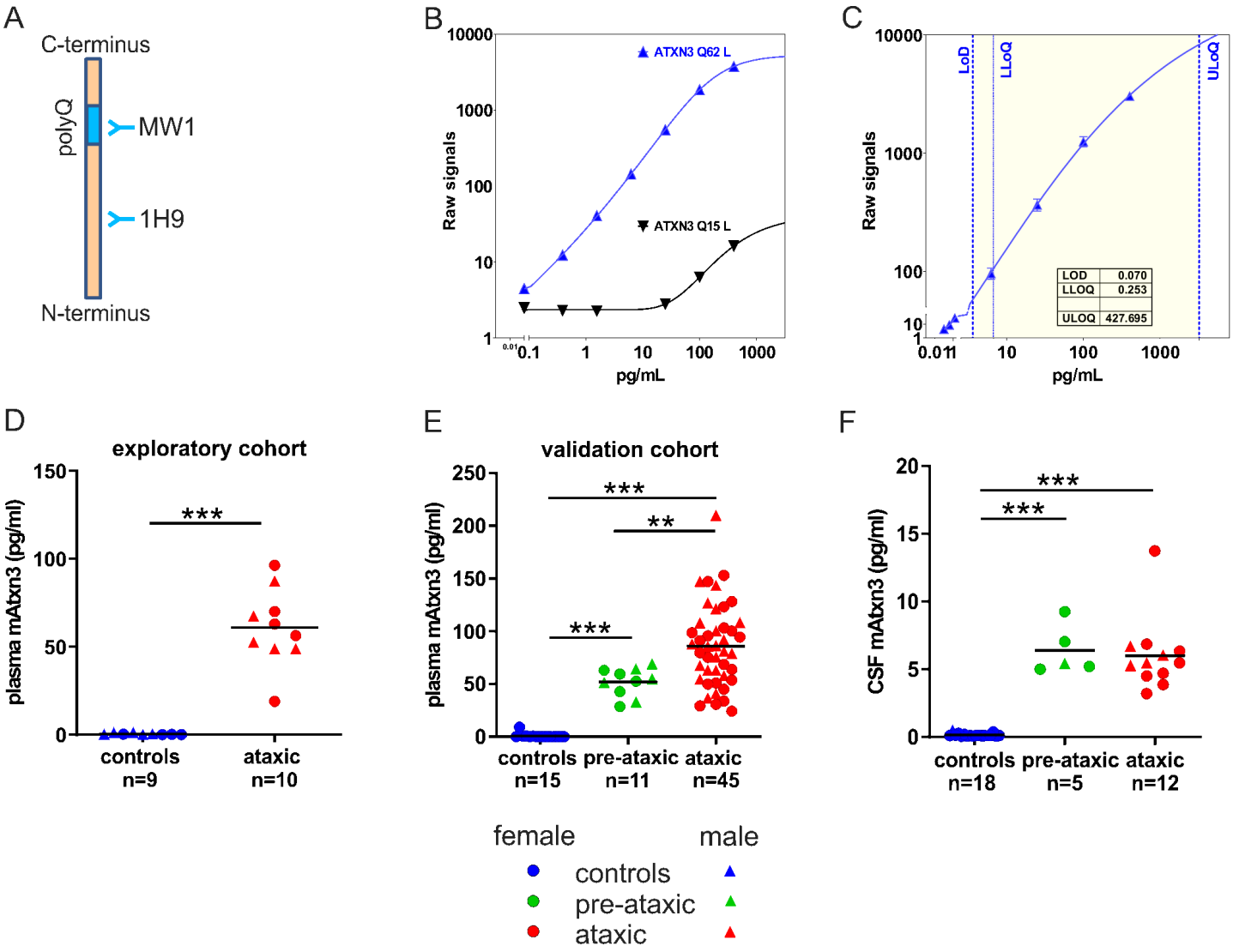

Figure 1

PolyQ-expanded Ataxin-3 is detectable across different fluid from SCA3 mutation carriers, including strong effect sizes in plasma. (A) Epitope binding sites of MW1 and 1H9 antibody within the ataxin-3 protein. (B) Detection of purified recombinant ataxin-3 proteins with the SMCTMimmunoassay showed specificity for polyQ-expanded ataxin-3 (ATXN3 62Q) over normal human ataxin-3 protein (ATXN3 15Q). (C) Determination of LOD (0.07 pg/mL), LLoQ (0.253pg/mL) and ULoQ (427.695 pg/mL) revealed a low picomolar detection threshold and an acceptable dynamic range. (D) In a small exploratory cohort a discrimination between healthy controls and ataxic mutation carrier was possible in plasma samples ($P < 0.001$). (E-F) In a larger validation cohort, polyQ-expanded ataxin-3 protein levels were measured in plasma and CSF and demonstrated a significant discrimination between healthy controls and mutation carriers (each $P < 0.001$). Only in plasma, a separation between pre-ataxic and ataxic mutation carriers was possible ($P = 0.009$). (D-F) Data were calculated with two-tailed Mann-Whitney U-tests and are Bonferroni-corrected. The number of analyzed cases per study group is included below the graphs.

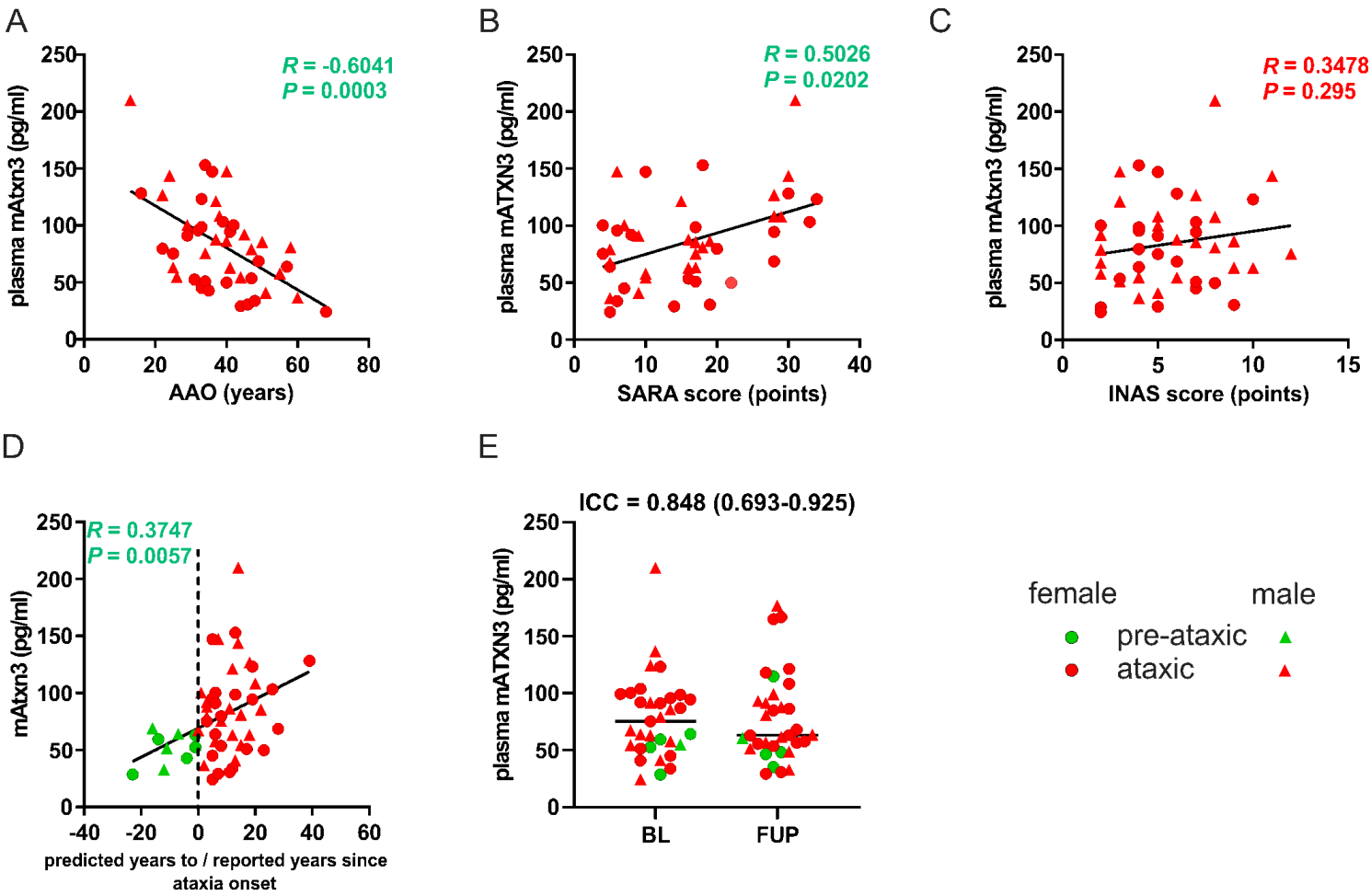

Figure 2

PolyQ-expanded ataxin-3 plasma levels correlate with clinical parameters. (A-C) Plasma polyQ-expanded ataxin-3 level correlated negatively with age at onset (AAO, P = 0.0003) (A) and positively with the clinical scores SARA (P = 0.02, B) and INAS (P = 0.04, C). (D) Cross-sectional correlation of predicted years to or reported years since ataxia onset (SARA >3) to polyQ-expanded ataxin-3 levels revealed a significant linear regression (P = 0.005). (E) Longitudinal analyses of 37 participants with one year follow up revealed a high stability of polyQ-expanded ataxin-3 during disease progression. Scatter blots show unadjusted values of 11 pre-ataxic and 45 ataxic study participants (for details see table 1). R and P values are age- and CAG-repeat-adjusted, generated from partial Spearman correlations including age and CAG-repeat as covariate. Stability analyses of ataxin-3 values over one year period were analyzed by intraclass variation (ICC) in 37 participants.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- additionalfile1.tif
- additionaltable1.docx

- additionalTable2.docx