The Ratio of Expanded to Normal Ataxin 3 in Peripheral Blood Mononuclear Cells Correlates with the Age at Onset in Spinocerebellar Ataxia Type 3

Spinocerebellar ataxia type 3 (SCA 3) is a rare devastating neurodegenerative disorder caused by the expansion of CAG repeats in exon 10 of the ataxin-3 (*ATXN3*) gene, resulting in the expression of polyglutamine (polyQ) expanded mutant protein (mATXN3) (reviewed in Paulson).[1] PolyQ aggregation in general has been shown to follow seeded growth polymerization kinetics with either the size or the concentration of an aggregation intermediate being critical for the fibrillization process.[2,3] Therefore, it is reasonable to argue that strategies aiming at decreasing the amount of aggregation prone mATXN3 species, or *ATXN3* in general, could provide direct therapeutic benefit. Using mouse and other model organisms, we and others have shown that decreased *ATXN3* does not lead to apparent morphological abnormalities or premature death.[4] Allele-specific exon skipping resulted in lower mATXN3 levels and aggregate load in a SCA3-YAC mouse model.[5] Similar results have been observed with various micro RNA approaches, suggesting that strategies to decrease *ATXN3* expression in human SCA3 patients would be safe and of direct therapeutic value.[6,7,8,9]

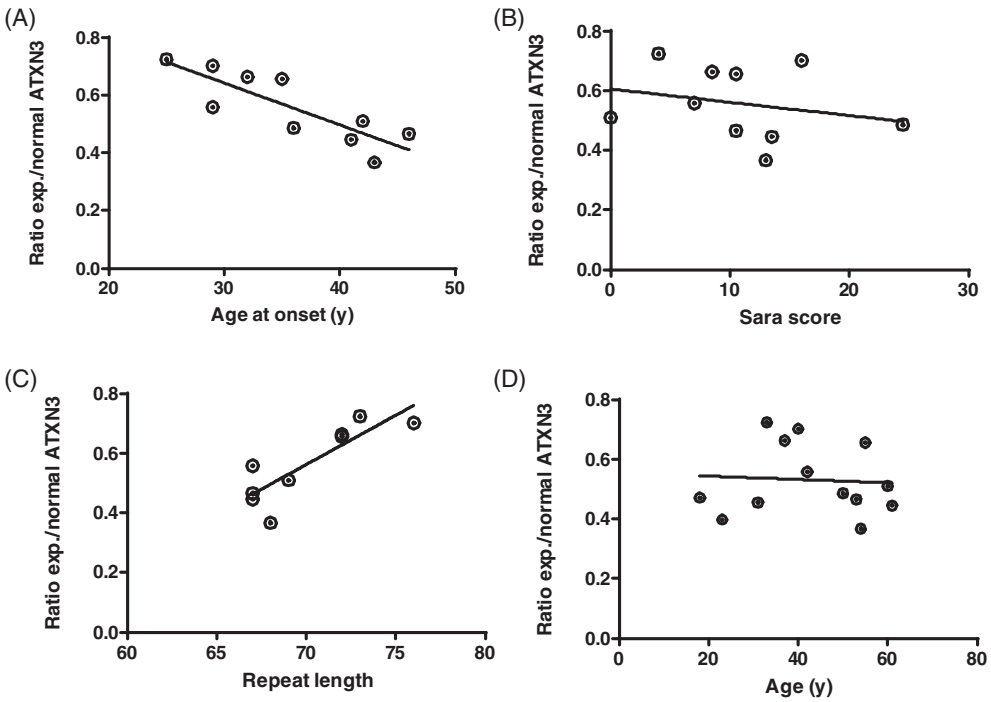

FIG. 1. Correlation of polyQ-expanded ataxin-3 protein levels with clinical parameters. (**A**) Ratio of expanded to normal *ATXN3* correlates with age at onset. The ratio of expanded to normal *ATXN3* is plotted against the age at onset of symptoms of the individual patients ($r^2 = 0.7001$, SE = 0.07096, $P = 0.0025$). (**B**) Ratio of expanded to normal *ATXN3* is not correlated to scale for the assessment and rating of ataxia (SARA) score. The ratio of expanded to normal *ATXN3* is plotted against the SARA score of the individuals ($r^2 = 0.05998$, SE = 0.1256, $P = 0.49530$). (**C**) Ratio of expanded to normal *ATXN3* correlates with repeat length. The ratio of expanded to normal *ATXN3* is plotted against the repeat length of the expanded allele from individual patients ($r^2 = 0.7224$, SE = 0.07141, $P = 0.0037$). (**D**) Ratio of expanded to normal *ATXN3* is not correlated with age. The ratio of expanded to normal *ATXN3* is plotted against the age of the individuals ($r^2 = 0.0046451236$, SE = 0.08835, $P = 0.0162$).

Key Words: ataxin 3, quantification, peripheral blood

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We developed a quantitative assay based on a fluorophore-labeled, highly specific antibody to *ATXN3* and recombinant human *ATXN3* purified to homogeneity, serving as standard to determine absolute amounts of *ATXN3* and observed considerable variation of normal and mATXN3 in human peripheral blood mononuclear cells (PBMC). Between 0.5 and 3 nanograms *ATXN3* were present per microgram protein (Supplementary Fig. 1). The well-known inverse correlation between CAG repeat length and age at onset was reflected in our cohort, suggesting that the cohort was representative despite the small number. No clear cut correlation with age, sex, or the severity of ataxia (scale for the assessment and rating of ataxia score) became apparent with absolute amounts of normal or mATXN3; similar to what has been observed recently (data not shown).[10,11] Independent of the absolute amount, however, the ratio of normal to mATXN3 correlated with the age at onset of motor symptoms (ie, the more mATXN3 relative to normal *ATXN3* was present in PBMC, the earlier the age at onset (Fig. 1). This phenomenon points to the importance of the individual relative proportion of expanded, dysfunctional protein, and fits with the idea of a toxic gain of function of mATXN3. As we observed no clear cut correlation with age and little variation with repeated sampling, measurement of *ATXN3* in PBMC appears as a feasible tool to determine the amount of both normal and mATXN3 in an individual over time and therefore, to evaluate the effect of compounds or molecular tools, which supposedly lower neuronal *ATXN3* expression in a clinical trial. It will be interesting to evaluate a putative change of *ATXN3* expression in PBMC after systemic (or intrathecal) application of a canonical drug or antisense-oligonucleotides, because we are concerned that the amount of *ATXN3* in cerebrospinal fluid (CSF) might be to low to measure, even with a highly specific and sensitive assay. Whether the relative amounts of *ATXN3* will develop into a useful biomarker for SCA3 (ie, whether this ratio has a prognostic value in individuals at risk before the onset of obvious motor signs) remains to be determined in larger longitudinal cohorts.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Whole-Exome Sequencing Identified Rare Variants in *PCDHGB1* in Patients with Adult-Onset Dystonia

Dystonia is a movement disorder characterized by intermittent or sustained muscle contractions causing abnormal movements and twisting postures. Despite an increased number of genes that cause dystonia, the etiology in most patients is still unknown.[1] Here, using whole-exome sequencing (WES),

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