Noninvasive Detection of Presymptomatic and Progressive Neurodegeneration in a Mouse Model of Spinocerebellar Ataxia Type 1

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Recent studies with a conditional mouse model of spinocerebellar ataxia type 1 (SCA1) suggest that neuronal dysfunction is reversible and neurodegeneration preventable with early interventions. Success of such interventions will depend on early detection of neuronal and glial abnormalities before cell loss and availability of objective methods to monitor progressive neurodegeneration. Cerebellar concentrations of *N*-acetylaspartate (NAA), *myo*-inositol, and glutamate as measured by magnetic resonance spectroscopy (MRS) correlate with ataxia scores of patients with SCA1, indicating their potential as reliable biomarkers of neurodegeneration. Here we investigated whether neurochemical levels are altered by early, presymptomatic disease and whether they gauge disease progression in a mouse model of SCA1. Cerebellar neurochemical profiles of transgenic mice that overexpress the mutant human ataxin-1 (the SCA1[82Q] line) were measured longitudinally up to 1 year by MRS at 9.4 T and compared to those of transgenic mice that overexpress the normal human ataxin-1 (the SCA1[30Q] line) and wild-type controls. Multiple neurochemicals distinguished the SCA1[82Q] mice from controls with no overlap at all ages. Six neurochemicals were significantly different in SCA1[82Q] mice at 6 weeks, before major pathological and neurological changes. Alterations in NAA, *myo*-inositol, and glutamate progressively worsened and were significantly correlated ($p < 0.0001$) with disease progression as assessed by histology (molecular layer thickness and an overall severity score). Therefore, the neurochemicals that correlate with clinical status in patients reflected progressive pathology in the mouse model. These data demonstrate that presymptomatic and progressive neurodegeneration in SCA1 can be noninvasively monitored using MRS.

Introduction

Hereditary spinocerebellar ataxias (SCAs) are a clinically and genetically heterogeneous group of movement disorders characterized by cerebellar neurodegeneration (Klockgether and Dichgans, 1997). The first SCA for which the mutation was discovered (Orr et al., 1993), SCA1, is caused by dominant inheritance of an expanded CAG repeat that results in a polyglutamine expansion in ataxin-1 (Zoghbi and Orr, 1995). Pathologically, SCA1 is characterized by loss of cerebellar Purkinje cells (PCs) and neurodegeneration within the brainstem and spinocerebellar tracts causing ataxia, dysarthria, and progressive bulbar dysfunction (Zoghbi and Orr, 1995). Neuronal and motor dysfunction is reversible in SCA1 by suppressing the expression of mutant ataxin-1 (Xia et al., 2004; Zu et al., 2004). However, successful im-

plementation of such interventions will depend on early detection and monitoring of cerebellar abnormalities by objective means.

We demonstrated that cerebellar neurochemical levels [*N*-acetylaspartate (NAA), *myo*-inositol, and glutamate] measured by proton magnetic resonance spectroscopy (¹H MRS) distinguish patients with SCA1 from controls with very high specificity and sensitivity (Öz et al., 2010). In addition, the levels of these neurochemicals correlated with scores on a standardized ataxia rating scale (Schmitz-Hübsch et al., 2006), indicating their potential as robust biomarkers of progressive disease. Before their applicability in preclinical and clinical trials, the pathological correlates of these MRS biomarkers need to be delineated, and their sensitivity to early and progressing pathology needs to be established. These goals can best be achieved in faithful mouse models of human neurodegenerative disease that allow MRS and histological assessment in the same brains. Therefore, here we studied a well characterized mouse model of SCA1 that reproduces the neuropathological and behavioral features of the human disease (Burright et al., 1995; Clark et al., 1997).

The SCA1[82Q] mice overexpress the mutant human ataxin-1 protein with an 82 glutamine stretch under the control of a PC-specific promoter (Burright et al., 1995). Ataxia develops as a result of neuronal dysfunction and atrophy, rather than cell loss, in these mice (Clark et al., 1997), making the model ideal for delineating the neurochemical correlates of PC dysfunction. Fur-

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thermore, expression of various neuronal genes are altered first (Lin et al., 2000), followed by pathological and subsequently neurological changes (Clark et al., 1997), as in many human neurodegenerative diseases. Hence this model is also ideal for testing the sensitivity of MRS biomarkers to presymptomatic abnormalities, but was never studied by noninvasive imaging before.

We hypothesized that MRS biomarkers would be sensitive to early and progressing disease in this ataxia model, even before the development of major pathological changes and ataxia. Therefore, we compared the cerebellar neurochemical profiles of *SCA1*[82Q] mice obtained by ^1H MRS at 9.4 T longitudinally to those of two control groups: *SCA1*[30Q] transgenic mice that overexpress the normal human ataxin-1 (Burright et al., 1995) and wild-type (WT) mice (background strain FVB). We further assessed the cerebella of the mice by histology and evaluated correlations between neurochemical levels and quantitative pathology scores in the same brains.

Materials and Methods

Study design. Three groups of male (unless otherwise noted) mice were studied: (1) *SCA1*[82Q] transgenic mice ($N = 14$) that overexpress the mutant human ataxin-1 with an 82 glutamine stretch; these animals belonged to the B05 strain described previously (Burright et al., 1995). (2) WT controls ($N = 21$, 2 females). Mice from the background strain (FVB) were used as the control group since the *SCA1*[82Q] mice were homozygous and did not have wild-type littermates. (3) *SCA1*[30Q] transgenic mice ($N = 8$, 4 females) that overexpress the normal human ataxin-1; these belonged to the A02 strain described previously (Burright et al., 1995). Four of these mice were homozygous and four heterozygous. This group represented a positive control.

MRS neurochemical profiles and histology data were obtained at ages of 6, 12, and 24 weeks and 1 year. These time points were selected based on well characterized pathological progression in these mice (Burright et al., 1995; Clark et al., 1997). To introduce interlitter variability, we studied 4 ± 2 mice from each litter starting at 6 weeks of age and assigned each randomly to 6, 12, or 24 week or 1 year follow-up. The animals were killed for histological evaluation at their assigned time points within 24 h of MR scanning. The study was designed this way to have a higher number of MRS measurements at the earlier time points, when neurochemical alterations were expected to be more subtle than late-stage disease. This design also provided 2–4 animals per group at each time point to compare the MRS and histology results in the same brains. Some of the WT mice were only studied by MRS: four WT mice were only scanned at 6 weeks, three WT mice only at 12 weeks, and four others only at 12 and 24 weeks. To establish test–retest reproducibility of neurochemical concentrations, we scanned four additional WT mice (16–18 weeks old) on 3 consecutive days.

Animal preparation. All experiments were performed according to procedures approved by the University of Minnesota Institutional Animal Care and Use Committee. Animals were induced with 3–4% isoflurane and a 1:1 mixture of O_2 : N_2O . Spontaneously breathing mice were fixed in a custom-built mouse holder and maintained anesthetized with 1.5–2% isoflurane while monitoring body temperature and respiration rate (SA Instruments) to ensure unchanging physiological status. Body temperature was maintained at 36–37°C with a circulating bath of warm water and a heating fan that received feedback from the rectal temperature probe. The typical scanning time for each animal was ~50 min to 1 h.

MR protocol. All experiments were performed on a 9.4 T/31 cm magnet (Magnex Scientific) interfaced to a Varian INOVA console (Varian) and equipped with a 15 cm gradient coil insert (450 mT/m, 200 μs) and strong second-order shim coils (Resonance Research). A quadrature surface radio frequency (RF) coil with two geometrically decoupled single-turn coils (10 mm diameter) was used as the MR transceiver. Following positioning of the mouse in the magnet, coronal and sagittal multislice images were obtained with a rapid acquisition with relaxation enhancement (RARE) sequence (Hennig et al., 1986) [repetition time (TR) = 4 s, echo train length = 8, echo time (TE) = 60 ms, slice thickness = 1 mm,

7 slices] to select a 5–7 μl volume of interest (VOI) centered on the midline in the cerebellum. The VOI was placed consistently during longitudinal studies by using anatomical landmarks and its size reduced in *SCA1*[82Q] mice with age to cover the same cerebellar region despite cerebellar atrophy. All first- and second-order shims were adjusted using FASTMAP with echo-planar readout (Gruetter and Tkáč, 2000). Localized ^1H MR spectra were acquired with a short-echo localization by adiabatic selective refocusing (LASER) sequence (TE = 15 ms, TR = 5 s, 256 averages) (Garwood and DelaBarre, 2001). Water was suppressed using variable power RF pulses with optimized relaxation delays (VAPOR) (Tkáč et al., 1999). Spectra were acquired and saved as single scans, which were individually frequency and phase corrected. Scans that showed evidence for motion were excluded and the remaining scans summed. Unsuppressed water spectra were acquired from the same VOI as a metabolite quantification reference. In addition, unsuppressed water spectra were acquired in a subset of animals from each group at a series of TE values (TE = 5–400 ms; TR = 30 s for full relaxation) to evaluate CSF contribution to the VOI (Ernst et al., 1993). No CSF contribution was found by fitting the integrals of these water spectra with a biexponential decay function, and hence no correction for CSF was necessary.

Metabolite quantification. The contribution of individual metabolites to the spectra was quantified using an automated deconvolution program (LCModel) (Provencher, 1993) as described previously (Tkáč et al., 2004; Marjanska et al., 2005; Öz et al., 2005). The following metabolites were included in the basis set: alanine (Ala), aspartate (Asp), ascorbate/vitamin C (Asc), glycerophosphocholine (GPC), phosphocholine (PCho), creatine (Cr), phosphocreatine (PCr), GABA, glucose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), glycine (Gly), *myo*-inositol (*myo*-Ins), lactate (Lac), NAA, *N*-acetylaspartylglutamate (NAAG), phosphoethanolamine (PE), *scyllo*-inositol (*scyllo*-Ins), taurine (Tau), and macromolecules (MM). The model metabolite spectra were generated using density matrix simulations (Henry et al., 2006) with the MATLAB software (MathWorks) based on previously reported chemical shifts and coupling constants (Govindaraju et al., 2000; Tkáč, 2008). The MM spectra were experimentally obtained from the mouse brain using an inversion recovery technique. Absolute metabolite concentrations were obtained relative to an unsuppressed water spectrum acquired from the same VOI. Metabolites quantified with Cramér–Rao lower bounds (CRLB, estimated error of quantification) >50% were classified as not detected, and metabolites quantified with CRLB $\leq$ 50% in at least 90% of the spectra were included in the neurochemical profile. Only three metabolites (Ala, Gly, and *scyllo*-Ins) were excluded from final analysis based on these strict criteria. If the correlation between two metabolites was consistently high (correlation coefficient < -0.5), their sum was reported rather than the individual values. Strong negative correlation was found in two cases, so that Cr + PCr and GPC + PCho (from here on will be referred to as tCr for total creatine and tCho for total choline), were included in the neurochemical profile. Therefore 15 statistically independent concentrations were evaluated.

Histology. Following the collection of the last MR spectra, brains were harvested and processed for histology. Mice were deeply anesthetized with sodium pentobarbital (100 mg/kg i.p.) and killed by transcardial perfusion with pH 7.4 PBS followed by 10% formalin. Brains were removed and postfixed in 10% formalin. Histology was performed in a blind manner (regarding the genotype and age of the animals) on paraffin-embedded sections using hematoxylin and eosin (H & E), Luxol fast blue-PAS, and immunohistochemistry for glial fibrillary acidic protein (GFAP) and calbindin. Luxol fast blue demonstrated no changes in myelin, even in the most severely affected mice, and GFAP was not a reliable quantitative measure of gliosis in this model due to animal-to-animal variation of staining intensity. Therefore, to seek associations between the MRS and histology measures, we used the molecular layer (ML) thickness at the primary fissure (Zu et al., 2004) and semiquantitative measures (severity scale) based primarily on H & E and in some cases calbindin staining. Much of the pathology in these models is related to shrinkage of PCs rather than neuronal loss (Clark et al., 1997); therefore, the measurement of the thickness of the ML, which contains the dendritic trees of the PCs, quantifies these atrophic changes better than neuronal counting. Similarly, hypertrophy of Bergmann glia is more

Figure 1. Localized proton MR spectra (LASSER, TE = 15 ms, TR = 5 s) and midsagittal T₂-weighted images obtained from one WT (upper row) and one SCA1[82Q] mouse (lower row) across their lifespan. The voxel position for the spectra is shown on each image. The spectra were processed identically, weighted with the same Gaussian function before Fourier transformation, and scaled based on neurochemical concentrations obtained by LCModel. The longitudinal alterations in NAA, glutamate, and myo-inositol in the spectra of the ataxia mouse are shown with arrows.

typical than hyperplasia. Therefore, a severity score that includes all aspects of pathology was the most effective method to quantify pathology. The following severity scale was used: 0, no pathological changes; 0.5, near normal but ML somewhat thinner; 1, mild changes including heterotopic PCs, vacuoles in PCs, thinning of ML, largely confined to the posterior lobules; 2, similar to 1 but more widespread; heterotopic PCs more numerous and often higher in the ML; 3, widespread ML thinning, numerous heterotopic PCs involving anterior lobules nearly as frequently as posterior, mild PC loss, primarily in the posterior lobules; 4, severe disorganization of cerebellar cortex with generalized severe atrophy of ML, frequent heterotopic PCs and PC loss.

Statistical analysis. Data from the different mouse groups were compared using two-way repeated-measures ANOVA, with fixed effects for experimental group, week of scan, and their interaction, and a random effect for the repeated measurements on the same mouse. Analyses were done using all available data (not just mice with data that were complete according to the design), in SAS using PROC MIXED, assuming approximately normally distributed outcomes. Model contrasts of means were used to test group comparisons at each week; *p* values were corrected for these multiple comparisons (three groups across four time points, resulting in 12 comparisons) using a step-down approach (Holm, 1979) for each metabolite. We used the Holm method because it does not assume that the tests are independent of each other. Additional *p*-value correction across the multiple metabolites was not carried out. Linear regression analyses were performed to evaluate the relationship between neurochemical levels and histological severity scores and ML thickness measurements.

Results

Longitudinal neurochemical changes are visible in individual mice

High-quality MR spectra (good signal-to-noise ratio, resolution, water/lipid/artifact suppression) were obtained from the cere-

bella of both control and ataxia mice across their lifespan (Fig. 1). Consistent placement of the VOI centered in the cerebellar midline and acquisition of data from the same tissue volume over time facilitated the reproducibility of the spectral quality and pattern. In the SCA1[82Q] mice, where cerebellar atrophy was apparent over time (Fig. 1), this was accomplished by reducing the VOI size relative to the control animals starting at 12 weeks. Test-retest reproducibility of cerebellar neurochemical concentrations was evaluated in four WT mice scanned on 3 consecutive days. The average coefficient of variation for six metabolites (NAA, tCr, tCho, Glu, myo-Ins, Tau) was ≤5%, for five metabolites (Asc, GABA, Glc, Gln, GSH) ≤10%, and in the remaining four metabolites (Asp, Lac, NAAG, PE) ≤25%.

As a result of the excellent test-retest reproducibility, progressive neurochemical alterations in SCA1[82Q] mice over time were discernible even in individual animals, as demonstrated by the NAA, Glu, and myo-Ins resonances in Figure 1.

Cerebellar neurochemical levels are altered before ataxia onset and in the absence of major pathological changes

The neurochemical profiles of the two control groups (WT and SCA1[30Q] mice) were very similar, except for lower Glc levels at 6 weeks (*p* < 0.05) and lower Tau levels (*p* < 0.05) at all ages in the cerebella of the SCA1[30Q] mice (Fig. 2). On the other hand, highly significant neurochemical alterations (Table 1) were detected in the cerebella of the SCA1[82Q] mice relative to both control groups starting at the earliest age studied. At 6 weeks, NAA and Tau were significantly lower and Gln, tCr, myo-Ins, and Glc significantly higher in the cerebella of SCA1[82Q] mice than

Figure 2. Cerebellar neurochemical profiles of the WT (open bars), the *SCA1*[30Q] (gray bars), and the *SCA1*[82Q] mice (black bars) at four ages. Error bars shown are $2 \times \text{SE}$ (95% C.I.). Metabolites that are significantly different ($p < 0.05$) in *SCA1*[82Q] mice than one or both control groups at a particular age are marked with a star, and the p values for the most significant alterations are listed in Table 1.

Table 1. Statistical significance of comparisons of the ataxia mice (*SCA1*[82Q]) with the two control groups: p values corrected for multiple comparisons using the step-down Bonferroni/Holm approach (Holm, 1979)

Comparison	Age (wks)	NAA	Glu	myo-Ins	tCr	Gln	Tau	Glc
<i>SCA1</i> [82Q] vs WT	6	<0.0001	n.s.	<0.0001	0.0014	0.0038	<0.0001	0.0271
<i>SCA1</i> [82Q] vs <i>SCA1</i> [30Q]	6	0.0001	n.s.	0.0060	<0.0001	0.0654	<0.0001	<0.0001
<i>SCA1</i> [82Q] vs WT	12	<0.0001	<0.0001	<0.0001	n.s.	n.s.	<0.0001	0.0075
<i>SCA1</i> [82Q] vs <i>SCA1</i> [30Q]	12	<0.0001	0.0041	<0.0001	n.s.	n.s.	0.0146	0.0001
<i>SCA1</i> [82Q] vs WT	24	<0.0001	<0.0001	<0.0001	n.s.	n.s.	<0.0001	0.0089
<i>SCA1</i> [82Q] vs <i>SCA1</i> [30Q]	24	<0.0001	0.0005	<0.0001	n.s.	n.s.	n.s.	0.0021
<i>SCA1</i> [82Q] vs WT	52	<0.0001	0.0006	<0.0001	n.s.	n.s.	0.0029	n.s.
<i>SCA1</i> [82Q] vs <i>SCA1</i> [30Q]	52	<0.0001	n.s.	<0.0001	n.s.	n.s.	n.s.	n.s.

n.s., Nonsignificant.

controls (Table 1). The concentrations of Asc and Lac were also different in the *SCA1*[82Q] cerebella than one or both control groups but with higher p values (0.02–0.04) (Fig. 2), and as such are interpreted as trends since p values were not corrected across the multiple metabolites.

In the histological evaluation, the WT animals received a severity score of 0 at all ages, while the *SCA1*[30Q] mice were scored 0 and 0.5 except for one mouse that was scored 1 (at 6 weeks) and another that was scored 2 (at 24 weeks, see below). At 6 weeks, the cerebella of the *SCA1*[82Q] mice showed minor pathological alterations, especially in the area covered by the MRS voxel containing the primary fissure. These alterations included vacuoles in PC, heterotopic PCs, and slight thinning of the molecular layer (Fig. 3). The *SCA1*[82Q] mice show no signs of ataxia at this age (Clark et al., 1997); therefore, the neurochemical alterations detected by MRS at 6 weeks were due to presymptomatic pathology.

The time courses of metabolite levels revealed two distinct trends (Fig. 4, Table 1). The levels of NAA, Glu, and *myo*-Ins were progressively more different from the control values as the *SCA1*[82Q] mice aged and disease progressed (Fig. 4, left). On the other hand, levels of tCr, Gln, and Tau were altered in the *SCA1*[82Q] mice at 6 weeks, but converged with the levels of one or both control groups as the animals aged (Fig. 4, right).

Alterations of NAA, Glu, and *myo*-Ins levels correlate with progression of pathology

The largest longitudinal changes in NAA, Glu, and *myo*-Ins levels in the cerebella of the *SCA1*[82Q] mice occurred between 6 and 12 weeks (Fig. 4). Glu levels were the same in all groups at 6 weeks, but became significantly lower in *SCA1*[82Q] mice than controls at 12 weeks (Table 1) and decreased further at 24 weeks. The NAA and *myo*-Ins levels, on the other hand, seemed to level off starting

Figure 3. Longitudinal histological changes in the ataxia mice. The area shown in the H & E-stained slides is indicated on the midsagittal cerebellum scheme. The bars indicate 100 μ m. The top slide shows a WT cerebellum at 12 weeks of age; the appearance of the WT cerebellum does not change up to 1 year. The bottom slides show sample cerebella from *SCA1[82Q]* mice at the four ages studied. The prominent pathological alterations are early formation of vacuoles (shown with arrowhead), heterotopic PCs (shown with arrow), and thinning of the molecular layer.

Figure 4. Time courses of metabolites in the cerebella of WT (open squares), *SCA1[30Q]* (gray triangles), and *SCA1[82Q]* mice (black circles). Data are shown up to 24 weeks because metabolite levels were not changed between 24 weeks and 1 year. The metabolites on the left hand column reflect disease progression in the *SCA1[82Q]* cerebella, while metabolites on the right hand column are different in the *SCA1[82Q]* cerebella than controls only at 6 weeks. Error bars shown are $2 \times$ SE (95% C.I.).

at 12 weeks. The pathological alterations in the *SCA1[82Q]* mice continued to progress until 24 weeks with the mice receiving a score of 2 at 6 weeks, 3 at 12 weeks, and 4 at 24 and 52 weeks. Therefore, the major alterations in NAA and *myo-Ins* levels preceded the pathological progression to the severe disease stage. When data from all perfused mouse brains were combined, robust correlations between the NAA, *myo-Ins*, and Glu levels and the pathology measures were apparent (Fig. 5). Note, however, that NAA and *myo-Ins* levels that correspond to severity scores 3 and 4 were very similar, demonstrating their leveling off at severe disease. At a severity score of 3, there is little PC loss in the area covered by the MRS voxel, while cell loss is prominent at a severity score of 4. Therefore, the NAA and *myo-Ins* changes in this neurodegeneration model were marking neuronal dysfunction and dendritic atrophy and not cell loss.

Tau and GSH were also positively correlated with the molecular layer thickness and negatively correlated with the severity score (data not shown). Although these correlations were less robust than those of NAA, *myo-Ins*, and Glu versus the histology measures, they were still significant ($p < 0.001$ for Tau and $p < 0.01$ for GSH vs both histology measures). The GSH correlations were particularly interesting since no group differences were observed for this metabolite across different ages,

but a significant decrease with age was observed for all groups, which likely was the reason for the observed correlation with the histology measures.

Ataxia mice are distinguished from controls at each age by particular metabolites

Since the neurochemical alterations in the *SCA1*[82Q] mice relative to controls were numerous at each age and highly statistically significant, plotting two altered metabolites at each age against each other separated the ataxia mice from controls with no overlap (Fig. 6). Furthermore, the progression of disease in individual mice could be monitored in such plots. For example, the mouse marked with an arrow in Figure 6 was an obvious outlier in the *SCA1*[30Q] group at 12 weeks, and at 24 weeks, its neurochemical levels were closer to the *SCA1*[82Q] mice than any of the control mice. This mouse was in fact the only control animal that received a severity score of 2 when killed at 24 weeks and interestingly had the lowest NAA and highest *myo*-Ins values in its group at 6 weeks (data not shown), but otherwise was no different from other group members.

Discussion

We have demonstrated in a mouse model of SCA1 that neurochemical levels measurable by ^1H MRS can be used to monitor early and progressive neurodegeneration. By using a 9.4 T scanner, together with state-of-the-art MR data acquisition and quantification methods, we were able to noninvasively and reproducibly measure the concentrations of 15 neurochemicals in a small brain region (Fig. 2) and monitor the onset and progression of neurochemical abnormalities in individual mice (Figs. 1, 6). A subset of the neurochemicals that correlated with ataxia scores of patients in our previous study (Öz et al., 2010) also displayed robust correlations with pathology scores in mice.

Neurochemical changes due to polyglutamine expansion in ataxin-1

The neurochemical alterations detected in *SCA1*[82Q] mice were primarily due to the expansion of the polyglutamine repeat in ataxin-1, rather than the overexpression of the human protein, based on the similar neurochemical profiles of the WT and *SCA1*[30Q] mice. Taurine was the only metabolite that was different between these two groups at all ages. Decreased Tau may be an indicator of the slight thinning of the molecular layer in the *SCA1*[30Q] cerebella (severity score 0.5) because the somata, dendrites, and dendritic spines of PCs contain the highest Tau levels in the cerebellar cortex (Ottersen, 1988). This would also be consistent with the correlation of Tau levels with the histology scores (data not shown). The *SCA1*[30Q] group also showed that mere overexpression of human ataxin-1 may lead to further pathology in some mice based on the two outliers that received severity scores of 1 and 2. Interestingly, high levels of normal

Figure 5. Correlation of MRS and histology findings. Cerebellar concentrations of NAA, *myo*-inositol, and glutamate are significantly correlated with both molecular layer thickness in the primary fissure and the global pathological severity score. The correlation coefficients are shown on each plot, and all linear regressions have a p value <0.0001 .

human ataxin-1 also result in neurodegeneration in a *Drosophila* model of SCA1 (Fernandez-Funez et al., 2000).

Parallel neurochemical changes in the SCA1 mouse model and patients

Most of the neurochemical alterations observed in the *SCA1*[82Q] mice in a longitudinal manner were the same as those we detected in a cross-sectional study of patients with SCA1. Namely, NAA and Glu were lower and *myo*-Ins higher than controls in all three brain regions studied in patients: the cerebellar vermis, cerebellar hemispheres, and the brainstem (pons) (Öz et al., 2010). Note that in two of these regions, levels of total NAA (tNAA, NAA + NAAG) were reported due to the cross-correlation of NAA and NAAG. The time courses of tNAA and its correlation to histology measures in the current study were identical to those of NAA alone, justifying the comparison of the mouse and patient findings. In addition to altered NAA, Glu, and *myo*-Ins, higher Gln and tCr levels than controls were also observed in the cerebella of patients. These alterations were only detected at the earliest time point in mice and the patients were studied at the early-to-moderate disease stage. Therefore it will be interesting to determine the natural history of these changes in patients and to determine whether Gln and tCr will decrease at later disease stages in patients as well. Although complicated by the slow progression of neurodegenerative diseases, longitudinal studies in

Figure 6. Separation of *SCA1*[82Q] mice (black circles) from controls (*SCA1*[30Q] in gray triangles and WT in open squares) at each age. Concentrations of selected metabolites obtained from cerebella of individual mice are plotted. The disease progression can be followed in individual mice, as demonstrated with an outlier *SCA1*[30Q] mouse (marked with an arrow).

patients are critical before utilization of the technique as an outcome measure in clinical trials. Another metabolite altered early in the *SCA1*[82Q] mice was Tau. However, brain Tau levels in rodents are much higher than humans, despite the similarity of the rest of the neurochemical profiles; therefore, Tau may not be an ideal biomarker that can be used in parallel in mouse models and humans.

Remarkably the three neurochemicals, NAA, *myo*-Ins, and Glu, that correlated strongly with disease progression as assessed by histology were the same ones that correlated most robustly with the ataxia scores in patients (Öz et al., 2010). Clearly, these associations do not establish causality; however, they demonstrate that these neurochemicals gauge disease reliably.

Implications of neurochemical changes for the neurobiology of *SCA1*

Two sets of MRS biomarkers were identified in the *SCA1*[82Q] mice: those that correlated with disease progression (Figs. 4, 5) and those that anticipated disease progression (Fig. 4). Among the first group, NAA is localized exclusively in neurons and is indicative of both neuronal cell number and viability (Clark, 1998; Dautry et al., 2000; Demougeot et al., 2001). In this study, alterations in NAA clearly mark neuronal dysfunction and dendritic atrophy, and not cell loss, because NAA levels did not change after severity score 3 when most cell loss occurred in the selected VOI. Mitochondrial NAA synthesis is correlated with oxygen consumption and ATP production in isolated rat brain mitochondria, suggesting that decreased NAA levels may reflect decreased mitochondrial energy production (Bates et al., 1996). Asp, the precursor of NAA, tended to be higher in *SCA1*[82Q] mice (Fig. 2) with uncorrected *p* values <0.03 at 6 weeks, which would be consistent with decreased mitochondrial NAA synthesis, but needs further investigation.

Increased *myo*-Ins levels were another marker of early and progressive pathology. *Myo*-Ins is primarily localized to glial cells based on culture work (Brand et al., 1993). Therefore increased

myo-Ins levels have typically been attributed to gliotic activity in neurological disorders (Pouwels et al., 1998; Kantarci et al., 2004; Öz et al., 2005; Vrenken et al., 2005). Gliosis was not a prominent feature in the *SCA1*[82Q] mice, even when examined by GFAP immunostaining. Therefore the progressive increase in *myo*-Ins levels may reflect the glial component that was relatively increased in the selected VOI because of shrinkage of the nonglial elements of the molecular layer. Alternatively, the increased *myo*-Ins levels may be related to one of its multiple functions in cells, such as serving as an osmolyte (Fisher et al., 2002).

The third marker of disease progression, Glu, was altered in the *SCA1*[82Q] cerebella after NAA and *myo*-Ins at 12 weeks (Fig. 4). Glu is primarily localized to neurons, with the highest levels present in glutamatergic neurons (Storm-Mathisen et al., 1992). While the Glu decrease in *SCA1*[82Q] mice may be surprising since the mutant gene expression and pathology is restricted to the GABAergic PCs, which have low levels of

Glu (Storm-Mathisen et al., 1992), secondary affects on associated cells are likely. Namely, PCs receive excitatory input from glutamatergic parallel and climbing fibers in the ML, which would be disrupted by PC dendritic pathology. In fact, the expression of five genes that code for regulators of glutamate signaling pathways in PCs are altered in the cerebella of *SCA1*[82Q] mice at 5 and 12 weeks of age (Serra et al., 2004).

The early increase in Gln, a metabolite that anticipates disease progression, indicates an imbalance between the Glu and Gln pools, which may also be due to a disruption of the Glu–Gln cycle between the presynaptic granule cells/climbing fibers and Bergman glia in the ML. For example, glutamate uptake into Purkinje cells appears reduced due to the downregulation of EAAT4 (Lin et al., 2000; Serra et al., 2004), a Purkinje cell-specific glutamate transporter. As a result, excess Glu may be taken up by Bergman glia and converted to Gln. The parallel reduction of Glu and Gln in the *SCA1*[82Q] mice with age (Fig. 4), on the other hand, likely corresponds to the progressive dendritic atrophy of PCs with a decrease in synaptic contacts by parallel and climbing fibers.

Total creatine was also higher in the *SCA1*[82Q] mice than controls only at 6 weeks. Our data indicated that the higher tCr levels in the *SCA1*[82Q] mice were due to increased PCr, which serves as an energy buffer in the cell. Increased levels of PCr early on in the disease may indicate decreased energy utilization, possibly due to compromised cerebellar development in the *SCA1*[82Q] mice. Namely, expression of mutant ataxin-1 is most detrimental during the first postnatal months of these mice since it destabilizes ROR α , a transcription factor critical for cerebellar development (Serra et al., 2006). Therefore, increased energy reserves at 6 weeks of age are consistent with reduced energy utilization during cerebellar development in these mice. Two other metabolites related to energy metabolism, lactate and glucose, may provide additional insight. Both were higher in the *SCA1*[82Q] cerebella relative to WT mice, possibly due to reduced oxidative metabolism and decreased glucose utilization. However, the interpretation of glucose and lactate findings is complicated by the

fact that isoflurane increases brain lactate (Valette et al., 2007) and blood and brain glucose levels (Kofke et al., 1987). Therefore, the changes we have seen may be indicative of a different response of the SCA1[82Q] mice to isoflurane than the controls. Together, the NAA, tCr, Glc, and Lac data strongly suggest alterations in energy metabolism in the cerebella of the SCA1[82Q] mice relative to controls, regarding both energy production and utilization.

In conclusion, these parallel MRS and histology data demonstrate that neurochemical levels measured by MRS can accurately gauge progressive neuronal dysfunction. They can therefore be used to noninvasively and reliably monitor disease progression and potentially response to therapy in preclinical and clinical trials.

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