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Principal Component Analysis of Cerebellar Shape on MRI Separates SCA Types 2 and 6 into Two Archetypal Modes of Degeneration

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Abstract

Although “cerebellar ataxia” is often used in reference to a disease process, presumably there are different underlying pathogenetic mechanisms for different subtypes. Indeed, spinocerebellar ataxia (SCA) types 2 and 6 demonstrate complementary phenotypes, thus predicting a different anatomic pattern of degeneration. Here, we show that an unsupervised classification method, based on principal component analysis (PCA) of cerebellar shape characteristics, can be used to separate SCA2 and SCA6 into two classes, which may represent disease-specific archetypes. Patients with SCA2 ($n=11$) and SCA6 ($n=7$) were compared against controls ($n=15$) using PCA to classify cerebellar anatomic shape characteristics. Within the first three principal components, SCA2 and SCA6 differed from controls and from each other. In a secondary analysis, we studied five additional subjects and found that these patients were consistent with the previously defined archetypal clusters of clinical and anatomical characteristics. Secondary analysis of five subjects with related diagnoses showed that disease groups that were clinically and pathophysiologically similar also shared similar anatomic characteristics. Specifically, Archetype #1 consisted of SCA3 ($n=1$) and SCA2, suggesting that cerebellar syndromes accompanied by atrophy of the pons may be associated with a characteristic pattern of cerebellar neurodegeneration. In comparison, Archetype #2 was comprised of disease groups with pure cerebellar atrophy (episodic ataxia type 2 ($n=1$), idiopathic late-onset cerebellar ataxias ($n=3$), and SCA6). This suggests that cerebellar shape analysis could aid in discriminating between different pathologies. Our findings further suggest that magnetic resonance imaging is a promising imaging biomarker that could aid in the diagnosis and therapeutic management in patients with cerebellar syndromes.

Keywords

Ataxia; Magnetic resonance imaging (MRI); Principal component analysis (PCA); Cerebellum; Biomarker

Introduction

Cerebellar ataxias are characterized by poor control of gait, speech, coordination, and eye movements [1]. Anatomically, cerebellar ataxias show progressive atrophy of the cerebellum that is often accompanied by atrophy of the brainstem, cerebral cortex, and other regions [2]. Thus, an anatomic biomarker could be an invaluable surrogate for following disease, particularly for patients of more advanced age as confounding medical issues may affect neurologic performance even on standardized ataxia rating scales.

Here, we use magnetic resonance (MR) shape characteristics to classify neurodegenerative ataxias into archetypal modes of degeneration. Specifically, we quantify shape based on the relative volumes of the individual lobules that comprise the total cerebellum. We hypothesize that principal component analysis (PCA) of cerebellar shape characteristics will separate different disease groups into different archetypes based on the differential patterns of cerebellar atrophy. Our central model is that the structure of the cerebellum is related to clinical phenotype and may well be more sensitive to the progression of disease because structure directly connects to potential underlying mechanisms. Indeed, we have previously

reported disease-specific anatomic differences in a subset of the cerebellar system in spinocerebellar ataxia (SCA) types 2 and 6 [3]. Presumably, these region-specific anatomic differences reflect disease-specific pathogenetic mechanisms. In this exploratory paper, we extend our analyses to the entire cerebellum to investigate the possible presence of archetypal modes of cerebellar neurodegeneration.

Methods

Subjects

Eleven patients with SCA2 (10 female/1 male) and seven patients with SCA6 (5 female/2 male) were compared against 15 neurologically normal controls (14 female/1 male). As part of a post-hoc analysis, three patients with idiopathic late-onset cerebellar ataxia (ILOCA; 2 female/1 male), one patient with SCA3 (1 male), and one patient with episodic ataxia type 2 (EA2; 1 male) were added (Table 1). All participants provided written informed consent. Age-adjusted volumes for the SCA2 subjects and matched controls were previously reported [4].

Ethics

This protocol was approved by the Institutional Review Board of University of California, Los Angeles, School of Medicine and the Institutional Review Board of The Johns Hopkins University, School of Medicine, and is in accordance with the declaration of Helsinki on ethical principles for medical research involving human subjects.

Clinical Evaluation

All participants completed a medical questionnaire including items evaluating course of illness, past medical history, family medical history, social history, and review of systems. The duration of disease was defined from the first self-reported symptom of ataxia. Participants underwent a videotaped, standardized neurological exam. They were assigned a functional staging score for ataxia (FSFA) from 0 to 6 that emphasized mobility. This is a subset of a neurological rating scale, Unified Ataxia Disorders Rating Scale (UADRS), which was described and validated in a cohort with Friedreich ataxia [5], and shown to correlate with morphometric changes of the pons and the total cerebellum in SCA2 [6].

MR Evaluation

Subjects were scanned on either a 1.5T GE Signa scanner with a 3D-SPGR sequence (two scans; sagittal, TR=24 ms, TE=4 ms, slice thickness=1.2 mm, FOV=240 mm, matrix 256×256) or a 3.0T Philips Integra MR scanner (three MPRAGE scans; TR=10.33 ms, TE=6 ms, voxel size 0.828125×0.828125×0.75 mm³). Available scans from a given scanning session were co-registered and averaged using the Brain Imaging Software Toolbox (Montreal Neurological Institute) in order to improve signal-to-noise ratio.

Volumetric Analyses

Regions of interest were manually delineated using Display software (Montreal Neurological Institute) by two raters who were blinded to the diagnosis of participants. Inter-rater reliability of the volumetric measurements was determined by having each rater analyze four subjects [two controls and two SCA2s; 23 regions per patient (left and right hemispheric lobules, lobules of the vermis, and the total cerebellum)] for determination of intraclass correlation coefficients (*icc.m*, Matlab Central File Exchange).

Regions of interest were visually identified based on Schmahmann's cerebellar atlas [7], a modification of the Larsell classification. The total cerebellar volume was defined to include

the cerebellar cortex, arbor vitae, corpus medullare, and the deep cerebellar nuclei. The cerebellar cortex was subdivided into anterior lobe (lobules I–V), simplex (VI), Crus I, Crus II, paramedianus (VIIB), biventer (VIII), tonsil/paraflocculus (IX), and flocculus (X). The corpus medullare (central white matter and the output nuclei of the cerebellum) was defined to include the superior cerebellar peduncles up to the juncture with the brainstem and included part of the middle cerebellar peduncle, as defined by a plane orthogonal to the peduncle at the level of the flocculus. The boundary between corpus medullare and the hemispheres was defined by virtual lines connecting adjacent fissure bottoms. The vermis was then delineated while maintaining the subdivisions of the declive (VI), tuber/folium (VII), pyramis (VIII), uvula (IX), and nodulus (X). In the presence of cerebellar atrophy, the volumetric measurements excluded the cerebrospinal fluid space between the lobules of the cerebellum.

Statistical Analyses

Inter-rater reliability of the volumetric measurements was assessed using intraclass correlation coefficient. To directly assess the effects of disease, a log-linear adjustment of the regional raw cerebellar volumes was performed to account for the exponentially decaying nature of neurodegeneration (rapid decrease in volume during the early stages of disease followed by a convergence to an asymptote). Age-adjusted regional volumes were calculated by using the rate of age-related neurodegeneration in the log(control volume) as the reference point for regions that correlated with age in controls. The relative regional volume (the ratio of age-adjusted log(regional volume)/age-adjusted log(total cerebellar volume)) was calculated as the primary endpoint to examine shape differences independent of individual size differences as well as to account for any potential differences between the magnetic resonance imaging (MRI) scanners. The shape characteristic was defined by relative regional volumes of each cerebellar subregion. When disease processes differentially affect different cerebellar subregions, this would result in a change in relative regional volume. Given our small and not normally distributed sample, the inter-group differences in relative regional volume were assessed using the Wilcoxon rank-sum test. The Bonferroni–Holm method was used to correct for effects of multiple comparison.

Principal Component Analysis

PCA is a mathematical procedure that uses an orthogonal transformation to convert a set of possibly correlated variables into a smaller or equal number of uncorrelated variables called principal components. The transformation is defined in such a way that the first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. PCA was performed on the relative regional volumes (*princomp.m*, MATLAB R2007a Statistics Toolbox). Inter-diagnosis differences in principal components were assessed using Hotelling's T-squared distribution test (*hotellingT2.m*, Matlab Central File Exchange).

Bootstrap Validation

In order to validate the hypothesis that PCA can correctly classify disease groups based on cerebellar shape characteristics alone, we performed a bootstrap validation, leaving out each subject (Subject_{bootstrap}) one at a time. The PCA space was defined without the anatomic information of Subject_{bootstrap}. The relative regional volumes of Subject_{bootstrap} were regressed against the anatomic scores of all subjects minus Subject_{bootstrap} within the PCA space to calculate their principal component scores. The ability to classify the calculated principal component scores of Subject_{bootstrap} into disease groups was tested by calculating the partial probabilities for membership of each Subject_{bootstrap} in a given diagnostic group according to the formula:

$$A_i = \frac{P_i}{\sum_k P_k} \quad (1)$$

$$P_i = 1 - \text{normcdf}(Z_i) \quad (2)$$

$$Z_i = \frac{\|X - \bar{\alpha}_j\|}{\sigma(\alpha_j)} \quad (3)$$

where A_i is the partial probability (the likelihood of resembling control, SCA2, or SCA6 based on cerebellar shape characteristics) of membership for diagnostic group i (control, SCA2, or SCA6). α is the set of elements projected onto the vector between the center of mass of the diagnostic group in the PCA space and the principal component scores of subjects (j) in diagnostic group i (Table 3).

For each subject, the magnitude of the vector between the subject's principal component scores and the center of mass of each diagnostic group (i) was computed (X). Z-score for diagnostic group i (Z_i) for each subject was computed for each disease group based on the magnitude of the vectors between the principal component scores of subjects (j) in diagnostic group i and the center of mass of the diagnostic group i . Probability (P_i) for diagnostic group i was defined as 1-normal cumulative distribution (*normcdf.m*, Matlab R2007a Statistics Toolbox) of Z_i , and partial probability (A_i) was calculated as the ratio of $P_i/\text{sum}(P_{\text{control}}, P_{\text{SCA2}}, \text{ and } P_{\text{SCA6}})$. Receiver operating characteristic (ROC) analysis was performed (*roc.m*, Matlab Central File Exchange) on partial probabilities (A_i) to determine the power of the PCA space to discriminate disease groups.

Results

Inter-rater Reliability

The mean intraclass correlation coefficient for inter-rater reliability was 0.991 as published in our previous work [4].

PCA Separates Disease Groups Based on Cerebellar Shape Index Alone

Relative regional volumes (the ratio of age-adjusted log (regional volume)/age-adjusted log(total cerebellar volume)) are listed in Table 2. The relative volume of corpus medullare in SCA2 subjects differed from the controls ($p=0.0009$), while Crus I trended towards differing ($p=0.08$). SCA6 did not differ from controls. SCA2 differed from SCA6 in the relative volume of corpus medullare ($p=0.006$) and nodulus ($p=0.04$). The MR images of the cerebellum of control, SCA2, and SCA6 subjects are displayed in Fig. 1.

Of the variability, 63.9% was explained by the first three principal components. The principal component coefficients (the relative "weight" of each cerebellar region in each principal component) are displayed in Fig. 2. Both SCA2 ($p=0.0001$) and SCA6 ($p=0.003$) differed from the controls within the first three principal components. SCA2 also differed from SCA6 ($p=0.002$; Fig. 3). Control subjects scanned on a 1.5T MRI ($n=10$) did not differ from controls scanned on a 3.0T MRI scanner ($n=5$) within the first three principal components ($p=0.27$).

Bootstrap Validation Shows Successful Classification of Disease Groups in the PCA Space

The partial probabilities (the likelihood of resembling controls, SCA2, or SCA6 based on cerebellar shape characteristics) for each subject was calculated via a bootstrap validation (Table 3). ROC analysis of the partial probabilities showed that for SCA2, sensitivity and specificity were optimized ($\alpha=0.05$) by a cut-off point at $A_{SCA2}=0.52$, with accuracy of 72.7% and an area under the curve (AUC) of 0.826 ($p<0.001$). For SCA6, sensitivity and specificity were optimized by a cut-off point at $A_{SCA6}=0.52$, with accuracy of 85.7% and an AUC of 0.852 ($p<0.001$).

Patients with Similar Clinical Phenotype Cluster Together in the PCA Space

In a post-hoc analysis, the principal component scores of one patient with SCA3, one patient with EA2, and three patients with ILOCA were calculated as per the method described in the “Bootstrap Validation” section. The SCA3 patient showed the highest partial probability for membership in SCA2 ($A_{SCA2}=0.89$), while the EA2 patient ($A_{SCA6}=0.85$) was closer to SCA6 (Table 4). All three patients with ILOCA showed the highest probability of resembling SCA6 phenotype ($A_{SCA6}=0.99, 0.70$, and 0.84 ; Fig. 4).

Discussion

This is the first study to investigate whether shape characteristics of the cerebellum can predict the diagnosis in unsupervised fashion. Even with the exclusion of the volumetric measurements for pons and the cerebral cortex, which are disproportionately atrophied in SCA2 but spared in SCA6 [2, 3], PCA was able to separate the three groups based solely on cerebellar measurements.

Different Clinical Phenotypes Are Associated with Different Cerebellar Shape Characteristics

We have previously shown that different regions of the cerebellum are differentially affected by the disease process in SCA2, which bestows a unique disease-specific shape characteristic [4]. Now, a bootstrap validation of SCA2 and SCA6 patients has confirmed that these two disease groups, which have complementary clinical phenotypes, also differ in anatomic characteristics. Clinically, SCA2 shows slowing of saccades but sparing of smooth pursuit. This pattern of clinical abnormalities differs from the clinical phenotype observed in SCA6, which shows abnormality of smooth pursuit and sparing of saccadic system [8]. Presumably, these complementary clinical phenotypes are secondary to differential disease-specific pattern of atrophy—SCA2 shows atrophy of the pons but sparing of the flocculus, while SCA6 shows atrophy of the flocculus but relative sparing of the pons [3].

We tested two subjects with known phenotype and found that they clustered with patients with similar anatomic characteristics. More specifically, we found that SCA3 associates with SCA2, while EA2 associates with SCA6 in the PCA space. Thus, SCA2 and SCA6 may represent two separate “archetypes” of cerebellar degeneration. Archetype #1 may represent a pattern of cerebellar neurodegeneration that is common to all cerebellar ataxias that are associated with atrophy of the pontine structure. SCA2 and SCA3 are characterized by severe atrophy of the pontine structure [3, 9]; however, even with the absence of volumetric measurements of the pons, we found that these two disease groups cluster together. One possible explanation is that the degeneration of the pons bestows unique characteristics to the shape of the cerebellum. The pons sends afferents to the cerebellum via mossy fibers then parallel fibers, which preferentially terminate in the declive, tuber/folium, and the uvula of the vermis [10–18]. In contrast, the pyramis and nodulus receive minimal input from the pons [11, 19, 20]. The region-specificity of the pontine afferents may give rise to a

characteristic change in the cerebellar shape as a result of anterograde trans-synaptic degeneration that may follow the degeneration of the pons.

Another possibility is that Archetype #1 may represent cerebellar ataxias that is caused by alteration of the physiology of inositol triphosphate receptor type 1 (ITPR1) of the endoplasmic reticulum (ER). It has been hypothesized that the polyglutamine-expanded forms of ataxin-2 (in SCA2) and ataxin-3 (in SCA3), but not the wild-type, bind to ITPR1 and potentiate mGluR1-mediated calcium release from the ER [21, 22].

Conversely, Archetype #2 may represent disease groups with pure cerebellar atrophy, in our case more specifically involving a mutation of CACNA1a. The EA2 patient resembled SCA6 anatomically, consistent with the fact that the two mutations are allelic and involve mutation of the gene encoding for the pore-forming subunit of CACNA1a [23]. Intriguingly, although patients with EA2 may eventually progress to an SCA6-like picture of pure cerebellar degeneration, this particular patient had no episodes of ataxia for several years, and the total cerebellar volume was normal. Nonetheless, the classification of disease based on the patient's location in the PCA space was still consistent with genotype.

PCA of Shape Characteristics Could Help Us Understand the Pathogenetic Mechanisms Underlying Sporadic Forms of Ataxia

The PCA of cerebellar shape characteristics showed that ILOCA subjects segregated near the SCA6 archetype. It is possible that ILOCA subjects are closer to an unidentified point of convergence in the PCA space that is unique to sporadic forms of ataxia. Alternatively, it may be possible that our ILOCA subjects and SCA6 subjects share similar pathogenetic mechanisms. The patients with sporadic forms of ataxia show a heterogeneous clinical phenotype without identifiable genetic mutation. While our particular set of ILOCA subjects did not vary widely in anatomic phenotype, a study of larger sample of ILOCA subjects may separate patients into different archetypal modes of degeneration. If the similarity in cerebellar shape is indeed a reflection of the similarity in pathophysiology, shape analysis of the cerebellum could aid in diagnosis, prognosis, and disease staging for sporadic forms of ataxia. This is particularly important for sporadic ataxia, which is the most common form of cerebellar ataxia, and the pathophysiology is less well understood.

Limitations

We chose PCA as an unsupervised method to demonstrate the robustness of our findings and its utility for the diagnosis of new, uncharacterized individuals. Certainly, dedicated inter-group comparison with methods such as multivariate analysis of variance (MANOVA) would provide better separation between the different diagnosis groups. Additionally, PCA of raw volumes of cerebellar lobules would also provide better inter-group separation due to the great loss of volume alone [total cerebellar volume loss: SCA2 (30% decrease); SCA6 (31% decrease)]; however, we deliberately sacrificed sensitivity to improve specificity. Because we used relative regional volumes (the ratio of age-adjusted log(regional volume)/age-adjusted log (total cerebellar volume)), any shape differences would be independent of individual size differences and would be less sensitive to any potential differences between the MRI scanners. Even with unsupervised dimensional reduction, the three diagnosis groups separate based on the shape of the cerebellum alone.

Although inter-rater reliability was negligible, the technical challenges associated with manual delineation of regions of interest and the reproducibility of the delineations cannot be ignored as a confounding factor in unsupervised disease classification. It is also theoretically possible that PCA separated individuals based on the MRI scanner differences alone, as SCA2 patients were scanned on a 1.5T while SCA6 patients were scanned on a

3.0T scanner; however, control subjects were indistinguishable on the basis of scanner type. Furthermore, any inter-scanner differences were mitigated by human manual delineation of regions of interest. Differences were also mitigated by performing PCA on the relative regional volume (the ratio of age-adjusted log(cerebellar subregional volume)/age-adjusted log(total cerebellar volume). Macrostructural distortion that is present in cerebellar subregions would also be likely to be present in overall total cerebellum.) rather than raw volumes. Additionally, our findings showed that SCA3 and EA2 patients clustered with SCA2 and SCA6, respectively, despite different scan acquisitions.

Due to the small sample size of our SCA3, EA2, and ILOCA patients, we could only infer the physiological basis of the archetypal separation. The observed trend must be validated by extending the analysis to include a more heterogeneous and larger sample of patients. Furthermore, the differential effect that the varying length of polyglutamine repeats and gender differences may have on the shape analysis must be examined further.

Conclusion

PCA of manually parcellated cerebellar lobules on structural MRI produces a shape index that is able to separate controls, SCA2, and SCA6 on cerebellar measurements alone. This relationship is specific to the cerebellum; pons and cerebral cortex were excluded from the PCA analysis. Lobule-specific characterization of the cerebellar neuro-degeneration could distill complex anatomic variability into clinically meaningful patterns. This could aid in elucidating the mechanisms underlying the pathogenesis of the disease, specifically in sporadic forms of ataxia where the pathophysiology is less well understood.

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Fig. 1.

SCAs show disease-specific pattern of regional cerebellar atrophy. Sagittal (a–c), coronal (d–f), and axial (g–i) views of the cerebellum. As compared to control (d) and SCA6 (f), SCA2 (e) shows significant atrophy of the corpus medullare (central white matters of the cerebellum and the deep cerebellar nuclei). Furthermore, as compared to SCA6 (f), SCA2 shows relative sparing of the posterior-inferior regions of the cerebellum

Fig. 2.

Principal component coefficients of the first three principal components. **a** An illustration of cerebellum with labeled subregions. Colormap indicates principal component coefficients or loadings. (The coefficient of each cerebellar subregion was classified into five equal-size intervals from minimum (coefficients) to maximum (coefficients) for each principal component [*dark blue*: positive coefficients (highest coefficient value); *red*: negative coefficients (lowest coefficient value)].) Principal component coefficients represent the relative “weight” of each original variable (relative cerebellar regional volume) in each principal component. The principal component coefficients are shown for the first three principal components (**b–d**)

Fig. 3.

PCA separates disease groups based on the pattern of cerebellar neurodegeneration. Plot of the first three principal components. (μ indicates center of mass for each disease group.)

Hotelling's T-squared distribution test showed that within the first three principal components, SCA2 ($p=0.0001$) and SCA6 ($p=0.003$) differed from the controls. SCA2 also differed from SCA6 ($p=0.002$)

Fig. 4.

SCA3 clusters with SCA2, while EA2 and ILOCA associate with SCA6 in the PCA space. Archetype #1 (*light green ellipse*) consists of SCA3 (*green circle*) and SCA2 (*white triangles*) in the PCA space. Archetype #2 (*light blue ellipse*) consists of EA2 (*pink circle*), three idiopathic late-onset cerebellar ataxia (ILOCA) subjects (*dark blue circles*), and SCA6 (*gray squares*). The principal component scores of patients with SCA3 ($n=1$), EA2 ($n=1$), and ILOCA ($n=3$) were computed by regressing the relative regional volumes of each subject against the anatomic scores of controls, SCA2, and SCA6. Based on the first three principal component scores, SCA3 showed the highest partial probability of resembling SCA2 ($A_{SCA2}=0.89$) while EA2 resembled SCA6 ($A_{SCA6}=0.85$). The three ILOCA subjects showed the highest probability of resembling SCA6 ($A_{SCA6}=0.99, 0.70, \text{ and } 0.84$)

Table 1

Clinical characteristics of subjects

	Male/female	Age (years) mean (std)	Duration of disease (years) mean (std)	FSFA	1.5T/3.0T (number of subjects)
Controls	1/14	53.1 (10.9)	N/A	0	10/5
SCA2	1/10	54.1 (10.6)	10.9 (5.5)	2.8 (1.1)	11/0
SCA6	2/5	55.7 (11.4)	10.1 (9.1)	2.4 (1.7)	0/7
EA2	1/0	40	30	0	1/0
SCA3	1/0	56	3	1	0/1
ILOCA	1/2	54.3 (8.0)	21.3 (14.2)	1.3 (1.5)	0/3

SCA2 and SCA6 did not differ from controls in age ($p=0.96$ and 0.57 , respectively). SCA2 did not differ from SCA6 ($p=0.58$) in age. The control subjects scanned on 1.5T MRI scanner did not differ in age from the controls scanned on 3.0T MRI ($p=0.74$). [Functional staging score for ataxia (FSFA) ranges from 0 to 6. This is a subset of a neurological rating scale, Unified Ataxia Disorders Rating Scale (UADRS), which emphasizes mobility. FSFA has been shown to correlate with morphometric changes of the pons and the total cerebellum in SCA2 [6].]

Table 2

SCA2 and SCA6 show region-specific pattern of cerebellar atrophy

	Control mean (std)	SCA2 mean (std)	SCA6 mean (std)
I–V	0.816 (0.015)	0.812 (0.016)	0.808 (0.014)
VI	0.831 (0.013)	0.822 (0.017)	0.819 (0.020)
Crus I	0.862 (0.008)	0.853 (0.007)	0.857 (0.006)
Crus II	0.828 (0.011)	0.832 (0.013)	0.826 (0.011)
VIIIB	0.775 (0.013)	0.775 (0.022)	0.785 (0.020)
VIII	0.827 (0.009)	0.820 (0.016)	0.809 (0.014)
IX	0.735 (0.015)	0.736 (0.025)	0.737 (0.020)
X	0.577 (0.017)	0.575 (0.015)	0.542 (0.029)
Corpus medullare	0.808 (0.007)	0.774 (0.013) ^a	0.809 (0.013)
Declive	0.623 (0.021)	0.626 (0.020)	0.589 (0.044)
Tuber/folium	0.586 (0.025)	0.586 (0.035)	0.540 (0.034)
Pyramis	0.632 (0.011)	0.648 (0.022)	0.622 (0.024)
Uvula	0.586 (0.010)	0.582 (0.012)	0.584 (0.014)
Nodulus	0.489 (0.015)	0.497 (0.012)	0.470 (0.020)

The mean age-adjusted relative regional volumes (the ratio of age-adjusted log(individual regional volume)/age-adjusted log(total cerebellar volume) of all subjects were calculated for each region for each disease group. Inter-group differences in relative regional volumes were assessed using Wilcoxon rank-sum test. Bonferroni–Holm method was used to correct for the effects of multiple comparisons

^aCerebellar regions that differ from controls ($p < 0.05$) after correction for multiple comparisons using the Bonferroni–Holm method. SCA2 differed from controls in corpus medullare ($p=0.0009$) and trended towards differing in Crus I ($p=0.08$). SCA6 did not differ from controls ($p>0.1$). SCA2 differed from SCA6 in corpus medullare ($p=0.006$) and nodulus ($p=0.04$).

Table 3

Bootstrap validation confirms that anatomic information can predict diagnosis

Duration of disease (years)	Sex	A_{Control}	A_{SCA2}	A_{SCA6}
SCA2				
1	F	0.04	0.01	0.95
6	F	0.65	0.08	0.27
8	F	0.01	0.71 ^a	0.29
9	F	0.01	0.35	0.65
9	M	0.08	0.83 ^a	0.09
10	F	0.05	0.85 ^a	0.10
12	F	0.08	0.86 ^a	0.06
12	F	0.07	0.61 ^a	0.32
14	F	0.06	0.88 ^a	0.07
18	F	0.01	0.76 ^a	0.24
21	F	0.06	0.89 ^a	0.05
Mean (std)		0.10 (0.19)	0.62 (0.33)	0.28 (0.28)
SCA6				
3	F	<0.01	<0.01	0.99 ^a
5	F	<0.01	0.02	0.97 ^a
6	M	0.15	0.14	0.71 ^a
7	F	0.04	0.32	0.65 ^a
10	F	<0.01	<0.01	0.99 ^a
10	F	<0.01	<0.01	0.99 ^a
30	M	0.73	0.16	0.12
Mean (std)		0.13 (0.27)	0.09 (0.12)	0.77 (0.33)

A bootstrap validation was performed by leaving out each subject (Subject_{bootstrap}) one at a time. PCA was then performed on all subjects minus Subject_{bootstrap}. The principal component scores of Subject_{bootstrap} were computed by regressing the relative regional volumes of Subject_{bootstrap} against the anatomic scores of all subjects minus Subject_{bootstrap}. Partial probabilities for membership of Subject_{bootstrap} in a given diagnostic group were determined based on the computed principal component scores. A_i is the partial probability of membership for diagnostic group i (control, SCA2, or SCA6)

^a Indicates subjects whose predicted diagnosis matched their actual diagnosis (partial probability was equal to or greater than the threshold determined by the ROC curve; SCA2=0.52 and SCA6=0.52). Based on the cerebellar shape characteristics alone, 72.7% (8/11) of SCA2 subjects were correctly classified as SCA2, while 85.7% (6/7) of SCA6 subjects were correctly assigned as SCA6

Table 4
Disease groups with similar clinical phenotype also show similar anatomic characteristics

	Age	Duration of disease	Sex	$A_{Control}$	A_{SCA2}	A_{SCA6}
SCA3	56	3	M	0.06	0.89	0.05
EA2	40	30	M	0.11	0.04	0.85
ILOCA #1	46	34	F	<0.01	<0.01	0.99
ILOCA #2	62	24	M	<0.01	0.16	0.84
ILOCA #3	55	6	F	0.22	0.08	0.70

Partial probability (A_i ; the likelihood of resembling controls, SCA2, or SCA6 based on cerebellar shape characteristics) of membership for diagnostic group i (control, SCA2, or SCA6) was computed as a post-hoc analysis as per the method described in the bootstrap validation section for patients with SCA3 ($n=1$), EA2 ($n=1$), and ILOCA ($n=3$). SCA3 clustered with SCA2 while EA2 associated with SCA6 within the first three principal components. This is consistent with their similarity in clinical phenotype. All ILOCA subjects showed the greatest probability of resembling SCA6