Spinocerebellar Ataxia in Monozygotic Twins

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Context: Although phenotypic heterogeneity in autosomal dominant spinocerebellar ataxia (SCA) has been explained in part by genotypic heterogeneity, clinical observations suggest the influence of additional factors.

Objectives: To demonstrate, quantitate, and localize physiologic abnormalities attributable to nongenetic factors in the development of hereditary SCA.

Design: Quantitative assessments of ocular motor function and postural control in 2 sets of identical twins, one with SCA type 2 and the other with episodic ataxia type 2.

Setting: University laboratory.

Main Outcome Measures: Saccadic velocity and amplitude, pursuit gain, and dynamic posturography.

Results: We found significant differences in saccade velocity, saccade metrics, and postural stability between each monozygotic twin. The differences point to differential involvement between twins of discrete regions in the cerebellum and brainstem.

Conclusions: These results demonstrate the presence of quantitative differences in the severity, rate of progression, and regional central nervous system involvement in monozygotic twins with SCA that must be owing to the existence of nongermline or external factors.

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The study of hereditary neurologic disease in monozygotic twins has been useful for estimating the contribution of nongenetic factors to disease phenotype, although similar studies are lacking in hereditary spinocerebellar ataxia (SCA). We previously reported a wide age range for onset and severity among sibling pairs and presumed monozygotic twins within 2 kindreds of SCA type 6 (SCA6) with identical CAG repeat size within each kindred. This suggested that nongenetic factors might play a role in determining disease severity. Nongermline effects are important to consider in SCA because of efforts to correlate genotype with phenotype, age of onset, and rate or progression of the disease. Moreover, recognizing nongermline effects may help identify approaches that could reduce disease severity. To document and characterize differences in the pattern of neurologic abnormalities, we performed quantitative studies of posture and ocular motor function in 2 sets of identical twins, one with SCA2 and one with episodic ataxia type 2 (EA2), where the twins in each set were discordant for age of onset and clinical severity of ataxia.

METHODS

CLINICAL DATA

The SCA2 twins 101A (firstborn) and 101B, first evaluated at age 65 years, were the products of a normal, full-term gestation, and delivery was noted to be monoplacental at birth. Both twins achieved normal motor milestones and had normal early childhood and adult development. At age 33 years, twin 101B began to feel stiffness of his legs and had difficulty walking down inclines and stairs. At age 55 years, he first noted difficulty with balance and had occasional muscle cramps. At age 60 years, he developed mildly slurred speech, frequent choking, and hand stiffness but no incoordination and was diagnosed as having type 2 diabetes mellitus. Twin 101A played sports until age 35 years and was not conscious of any gait problems until he was 65 years old. In retrospect, he may have had difficulty with balance on ice and walking down stairs at age 55 years. At age 62 years, his wife began to notice that he had imbalance, and the following year he developed rare choking and slurred speech.

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The EA2 twins 63A (firstborn) and 63B, first evaluated at age 43 years, were the products of a normal, full-term gestation, and delivery was noted to be monophasic at birth. Early development was normal for both twins. At age 6 to 7 years, both developed migrainelike episodes of vomiting associated with vertigo and syncope and later developed throbbing headaches and imbalance that frequently occurred with stress. Twin 63A was believed to have more frequent and severe migrainelike episodes. At age 20 years, they both developed mild, progressive imbalance. By age 30 years, the family noted that twin 63A had greater dysarthria, hand incoordination, and gait unsteadiness compared with twin 63B. Twin 63A sustained a concussion in a bicycle crash at age 33 years.

All procedures were conducted with informed consent in accordance with the institutional review board at the University of Minnesota, Minneapolis.

GENETIC STUDIES

Genomic DNA extracted from whole blood from each twin (Puregene System; Gentra Systems Inc, Research Triangle Park, NC) was used for genetic studies. Twins 101A and 101B were members of a known kindred with SCA2 and were screened by polymerase chain reaction using primers DAN1 and UH136 to confirm the presence of identical SCA2 CAG repeat alleles of 23/36 (wild-type/expanded). Twin 63A had normal-sized alleles for SCA1, SCA2, SCA3, SCA6, and SCA8 ataxia genes during routine screening for SCA (University of Minnesota Laboratories, Minneapolis, and Athena Diagnostics, Worcester, Mass).

Although the clinical diagnosis for twins 63A and 63B is EA2 and 63A had a positive response to acetazolamide sodium, mutations in exons 6, 11, 16, 22, 23, 27, 30, 32, 33, 37, 39, and 40 of the gene CACNA1A, which all reported mutations associated with EA2 have been found, were ruled out by dyeoxy sequence analysis of both DNA strands.7,8

For determination of zygosity, the polymerase chain reaction with fluorescent-labeled primers was used to amplify 10 highly polymorphic loci (3 located within genes THO1, FGA, and vWA and 7 in anonymous segments D2S1338, D3S1358, D8S1179, D16S539, D18S51, D19S433, and D21S11), which were analyzed using capillary electrophoresis (ABI Prism model 310; Applied Biosystems, Foster City, Calif.). Genotypes were determined using the Genotyper 2.5 software package (Applied Biosystems). Concordance for all 10 loci predicts that the twins are monozygotic with a greater than 99.98% probability, regardless of the allelic pattern observed (calculations based on allele frequencies observed in 4 major racial groups by the University of Utah DNA Diagnostic Laboratory, Salt Lake City).

OCULAR MOTOR RECORDING AND POSTUROGRAPHY

The horizontal and vertical components of eye movements were recorded using the magnetic search coil technique. Patients were seated in a chair, with the head held stationary, and tracked a computer-guided laser target that was rear projected onto a screen.1 For saccade testing, the target light was displaced from 10° and 19.5° off center to the same distance on the other side of center (in both the horizontal and vertical planes), giving stimulus amplitudes of 20° and 39°. For pursuit tracking, the target light moved sinusoidally 19.5° to either side of center at 0.1 and 0.2 Hz.

The eye signals were sampled at 1000 Hz, and analyses were performed with Matlab routines to estimate the amplitude, velocity, and duration of saccades as well as the amplitude, gain, and phase of the slow (nonsaccadic) component of pursuit eye velocity. Results for the twins were compared with those of 6 age-matched, healthy controls, whose values did not differ from previously studied healthy subjects in the age range of 40 to 65 years.9-11

Postural stability was evaluated using the EquiTest protocol (NeuroCom International Inc, Clackamas, Ore).12 There were 3 trials for each of 6 conditions. The platform was stationary for 1 to 3 and sway referenced for 4 to 6. The visual surround was sway referenced for conditions 3 and 6 and stationary for the other conditions. The eyes were closed for conditions 2 and 3 and open for all others. The equilibrium score that was calculated for each trial is the peak-to-peak amplitude of the estimated angle of the subject’s center of mass, normalized to 12.5° (mean sway angle of control subjects at their limit of stability) and expressed as a percent. A score of 100 means no sway at all. A score of 0 means the patient fell into the safety harness.

Statistical analyses were performed with the Systat computer program (SPSS Inc, Chicago, Ill) and included a multiple multivariate analysis of variance, univariate F statistics, and nonparametric statistics (Mann-Whitney and Kolmogorov-Smirnov tests). Posturography scores were compared with those of a panel of 93 healthy subjects.12,13 Pursuit gain (ratio of peak eye velocity to peak stimulus velocity) was computed for 5 to 10 stimulus cycles, and the values were normalized to the mean value for healthy subjects. The normalized values for both horizontal and vertical pursuit were pooled for each patient and 2-tailed t tests were performed. Normalized velocity histograms for pursuit were compared with the Kolmogorov-Smirnov test.

RESULTS

CLINICAL NEUROLOGIC FINDINGS

Twin 101B had slight restriction of gaze upward and laterally and mildly slowed horizontal saccades, whereas twin 101A had more obvious restriction of upward gaze, hypometric upward saccades, but no clinically visible slowing of horizontal saccades. Both twins had mild spasticity in the upper extremities, mild incoordination of arms, and mild truncal instability, but only twin 101B had incoordination of the legs. Twin 101B walked with a wider base and had more difficulty with tandem gait than did twin 101A. Twin 101B had reduced vibratory and position sense in his feet.

Twin 63A and 63B had downbeat nystagmus in the primary position and in lateral gaze, poor visual pursuits, and dysmetric saccades. Both had mild dysarthria and mild limb incoordination, although twin 63A had greater arm incoordination. Twin 63B had only mild widening of gait base and mild impairment of tandem gait, whereas twin 63A was more severely affected.

OCULAR MOTOR STUDIES

The eye movements had significant abnormalities in all 4 patients. Some features were qualitatively distinct for the different pairs of twins. The EA2 twins had coarse, gaze-evoked, horizontal and downbeat nystagmus (Figure 1A), whereas the SCA2 twins had no nystagmus (Figure 1B) and slower eye movements. Other features were quantitatively different for one twin compared with the other. The Table shows results for pursuit gain and initial saccades directed to the target.
Twins 101A and 101B had abnormal pursuit and saccades and an increase in frequency of microsquare wave jerks (Figure 1B) compared with healthy subjects, although the most severe abnormality was slowed horizontal saccades, as previously reported for SCA2.14 Twin 101A had a greater impairment of saccades and pursuit than did twin 101B. The initial saccades (that were directed to the target light) of twin 101A had a significantly lower velocity compared with those of twin 101B for the 39° horizontal target (Figure 2B) and showed a trend for lower velocities for all other targets (Table). Figure 3B shows that the initial saccades of twin 101A were also more dysmetric than the saccades of twin 101B. The saccadic gains for twin 101A were significantly lower for the 39° up target and higher for both the 20° up and horizontal targets. The gains for the other vertical targets showed similar trends.

Pursuit was also significantly worse for twin 101A compared with twin 101B. The pursuit gain was significantly lower for twin 101A compared with his brother (Table). In addition, eye velocity was lower throughout the stimulus cycle. Figure 4 shows the normalized, cumulative eye velocity histogram for a horizontal target at 0.2 Hz. The histogram is based on 9 stimulus cycles for each twin and 29 cycles for each of 3 healthy subjects. The ordinate values were normalized throughout the trial (1 indicates 100% of velocities over a trial), and the abscissa values were normalized to peak target velocity (designated as 1). Rightward (up) and leftward (down) velocities were averaged together. Both twins had probabilities greater than normal (above the lightly shaded region) for velocities less than 1. In other words, eye velocity was less than normal throughout the stimulus cycle, representing a greater percentage of the total range of eye velocities. The deficit was greater for twin 101A for horizontal compared with vertical pursuit but was significant for both directions ($P<.01$; Kolmogorov-Smirnov test).

**EA2**

Both EA2 twins showed significant abnormalities compared with healthy subjects in saccade velocity, saccade metrics, and pursuit gain. Figure 2A shows that the velocity of horizontal saccades for both twins 63A and 63B was frequently, but not always, lower than normal, and there were significant differences between the twins (Table). However, for twin 63A the amplitudes of the initial saccades directed to the 39° horizontal target (solid circles in Figure 2A) were significantly lower compared with twin 63B (open circles in Figure 2A). Also, the vertical saccades for twin 63A were significantly more dysmetric compared with those of her sister: lower amplitudes for upward (20° and 39°) and downward (39°) targets and higher for the 20° downward target (Table).

The saccade dysmetria is further illustrated in Figure 3, which shows the saccade gain for both twins compared with the normal range (shaded region). Twin 63A had lower gains for the 39° horizontal and 20° up targets, and there was a trend for lower gains for the 20° horizontal and 39° vertical targets. The upward and downward saccades to the 20° target showed the most significant differences between the twins. For downward saccades to the 20° target, twin 63A had higher amplitudes because the saccades were hypermetric compared with healthy subjects. The ratios of the mean upward to mean downward saccade gains for the 20° target were 0.47 for twin 63A and 0.96 for twin 63B. A statistical analysis on the ratio of consecutive pairs of upward and downward saccades showed these ratios to be different ($P<.006$). The ratios for saccades to the 39° target were not significantly different (0.84 and 0.86 for twins 63A and 63B, respectively).

In addition to the saccadic abnormalities, the gain for pursuit was significantly reduced (Table) for twins 63A and 63B compared with healthy subjects, but there was no significant difference between them. Also, the cumulative eye velocity histogram did not show any difference between the twins. However, despite the similarities in pursuit tracking, twin 63A showed significantly slower and more dysmetric saccades in both directions compared with her twin sister.

**POSTUROGRAPHY**

For both sets of twins, postural sway was greater than for healthy controls (Figure 5, gray regions), but there were significant differences between twins in both sets (Figure 5, solid and open bars).
SCA2

Twins 101A and 101B both showed significantly lower equilibrium scores (greater sway) compared with healthy subjects for some (P < .02) but not all 6 test conditions. Both twins had low scores for condition 2, indicating that the diabetes of twin 101B did not contribute to the postural instability for this part of the posturography test. However, when compared with each other, twin 101A had lower scores for conditions 1 and 4 and showed a trend for lower scores for conditions 3 and 5 compared with twin 101B, whereas twin 101B had lower scores for condition 6. These differences imply that twin 101A had a greater vestibulospinal deficit (condition 5) and a greater impairment with visual-vestibular interactions (conditions 1 and 4) vis-à-vis the control of postural stability.

EA2

The equilibrium scores for twin 63A were significantly lower compared with normal scores for all test conditions (P < .03). For twin 63B, the scores were slightly lower than normal for conditions 1 to 3 (P < .05), and there was a trend for lower scores for conditions 3 and 5. Comparing the 2 twins with each other, twin 63A had significantly lower scores for conditions 1, 4, and 5 and showed a trend for lower scores for all the other conditions. Twin 63A also had latencies of the long-loop reflexes in both legs that were greater than normal and greater than twin 63B (for forward movements of the platform) (Table). These differences imply a greater deficit in vestibulospinal reflexes (condition 5) and visual-vestibular interactions (conditions 1 and 4), similar to the deficits in the SCA2 twins.

COMMENT

We used quantitative techniques to demonstrate significant differences in eye movements and postural stability in 2 sets of monozygotic twins. Bedside neurologic examination and quantitative analysis of ocular motor function indicated that SCA2 twin 101A had more severely affected eye movements than his twin. The more dysmetric and slower saccades and more abnormal pursuit in twin 101A could be owing to a greater involvement of the pontine gaze areas (including the saccadic burst neurons) in addition to the cerebellum. Twin 101B had a greater problem with posture control than twin 101A. The posturography results were consistent with the history and clinical findings, indicating that he had an earlier onset and was more severely affected by balance and gait problems. Although twin 101A had slightly greater postural instability for some test conditions, he performed at the lower limit of normal for condition 6. In contrast, twin 101B was severely abnormal for condition 6, indicating more severely impaired vestibulospinal pathways in combination with less effective suppression of inaccurate visual information compared with twin 101A. In addition, twin 101B showed a trend for increased latencies of the long-loop reflexes of both legs. This could be due in part to the diabetes...
mellitus in twin 101B and would contribute to symptoms of postural instability.

Similarly, the history and quantitative analyses showed the EA2 twin 63A to be more severely affected than her sister. Twin 63A had more dysmetric saccades and greater abnormalities of postural sway compared with twin 63B. The saccadic abnormalities could be owing to a more severe involvement of the posterior midline areas of the cerebellar cortex (lobules VIc and VII, the oculomotor vermis), although the deep cerebellar nuclei (ie, the fastigial nucleus) could also be involved. The greater postural abnormalities indicate a more severe involvement of vestibulospinal pathways and the cerebellar nodulus and uvula. Thus, the physiologic measurements indicate differences between monozygotic twins with SCA with respect to the severity of involvement of certain brain regions in the degenerative disease process.

This study confirms the existence of nongermline factors that have differentially affected each member of 2 sets of monozygotic twins with SCA. Earlier age of onset itself cannot be directly responsible for the greater severity of many of the neurologic features because twin 101B had earlier age of onset but less severely abnormal eye movements. Several genetic mechanisms could be responsible for the differences in severity, age of onset, and regional involvement of central nervous system structures for identical twins affected with hereditary ataxia. The SCA2 mutation consists of an expansion of an unstable trinucleotide repeat that encodes a pathologic, elongated polyglutamine tract. Somatic mosaicism of trinucleotide repeat expansions has been documented in several trinucleotide repeat disorders. Stochastic occurrence of such mosaicism in affected tissues might account for some clinical and physiologic differences between the twins. Other possible nongermline genetic factors that also could play a role in modulating the effect of mutations in both sets of twins include the occurrence of somatic mutations, differential heteroplasmy of
disease-modifying mitochondrial mutations, and, in the case of female twins 63A and 63B, differential Lyonization of heterozygous X chromosome modifier loci. Finally, although a $\text{CACNA1A}$ mutation in twins 63A and 63B has not yet been identified, unexplained variable penetrance within EA2 families bearing the same $\text{CACNA1A}$ mutation is well recognized.

Other medical and environmental factors, including the discordance for diabetes mellitus, differences in diet, consumption of alcohol, and other unrecognized childhood infections or head trauma, might also alter disease severity. It is likely that diabetes mellitus plays a role in the development of peripheral neuropathy in twin 101B. However, SCA2 is also associated with peripheral neuropathy. Although the trend for greater latencies of the long-loop reflexes in twin 101B might be explained by a deficit in peripheral nerve function owing to diabetes, the much earlier age of onset of gait symptoms (which preceded the clinical onset of diabetes by 20 years) and the lack of any difference between the twins in sway for posturography test condition 2 make this unlikely. Moreover, in other tests, twin 101B performed better than his twin, arguing against a harmful effect of diabetes. Finally, greater severity of cerebellar abnormalities in twin 63A may relate to her greater frequency of attacks of migraine and ataxia symptoms, supporting the importance of cessation of attacks with acetazolamide.

In summary, the present results have (1) documented the existence of nongermline or external factors that alter the severity and rate of progression of degenerative cerebellar ataxia and that may complicate efforts at making genotype-phenotype correlations; (2) given a precise characterization and quantification of physiologic differences between twins that manifest discordant clinical abnormalities that are difficult to quantify; and (3) identified potential anatomic sites that might give rise to the functional differences. Additional studies of hereditary, neurologic disorders in monozygotic twins should give further insight and help to identify factors important in the pathogenesis of degenerative diseases.
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