

Analysis of Cerebral Shape in Williams Syndrome

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Background: As a neurobehavioral disorder with a specific neurocognitive profile and a well-defined genetic etiology, Williams syndrome (WMS) provides an exceptional opportunity to examine associations among measures of behavior, neuroanatomy, and genetics. This study was designed to determine how cerebral shape differs between the brains of subjects with WMS and those of normal controls.

Subjects: Twenty adults with clinically and genetically diagnosed WMS (mean \pm SD age, 28.5 \pm 8.3 years) and 20 healthy, age- and sex-matched controls (mean \pm SD age, 28.5 \pm 8.2 years).

Design: High-resolution structural magnetic resonance imaging data were used for shape-based morphological analysis of the right and left cerebral hemispheres and the corpus callosum. Statistical analyses were performed to examine group differences.

Results: Both right and left cerebral hemispheres of subjects with WMS bend to a lesser degree in the sagittal plane than normal controls ($P < .001$). The corpus callosum also bends less in subjects with WMS ($P = .05$). In addition, subjects with WMS have decreased cerebral ($P < .001$) and corpus callosum ($P < .001$) midline lengths.

Conclusions: Subjects with WMS have significantly different cerebral shape from normal controls, perhaps due to decreased parieto-occipital lobe volumes relative to frontal regions. The similar observation in the corpus callosum may be associated with a decreased size of the splenium in WMS. These findings may provide important clues to the effect of genes in the WMS-deleted region on brain development.

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WILLIAMS syndrome (WMS) is a rare genetic disorder caused by a hemizygous deletion on the long arm of chromosome 7.¹⁻⁵ It is characterized by a variety of physical manifestations, including infantile hypercalcemia, supra-aortic stenosis, other cardiac and vascular problems, as well as delayed motor and cognitive development.⁶⁻⁸ Adolescent and adult individuals with WMS also characteristically manifest an unusual profile of neurocognitive strengths and weaknesses. In the context of general cognitive impairment and difficulties in problem solving, language is relatively spared in WMS. Visuospatial abilities are dramatically impaired, however, with drawings and block design exhibiting fractionated attention to detail; in contrast, face processing is remarkably spared, remaining at the level of normal controls.⁹⁻¹⁴ This cognitive profile of peaks and valleys of abilities suggests that the deleted genes associated with WMS may

lead to uneven effects on brain development and function.¹⁰

Previous neuroimaging studies suggest that subjects with WMS have whole-brain volumes that are reduced by approximately 13% when compared with normal controls, though cerebellar volume is typically preserved.¹⁵⁻¹⁷ Recent findings using higher resolution scans show that the reduction in cerebral volume is not uniform throughout the brain, but instead follows a topographic pattern that suggests a neuroanatomical substrate for some neurobehavioral features occurring in this condition. For example, compared with normal controls, the occipital lobe is disproportionately reduced in WMS, particularly on the right, while there is a proportional increase in the volume of the superior temporal gyrus (STG), cerebellum, and the frontal lobe.¹⁷ The pattern from these data indicates that subjects with WMS may have gross differences in brain morphology compared with individuals with normal brain development.

SUBJECTS AND METHODS

SUBJECTS

Twenty subjects with WMS (13 women and 7 men; mean age, 28.5 ± 8.3 years; age range 19-44 years) and 20 normal controls individually matched for age and sex (13 women and 7 men; mean age, 28.5 ± 8.2 years; age range 19-48 years) were studied. Each subject gave informed consent for their participation in the study. All subjects underwent a battery of cognitive probes, neurophysiological studies, magnetic resonance imaging (MRI), and molecular genetics studies that were provided within the context of a multisite research program based at the Salk Institute for Biological Studies, La Jolla, Calif.

The diagnosis of WMS was confirmed genetically in all subjects²⁰ with WMS using fluorescent in situ hybridization probes for *elastin*, a gene found in the critical 7q11.23 deletion region.^{1,3,5} In addition, diagnosis of WMS was performed clinically, either by a medical geneticist or other physician familiar with this condition. All diagnoses were further confirmed using the WMS diagnostic scoresheet, developed by the Medical Advisory Board of the Williams Syndrome Association, Clawson, Mich. Subjects were excluded if they had any other neurological or neuropsychiatric conditions that were not typically associated with WMS. Fourteen of the subjects with WMS and their age-matched controls were part of our earlier whole-brain volumetric study.¹⁷

IMAGING

Magnetic resonance images of each subject's brain were acquired using a 1.5-T GE-Signa Scanner (General Electric Co, Milwaukee, Wis). The images were acquired in the sagittal plane with a volumetric 3-dimensional radio frequency spoiled gradient echo protocol. The scan parameters were: time to repeat, 24 milliseconds; echo time, 5 milliseconds; flip angle, 45° ; number of excitations, 2; matrix size, 256×192 ; field of view, 24 cm; and slice thickness, 1.2 mm. All but 2 of the 40 scans were acquired at the University of California, San Diego, Medical Center. The remaining 2 scans, both controls, were acquired using an identical scanner and pulse sequence at Stanford University Medical Center, Stanford, Calif. Image processing and analysis were performed at the Stanford Psychiatry Neuroimaging Laboratory, Stanford.

All scans were imported into the program *BrainImage 3.X*²⁰ for semiautomated removal of nonbrain tissue. Subsequent manipulations and measurements were also performed in the *BrainImage* environment. All raters were blinded to the group identity of the subjects.

The calculation of bending angle was performed identically for both cerebral and corpus callosum regions of interest (ROIs) using a semiautomated computer algorithm

based on the "curved line" method^{21,22}; this computer implementation has been used in previous studies of corpus callosum morphology in our laboratory.²³ The algorithm determines the *midline* of the ROI, defined by the midpoints of lines drawn perpendicular to the ROI surface (**Figure 2**). *Bending angle* is defined as the angle whose vertex is the midpoint of the ROI midline, and whose nodes are the most anterior and posterior points on the midline. The length of the midline, and therefore the length of the structure, is automatically calculated with this algorithm.

CORPUS CALLOSUM MEASUREMENTS

The drawing of ROIs and measurement of each brain was performed based on a previously established protocol.²³ In brief, each brain was rotated in a multiplaner viewer in *BrainImage* until the best midsagittal view was acquired. The determination of the best midsagittal slice was based on the clarity and distinction of the corpus callosum, cerebellar vermis, cerebral aqueduct, and spinal cord. The ROIs were then hand drawn around each corpus callosum, and the bending angle algorithm was applied. Interrater reliability for midline length and bending angle in datasets¹⁰ was 0.98 and 0.93, respectively, as determined by the intraclass correlation coefficient.

CEREBRAL MEASUREMENTS

A 3-dimensional Talairach-based stereotaxic grid was applied to each brain.^{19,24,25} This grid is proportional, adjusting to the size and shape of each individual brain. The sagittal slice exactly one fourth of a Talairach sector away from the midsagittal line (approximately 5 mm) was extracted for both the left and right hemispheres. Because these slices were chosen in Talairach space, they were parallel to the midsagittal plane and in proportionally the same neuroanatomical location in each brain analyzed, just medial to the head of the caudate nucleus.

On each of the selected bilateral sagittal slices, the posterior fossa was circumscribed using methods based on a previously validated protocol.²⁶ The corpus callosum also was removed from each of the cerebral slices. The bending angle algorithm was then applied to the cerebral ROIs as described for the corpus callosum above. The reliability for midline lengths and bending angles of the right and left cerebral regions was 0.98 or higher as defined from 10 datasets.

DATA ANALYSIS

To ensure the validity of using parametric statistics, all data were first visually inspected for normality. Analyses of variance and covariance were then performed. Because initial analyses suggested an association between age and both bending angle and midline length, age was used as a covariate in all calculations. A 2-tailed *P* value of .05 or less was used as the significance level for all analyses.

The initial study of brain shape described here represents a follow-up to our most recent description of the WMS brain.¹⁷ In particular, the analyses are based on the consistent observation that the occipital lobe and cerebellum of individuals with WMS fail to conform to a standard stereotaxic grid used in our neuroimaging labora-

tory as well as others.¹⁷⁻¹⁹ This is the first study designed to quantify these previous qualitative observations in WMS (**Figure 1**) by directly measuring cerebral shape differences in WMS, as well as differences in the shape of the cerebrum's most prominent white matter structure, the corpus callosum.

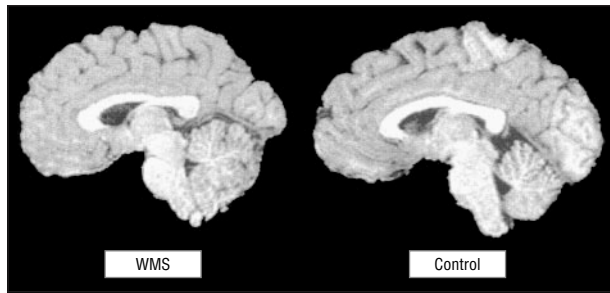


Figure 1. Midsagittal magnetic resonance imaging slice of a subject with Williams syndrome (WMS) and an age- and sex-matched control, demonstrating the qualitative cerebral shape differences in WMS. Note the significantly decreased bend of the cerebrum and the decreased size of the posterior cerebral regions in the WMS brain.

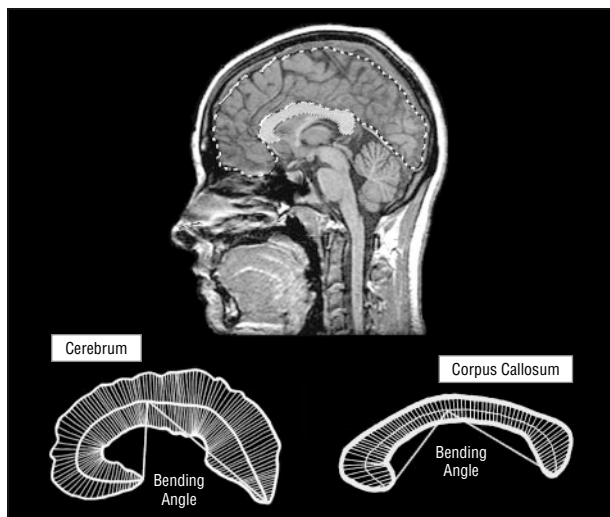


Figure 2. The curved line method of shape analysis. Multiple lines are drawn perpendicular to the surfaces of the corpus callosum or cerebrum (the number of lines drawn by the computer version is limited only by the resolution of the scan). The midline is defined as the line bisecting all of these lines. Bending angle is defined as the angle with a vertex at the point of bisection of the midline and nodes at the most anterior and posterior points on the midline. When measuring cerebral bending angle and midline length, the region of interest is not drawn on the midsagittal slice (as shown here for simplicity) but on a slice one fourth of a Talairach sector away from the midsagittal plane (adapted from Allen et al).

RESULTS

CORPUS CALLOSUM

As shown in **Figure 3A**, the corpus callosum bending angle is significantly larger in subjects with WMS than in controls ($F=17.45$, $P<.001$). The corpus callosum midline length, however, tends to be much smaller in subjects with WMS ($F=22.04$, $P<.001$) (**Figure 4A**). Because the midline length of the corpus callosum could affect the bending angle, an analysis of covariance was performed using midline length and age as covariates. This analysis suggested that the WMS corpus callosum bending angle was still larger after covarying for length and age ($F=4.2$, $P=.05$).

CEREBRAL MEASUREMENTS

In congruence with corpus callosum bending angle measurements, the analyses showed that subjects with WMS

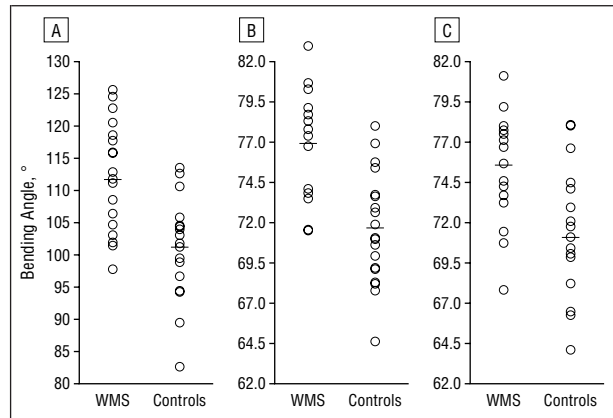


Figure 3. Bending angle measurements of the corpus callosum and cerebrum in a group of 18 patients with Williams syndrome (WMS) and 18 age- and sex-matched controls. A, Corpus callosum; B, left cerebrum, and C, right cerebrum.

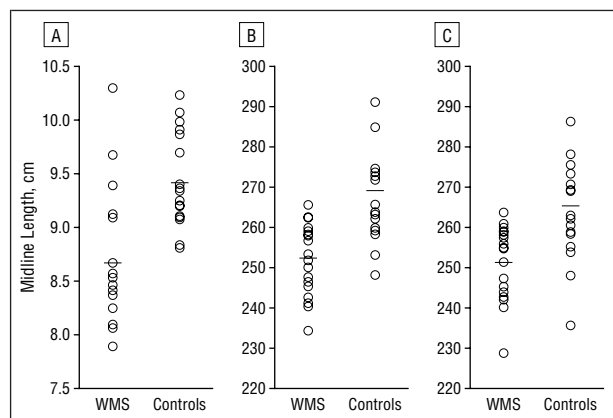


Figure 4. Midline length measurements of the corpus callosum and cerebrum. A, Corpus callosum; B, left cerebrum; and C, right cerebrum.

have cerebral bending angles significantly larger than normal for both the left (analysis of variance, $F=25.7$, $P<.001$) and right ($F=14.1$, $P<.001$) cerebral hemispheres (**Figure 2B-C**). Analyses of covariance covarying for age and midline length also indicated significant group differences ($F\geq 7.5$, $P=.009$).

Paralleling corpus callosum findings, we found cerebral midline length to be smaller in the WMS group for both left and right hemispheres ($F\geq 17.9$, $P<.001$ for both hemispheres). All results are summarized in the **Table**.

COMMENT

Global measures of cerebral shape in persons with WMS differ significantly from those of normal controls. Specifically, the overall length of both cerebral hemispheres is significantly smaller in the WMS group. In addition, WMS brains bend in the sagittal plane less than control brains. This group difference in bending angle could come from several sources: either the frontal lobe or the parieto-occipital lobe could be straighter, or brain volume could be disproportionately reduced in particular regions. Since previous studies have indicated that the volume of the frontal lobe is relatively spared in WMS,¹⁵

Summary of Experimental Findings*

Measurement	WMS	Control	Anova <i>P</i>	Ancova <i>P</i>
Corpus callosum				
Bending angle, degrees	112.1 ± 8.4	101.8 ± 7.9	<.001	.05†
Midline length, cm	8.67 ± .59	9.43 ± .43	<.001	<.001‡
Cerebrum				
Left cerebral bending angle, degrees	76.9 ± 3.1	71.7 ± 3.4	<.001	<.001†
Right cerebral bending angle, degrees	75.4 ± 3.1	71.4 ± 3.7	<.001	.01†
Left cerebral midline length, cm	252.91 ± 9.0	267.85 ± 10.87	<.001	<.001‡
Right cerebral midline length, cm	251.43 ± 8.85	265.30 ± 11.67	<.001	<.001‡

*All the measured structures show decreases in midline length in William syndrome (WMS) and increases in bending angle when compared with normal controls. Unless otherwise indicated, data are mean ± SD. ANOVA indicates analysis of variance; ANCOVA, analysis of covariance.

†Covaried for age and midline length of the corresponding structure.

‡Covaried for age.

we believe that the basis of the shape differences lies in variation in the parieto-occipital region.

The measurement of corpus callosum shape in WMS parallels the cerebral findings. The corpora callosa of WMS subjects are shorter and tend to curve less than those of normal controls, though the group differences are smaller than that observed in the cerebrum. Though our results of shorter corpora callosa are in accord with previous findings, an earlier study found no significant shape differences between the corpora callosa of subjects with WMS and those of normal controls.²⁷ This discrepancy is most likely due to different metrics: bending angle in this study vs circularity in the previous study, the latter representing a ratio of corpus callosum length over height. Though both measures are sensitive to disproportionate differences in shape, bending angle is more sensitive to small shape differences near the anterior and posterior extremes. Since the rostral fifth of the corpus callosum has been found to be relatively preserved in subjects with WMS,²⁷ it is possible that shape differences are due to a reduction in the size of the splenium. Our laboratory's preliminary measurements of corpus callosum size have indicated that both the splenium and the isthmus (posterior body) are reduced in WMS.²⁸ The finding of decreased splenium size in WMS supports both the neuroanatomical evidence of decreased white matter and occipital lobe volume¹⁷ as well as the neurobehavioral findings of visuospatial deficits in this condition because it is the splenium that connects bilateral parieto-occipital lobe regions.²⁹

Other investigators have used the "mean callosal curvature," a ratio of the bending angle divided by the midline length, to subtract out a possible effect of midline length on bending angle.²¹ However, such a measure may actually amplify differences between 2 groups, increasing the chances of finding a significant difference. In addition, by combining bending angle and midline length into 1 ratio, this measure makes it difficult to determine which of the 2 variables is contributing more to an observed difference between groups. Nevertheless, to provide data that can be compared across studies, an analysis of variance of mean callosal curvature (bending angle over length) was conducted. This analysis indicated a significant difference between subjects with WMS and normal controls ($F=28.0, P<.001$). The difference in mean cerebral cur-

vature also was significant for both left and right hemispheres ($F=49.6, P<.001$; $F=25.01, P<.001$, respectively).

As a syndrome with a proven genetic origin, it is likely that the neuromorphologic variations observed in WMS are caused by aberrant brain development. Both the corpus callosum and the cerebral hemispheres develop in a rostrocaudal direction.^{30,31} Premature termination of brain development on the rostrocaudal axis could produce cortical shapes much like that in the subjects with WMS described here. Furthermore, there are several genes in the 7q11.23 region that are differentially expressed in the brain, including syntaxin, *CYLN2*, *LIM-kinase1*, and *WBSCR11*.³²⁻³⁵ Hemizygosity for *LIM-kinase1*, for example, has been correlated with visuospatial impairment for both subjects with WMS and subjects with microdeletions of only the *ELN* and *LIM-kinase* genes.³⁶ Though the function of *LIM-kinase1* is unknown, proteins with LIM domains are implicated as developmental regulators of cell differentiation.³⁷

Another gene in the WMS critical region, *FZD9* (formerly known as *FZD3*, the human homologue of *Drosophila's* frizzled gene), is expressed strongly in adult brains and seems to play a key role in global brain development.³⁸ *FZD9* is related to the *Wnt* gene family, the genes of which encode for secreted signaling glycoproteins and are known to be involved in controlling early cell development, tissue differentiation, segmentation, and dorsal-ventral polarity.³⁹ A gene with such properties is a likely candidate for controlling the development along the anterior-posterior axis. Indeed, recent findings have found that the mouse homolog of *FZD9*, called *Fzd9*, is highly expressed in the central nervous system during its development and is expressed most strongly in the telencephalon.⁴⁰ Furthermore, the pattern of expression during development varies along the rostrocaudal axis. The *FZD9* gene has also been implicated in the development of midbrain and cerebellar structures.³⁸ Further studies focused on associations among neuroanatomy, neuropsychology, and neurogenetics in WMS are likely to reveal important information regarding the neurobiological origins of the WMS phenotype.

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