Cerebellar Volume in Offspring From Multiplex Alcohol Dependence Families

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Background: *Increased susceptibility for developing alcohol dependence (AD) might be related to structural differences in brain circuits that influence the salience of rewards and/or modify the efficiency of information processing. The role of the cerebellum in regulating cognitive functions is being increasingly recognized along with its well-known influence on motor performance. Additionally, developmental changes in cerebellar volume during adolescence have been reported.*

Methods: *Magnetic resonance imaging was used to measure the cerebellum in 17 high-risk adolescent and young adult offspring from multiplex alcohol dependence families and 16 control subjects matched for gender, age, and IQ.*

Results: *High-risk (HR) adolescents/young adults showed increased total cerebellum volume and total grey in comparison with control subjects. Age-related decreases in total grey volume were seen with age, a pattern that was not seen in HR offspring.*

Conclusions: *Offspring from multiplex families for AD manifest genetic susceptibility by having larger cerebellar volume, which seems to be related to lesser grey matter pruning for age. Larger cerebellar volumes in adult obsessive compulsive disorder (OCD) patients have been reported. This suggests a possible similarity in structural underpinnings for alcohol dependence and OCD.*

Key Words: Cerebellum, high-risk offspring, alcohol dependence, MRI

There is ample evidence that the offspring of alcoholic parents are at increased risk for developing alcohol dependence (Goodwin et al 1973; Heath et al 1999; Pickens et al 1991). Twin and adoption studies have demonstrated significant heritability and suggest the presence of significant genetic mediation of this greater susceptibility. Children/adolescents from multiplex families in which multiple cases of alcohol dependence are segregating carry an especially high lifetime risk for developing alcohol dependence. Longitudinal studies that are ongoing in our laboratory document that offspring from multiplex families are at increased risk for developing some form of child/adolescent psychopathology (Hill et al 1999a) and have an earlier age of onset to begin drinking with greater consequences when they drink (Hill and Yuan 1999; Hill et al 2000a). Additionally, they exhibit subtle developmental delays in neurological capacity (Hill et al 1999b, 2000b).

Age-related improvement in postural balance is a feature of child/adolescent development that has been documented in many laboratories (Hill et al 2000b; Shumway-Cook and Woollacott 1985; Usui et al 1995). A longitudinal follow-up at annual intervals during childhood and adolescence has demonstrated that high-risk children exhibit greater body sway than control subjects and seem to be delayed in the acquisition of age-appropriate postural control (Hill et al 2000a), confirming earlier reports based on cross-sectional data suggesting that children of alcoholics show greater body sway (Hegedus et al 1984; Hill and Steinhauer 1993; Lester and Carpenter 1985; Lipscomb et al 1979). Additionally, high-risk offspring show differing developmental trajectories of age-related P300 amplitude (Hill et al 1999b).

Neuropsychological performance for offspring of alcohol-dependent individuals has been reported to be diminished for both verbal (Knop et al 1985) and nonverbal problem-solving skills, including Block Design (Sher et al 1991) and Halstead Category errors (Drejer et al 1985). In comparison with control children, high-risk offspring have been reported to have lesser school achievement (Knop et al 1985; Marcus 1986; Sher et al 1991) although some studies have found no differences between offspring of alcohol-dependent parents and control subjects (Reich et al 1993; Vitaro et al 1996) or have reported only limited achievement deficits (Hill et al 1999a). Together, the neuropsychological, event-related potential (ERP), and postural results suggest that subtle differences in neurocognitive capacity might be characteristic of offspring from alcoholic families. With the increased recognition that the cerebellum has a role in cognitive processing as well as motor performance, it seems that the cerebellum is a good candidate structure for developmental studies.

Although neuroimaging studies have become quite common in the alcoholism literature, the emphasis has been either on documenting brain pathologies in long-term adult alcoholics (Sullivan et al 2003) or in outlining consequences of adolescent substance abuse (DeBellis et al 2000); DeBellis et al have reported that alcohol-dependent adolescents have significantly smaller hippocampal volume in comparison with age- and gender-matched control subjects. Similarly, Brown et al (2000) have shown that alcohol-dependent adolescents show significantly poorer neuropsychological performance, including lower Wechsler Intelligence Scale for Children–Revised (WISC-R) Vocabulary, Information, Similarities, and Coding scores, and reduced reproduction retention rates in comparison with control subjects. These observed differences in alcohol-dependent youth could be the result of alcohol use or, alternatively, might be neurobiological markers of vulnerability that are present before alcohol initiation. Finally, a previous report based on data from the present sample revealed amygdala differences in these high-risk offspring with minimal alcohol exposure (Hill et al 2001).

On the basis of the longitudinal data suggesting that high-risk children experience developmental delays in acquiring age-

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Received August 4, 2005; revised January 10, 2006; accepted January 13, 2006.

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2006.01.007

appropriate postural control (Hill et al 2000b) and age-appropriate P300 (Hill et al 1999b), we hypothesized that brain areas that are changing rapidly during adolescence might show volumetric differences between high- and low-risk children/adolescents. Both progressive and regressive processes are characteristic of adolescence (Cowan et al 1984; Giedd et al 1996a, 1996b, 1999; Jernigan and Sowell 1997; Jernigan and Tallal 1990; Jernigan et al 1991; Paus et al 1999; Pfefferbaum et al 1994; Thompson et al 2000). In contrast to the decrease in cortical grey matter that is seen in association with developmental changes up to the age of 30, there is evidence for continued growth during the same developmental period in subcortical limbic structures, including the septal area, hippocampus, and amygdala, areas that show an increase in volume (Jernigan and Sowell 1997). Previous analyses of this data set targeted the amygdala and hippocampus for volumetric analysis with a region of interest (ROI) technique, finding smaller volume of the right amygdala (Hill et al 2001). This result suggests a slower rate of growth for this limbic region in offspring at higher risk for developing alcohol dependence as a result of their familial/genetic background (Hill et al 2001).

Because the cerebellum contributes to neurocognitive and motor functioning (Desmond and Fiez 1998), we hypothesized that cerebellar volumes might differ between children at high or low risk for alcoholism. The cerebellum, like the prefrontal cortex, reaches maturity rather late, with changes occurring into the late 20s and early 30s (Diamond 2000). Moreover, cerebellar volumes have been found to differ from control subjects in patient samples of children with autism (Townsend et al 2001), attention-deficit/hyperactivity disorder (ADHD) (Castellanos et al 2002), and schizophrenia (Jacobsen et al 1997). Also, longitudinally obtained magnetic resonance images (MRIs) of normal children and children with psychopathology have now documented developmental changes in cerebellar volume (Castellanos et al 2002), although the direction and amount of this change are not well understood.

Methods and Materials

Subjects

A total of 33 adolescent/young adult male participants (17 high-risk participants and 16 low-risk control subjects) were studied. The high-risk group and a subset of the control subjects ($n = 22$) were members of the larger longitudinal cohort of offspring from high-density for alcoholism pedigrees initiated in 1990. The high-risk offspring were from multiplex alcoholism families selected through the presence of a pair of adult alcoholic brothers. As a result, each high-risk offspring had an average of four first- and second-degree relatives who were alcoholic. Low-risk offspring from the larger longitudinal cohort were identified through their families who were selected for absence of axis I psychopathology. Low-risk historical control subjects ($n = 11$) were similarly selected for absence of axis I psychopathology.

Participants who were members of the longitudinal cohort had been followed an average of 7.3 years (± 2.1 SD) at the time the scans were performed. Yearly evaluations included a clinical interview to determine diagnostic status and collection of neuropsychological test data. Yearly evaluations also included ERP and sway assessments. Because a subset of the control subjects were not part of the longitudinal study, sway and ERP data were not available for them. Accordingly, the present report does not include these data. Participants who were members of the longitudinal cohort were evaluated for neuropsychological performance on the occasion of their initial follow-up visit to our laboratory. This included administration of subtests from the WISC-R and administration of the Peabody Picture Vocabulary Test (PPVT-R) for determination of IQ. The PPVT-R was used as an indicator of overall intelligence, because it does not require reading skill for completion. The WISC-R subtests given at the baseline evaluation included Vocabulary and Block Design.

Table 1. Demographic Characteristics of the Adolescent/Young Adult Males

	High-Risk		Low-Risk		Statistic	
	Mean	SD	Mean	SD	*t*	*p*
Age	17.6	2.9	17.5	2.2	.20	ns
SES	40.7	10.6	41.3	8.8	.19	ns
IQ	110.1	16.2	113.4	12.4	.51	ns
Height (cm)	180.3	8.9	178.2	7.6	.47	ns
Weight (kg)	84.6	17.4	75.5	16.8	1.54	ns

Degrees of freedom = 1,31. SES, socioeconomic status.

Because of the known variability in volume of brain structures during development (Giedd et al 1996a, 1996b; Pfefferbaum et al 1994), a yoked control design was used in which a control subject was selected on a case-by-case basis to match each high-risk case with gender and age (within 6 months) as matching characteristics. Where an appropriate gender- and age-matched control subject was not available from the longitudinal follow-up, an historical control was included from the files of the co-author (MSK). Control subjects were selected with the intent of equalizing groups for IQ, socioeconomic status (SES), and handedness (all but one subject was right-handed). Handedness was determined with the subject's stated hand preference and confirmed by noting the number of handedness items scored for each hand with the Revised Physical and Neurological Examination for Subtle Signs (PANESS) inventory (Denckla 1985) where 13 items are used to define right-handedness. Socioeconomic status was determined with the Hollingshead Four Factor Index (Hollingshead 1975), which uses both education and occupation to determine the appropriate SES. As may be seen in Table 1, the high- and low-risk groups were well-matched on age, SES, IQ, height, and weight. Only a single subject was left-handed.

Clinical Evaluation

All children/adolescents (8–18 years old) and their accompanying parents were separately administered the Schedule for Affective Disorders and Schizophrenia for School-aged Children (K-SADS) (Chambers et al 1985). The interview was performed by trained clinical interviewers (M.A. in Psychology) with diagnostic reliability of 90% or greater. Also, the mother was queried extensively regarding alcohol, drug, and cigarette use during pregnancy, as previously described (Hill et al 2000c; Steinhauer and Hill 1993).

A 3rd- or 4th-year resident who was specializing in child psychiatry in an integrated general and child psychiatry program conducted an unstructured interview independently with both the child and the parent. Both the interviewer and the resident in psychiatry were blind to the risk status of the subject's family. A "best-estimate" consensus diagnosis between the interviewer and the psychiatrist was determined for all axis I DSM-III disorders. (DSM-III was the diagnostic system in place at the initiation of the longitudinal follow-up and has been used throughout follow-up for consistency. One goal of the study is to assess change and persistence across childhood and adolescence, necessitating use of

Table 2. Percentage of Cases With a Lifetime Diagnosis, by K-SADS Interviews ($n = 33$)

Diagnosis	High-Risk	Low-Risk
Alcohol or Drug Dependence[a]	29.4	0
ADHD	35.3	0
Anxiety Disorders[b]	35.3	12.5
Depression	17.6	0
Oppositional Defiant Disorder	41.2	6.3
Conduct Disorder	23.5	0
Any Diagnosis	76.5	13.5

Note that the high-risk children are enrolled in a longitudinal follow-up and have received annual diagnostic procedures. Some of the low-risk control/subjects ($n = 5$) were similarly evaluated at yearly intervals. The remainder were evaluated a single time before being scanned and are historical controls from the files of one of the co-authors (MSK).

All diagnoses were based on DSM-III (K-SAD interviews for 8–18-year-olds) or DSM-IV (Composite International Diagnostic Interview [CIDI] for 19 years and older). Diagnoses were lifetime up to the time of the scan. It was not possible to apply DSM-IV criteria to the K-SADS responses on file for all diagnoses. Repeating the diagnostic evaluation for attention-deficit/hyperactivity disorder (ADHD) did reveal a lesser number of cases by DSM-IV than DSM-III, however.

[a]Five cases met criteria for alcohol or drug dependence by the time the magnetic resonance imaging (MRI) scan was done: Case 1: DSM-III diagnosis of alcohol dependence by K-SADS interview at age 15; MRI scan age 20; Case 2: DSM-III diagnosis of drug dependence by K-SADS interview at age 16. DSM-IV diagnosis of alcohol dependence by CIDI interview at age 22; MRI scan age 22; Case 3: DSM-III diagnosis of alcohol dependence by K-SADS interview at age 18. DSM-IV diagnosis of drug dependence by CIDI interview at age 21; MRI scan age 21; Case 4: DSM-III diagnosis of drug dependence by K-SADS interview at age 18; MRI scan age 18; and Case 5: DSM-III diagnosis of drug dependence by K-SADS interview at age 15; MRI scan age 15.

[b]Anxiety disorders included simple phobia, overanxious disorder, separation anxiety, and social anxiety.

the same diagnostic system over a 15-year period of follow-up). Those individuals remaining in the follow-up at age 19 were evaluated semi-annually. Evaluation included the administration of the Composite International Diagnostic Interview (CIDI), an instrument that allows the interviewer to obtain DSM-III and -IV and International Classification of Diseases (ICD 9) diagnoses (Janca et al 1992). Lifetime diagnoses at the time of the MRI scan for the 33 individuals who participated in the MRI study may be seen in Table 2.

MRI Acquisition

The MRI scans were performed at the University of Pittsburgh Medical Center Magnetic Resonance Research Center with a Signa 1.5-T system (GE Medical Systems, Milwaukee, Wisconsin). A sagittal scout series verified subject position, cooperation, and image quality. A three-dimensional, spoiled gradient-recalled acquisition in the steady state pulse sequence was used to obtain 124 contiguous images with slice thickness of 1.5 mm in the coronal plane (echo time = 5 msec, repetition time = 25 msec, flip angle = 40°, acquisition matrix = 256 × 192, number of excitations = 1, field of view = 24 cm). Coronal sections were obtained perpendicular to the anterior commissure/posterior commissure line to provide a more reproducible guide for image orientation. Axial proton density and T_2-weighted images were obtained to enable exclusion of structural abnormalities on the MRI scan. All subjects tolerated the procedure well. No sedation was used.

ROI Analysis

The imaging data were transferred from the MRI unit to a computer workstation in the neuroimaging laboratory and ROIs were drawn with the Neural Net program from BRAINS2 (Magnotta et al 2002), which provides valid and reliable volume measurements of specific structures by using a semiautomated segmentation approach. The Neural Net program requires the operator to define the landmarks and boundaries for the structure of interest so that training classes can be established. Once the training classes are available, the algorithm is applied to the scans to be measured. The Neural Net program has good reliability and validity in comparison with manual tracings (Pierson et al 2002). Inter-rater reliability within this laboratory is $> .96$. Nevertheless, tracings provided by Neural Net can be trimmed for maximal accuracy. To do this, ROI manual tracing are performed in the sagittal plane; BRAINS2 provides separate windows for coronal, axial, sagittal, and three-dimensional views and allows the operator to adjust the characteristics of ROI intersections with other planes. All anatomical measurements were made by two trained and reliable raters (SM and JN) who were blind to subject information (see Figure 1A and 1B). These individuals were supervised by one of the authors (KP), who reviewed all tracings and final measurements. Interrater reliabilities for this laboratory and detailed descriptions of the ROI methodology have been described previously (Prasad et al 2005).

Specific boundaries and landmarks for the cerebellum followed the guidelines established for cerebellum with the Neural Net algorithm (Pierson et al 2002). Specifically, at the most posterior point of the fourth ventricle in the coronal plane, the midline was determined by using the vertex of the fourth ventricle and the midline of corpus medullare. This established the boundary between right and left cerebellum. Slices were determined to be right or left with the convention that midline slices were considered to be part of the right cerebellum. The cerebellar peduncles were excluded from the corpus medullare ROIs at the point at which they emerged from beyond the grey matter of the cerebellar cortex, as viewed in the axial plane. The vermis was included in the cerebellum measurements and included in the right and left cerebellar measurements with the midline as the boundary for inclusion in one or the other hemisphere.

Segmentation was done to optimize the κ-value obtained with successive iterations by one of the authors (JS). The BRAINS2 program allows for successive maximization of tissue into grey and white matter. After tissue plugs are selected, the separation of tissue classes is performed with discriminant function analysis. Once a best-fitting function is found with training classes, the function is applied to the entire image, including the training classes, providing a straightforward way to verify how well the discriminant function classifies the tissues. The predicted classification on the basis of the training samples is compared with the a priori labeled grey matter, white matter, and cerebrospinal fluid (CSF) plugs, and a κ-statistic is applied. A κ of 1.00 would indicate that the discriminant function correctly classified every training class sample into the correct tissue type. In practice, κ-values in the range of .92–.98 are considered to be acceptable. Visual inspection is also routinely used to confirm the accuracy of the κ-values.

Cerebellar volumes were analyzed uncorrected and corrected for intracranial volume. Intracranial volume was measured by including both cerebral hemispheres, brainstem, and the CSF surrounding these structures (Prasad et al 2005). Intracranial volumes were calculated by summing areas of successive coronal slices, including grey and white matter and CSF volumes, and multiplying by slice thickness.

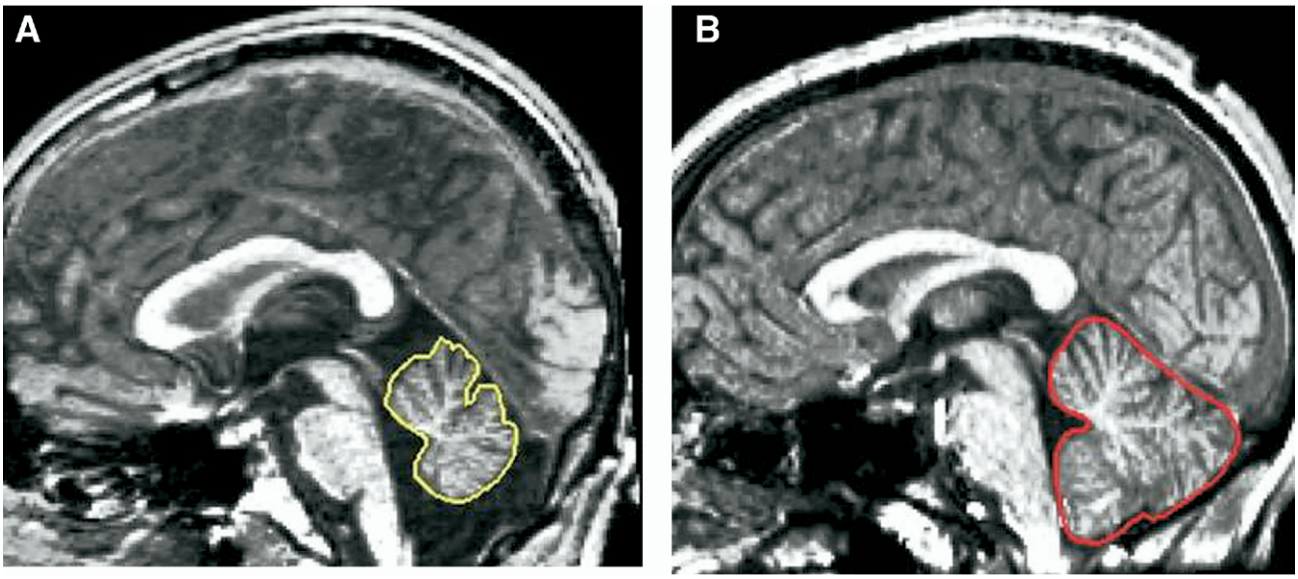

Figure 1. (A) Scan of a low-risk control subject with cerebellum and vermis outlined in **yellow**. **(B)** Scan of a high-risk subject with cerebellum and vermis outlined in **red**.

Results

The risk groups were well-matched for age, IQ, height, weight, and SES, as may be seen in Table 1. Intracranial volumes were similar for the high-risk group (1577.89 ± 98.94 cm^3) and for the low-risk control subjects (1522.73 ± 136.78 cm^3). Mothers of the high- and low-risk offspring were free of heavy use of alcohol or drugs during pregnancy; however, the high-risk children were more likely to have a lifetime childhood/adolescent disorder than control subjects, consistent with our previously published data from the larger sample (Hill et al 1999a).

Cerebellar volume was greater for high-risk subjects than low-risk control subjects (157.8 ± 14.4 cm^3 versus 147.89 ± 15.2 cm^3). Cerebellar volume (right, left, and total) by risk group was statistically analyzed uncorrected and corrected for intracranial volume (ICV). Analysis of the uncorrected values showed marginally significant differences for left cerebellar volume [$t(31) = 1.96$, $p = .059$] and total volume [$t(31) = 1.92$, $p = .063$] Although a significant difference between groups was not seen for age, IQ, SES, weight, or height (Table 1), to evaluate the possible effect of these variables on the obtained results, a General Linear Model (GLM) (SPSS version 12; SPSS, Chicago, Illinois) analysis was performed with age, IQ, SES, body mass index, and ICV as covariates. This analysis revealed that only ICV seemed to contribute a significant effect to the model. With ICV as a single covariate, differences between groups remained marginal, showing only a statistical trend. Figure 1A illustrates the outline of the cerebellum at a midsagittal section for a low-risk control subject and Figure 1B displays the cerebellar ROI at the same slice for a high-risk participant.

Analysis of uncorrected grey volumes revealed highly significant differences for total grey [$t(1,30) = 3.47$, $p = .0008$], for right hemisphere grey [$t(1,30) = 3.40$, $p = .001$], and for left hemisphere grey [$t(1,30) = 3.24$, $p = 001$]. Group differences in total grey were also tested with a GLM approach. With age, IQ, body mass index, SES, and ICV as covariates, the overall F-value for the corrected model was 6.38 [$df = 6,25$]; $p < .001$. A significant effect for group was seen [$F(1,25) = 15.69$, $p = .001$]. Also, a significant effect for age was seen [$F(1,25) = 6.48$, $p = .02$]. Differences in total grey volume by risk group may be seen in Figure 2.

To assess the likelihood that these group differences might reflect differing developmental growth curves, a series of regressions of grey volume on age were performed and tested with analysis of variance. The relationship between age and total cerebellar grey volume for the total sample ($n = 32$) showed a significant effect for age, with total grey decreasing with age. A maximal R^2 of .12 [$F(1,31) = 4.10$, $p = .05$] for the entire sample was seen with an inverse model (curve fitting was done to find the most appropriate model).

Image segmentation with adequate κ-values was achieved for 32 of the scans (16 high-risk and 16 low-risk). With the segmented images, a regression analysis of total cerebellar grey with age revealed an age-related decline in grey matter that was especially prominent in the normal control subjects [$R^2 = 55$; $F(2,13) = 7.99$, $p = .005$]. Unlike the low-risk control subjects,

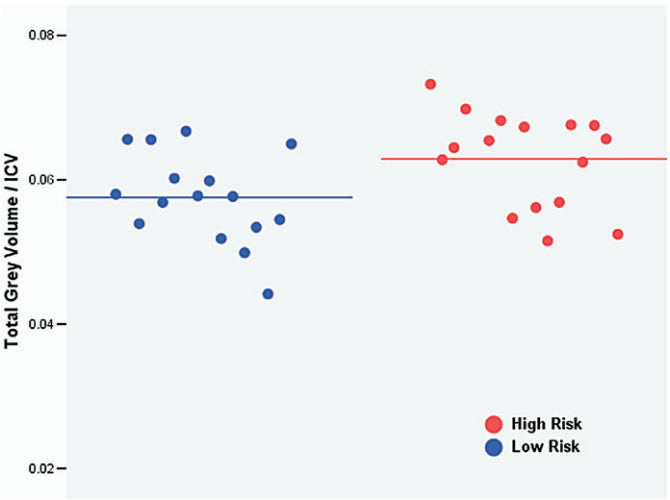

Figure 2. Total grey volume is plotted by risk group, illustrating the greater mean volume of grey for the high-risk offspring.

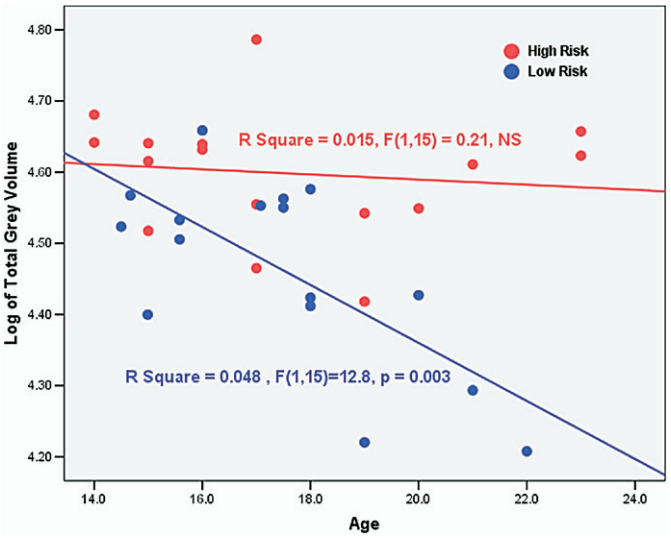

Figure 3. Total grey volume is plotted by age for the control sample ($n = 16$), represented in **blue circles**. Total grey volume plotted by age for the high-risk sample ($n = 16$), represented by **red circles**, is much less prominent and not statistically significant.

the quadratic regression on age for cerebellar grey obtained for high-risk offspring revealed a lesser change with age [$R^2 = 21$, $F(2,14) = 1.90$, p = ns] (a log transformation was used to test group differences in slopes by age). Comparison of these slopes (Figure 3) reveals a steeper slope in the low-risk control subjects than in the high-risk children/adolescents that was statistically significant [$F(1,31) = 5.58$, $p = .03$].

Discussion

The principal finding of this study is that offspring from multiplex alcoholism families have greater cerebellar grey volume in comparison with normal control subjects with whom they had been matched for age, gender, IQ, SES, and handedness (Table 3). On the basis of prior neuroimaging studies, it was expected that grey matter density would show a sustained loss starting around puberty (Gogtay et al 2004). Further analysis of the segmented images revealed significant age-related changes in grey matter, with younger adolescents having larger cerebellar grey volume and older adolescent/young adults having smaller volumes. Because the present sample was cross-sectional, additional work is needed in a prospective sample to see whether group differences in age-related changes in grey matter can be replicated.

This tendency for reduced volume to be seen with age suggests that pruning of grey matter in the cerebellum might be an important developmental change that occurs during adolescence; however, because grey matter density seen on MRI is an indirect measure of vasculature, glia, and neurons along with their synaptic and dendritic processes, other interpretations are plausible. Changes in grey matter that are assumed to be due to a loss of grey might actually be glial changes or alterations in vascularity (Paus 2005). Nevertheless, greater grey matter thinning has been related to improved performance on the WISC Vocabulary subtest (Sowell et al 2004). Because the question of what grey matter loss might reflect has not been adequately resolved, we are inclined to speculate that reduction in grey matter during adolescence is likely due to pruning, although this clearly remains an assumption. The smaller decline in cerebellar grey with age seen in the high-risk offspring suggests a possible neurological basis for the developmentally slower acquisition of postural control seen in high-risk offspring (Hill et al 2000a).

In addition to the greater volume of cerebellar grey seen in these high-risk children was a tendency for total volume of the cerebellum to be larger. These findings stand in contrast to studies of male and female children with childhood-onset schizophrenia (Jacobsen et al 1997); female (Castellanos et al 2001), male (Berquin et al 1998), and mixed samples (Castellanos et al 2002) children with ADHD, and adult bipolar patients (Delbello et al 1999) where decreased volume of the cerebellum has been reported. Because smaller tissue volume of brain structures is often seen in association with clinical presentation in pediatric cases, finding a larger volume for the cerebellum of youngsters at higher genetic risk for alcohol dependence might not have an intuitive appeal as a marker for clinical risk. Observations in adult samples, however, have revealed greater tissue volume in association with clinical states. Neuroimaging of a consecutive series of adult obsessive compulsive disorder (OCD) patients has revealed relative increases in grey matter volume in the anterior cerebellum (Pujol et al 2004). This study used SPM2, a program that uses a good clean-up protocol to strip brain and process volumetric data in native space. An earlier study did not find an increase in cerebellar volume with voxel-based morphometry (SPM 99 [Institute of Neurology, London]) but found decreased grey matter density in the left cerebellum (Kim et al 2001); however, SPM 99 has the drawback of using a poorer brain stripping paradigm and bias correction component than SPM2. Functional imaging studies have been consistent in showing increased resting cerebral blood flow in the cerebellum of OCD patients (Busatto et al 2000; Nakao et al 2005). These data suggest that the increased volume of the cerebellum seen in morphological studies of OCD patients might well reflect a decrease in functional efficiency of the cerebellum in OCD patients. Similarly, the increased volume of the cerebellum

Table 3. Brain Volumes of High- and Low-Risk Control Children/Adolescents

	High-Risk ($n = 17$)		Low-Risk ($n = 16$)		Statistic	
Structure (cm^3)	Mean	SD	Mean	SD	t	p
Total Left Grey	49.84	5.26	43.56	5.71	3.24	.001
Total Right Grey	50.91	4.10	44.85	5.82	3.40	.001
Total Grey	99.67	8.78	87.46	11.01	3.47	.0008
Total Left	80.42	8.23	74.82	8.20	1.96	.059
Total Right	78.95	6.64	74.50	7.44	1.81	.08
Total Cerebellum	157.80	14.36	147.89	15.18	1.92	.063
Total Intracranial	1569.66	96.05	1522.73	136.78	1.13	ns

All p values are two-tailed tests. Degrees of freedom, $n = 31$ for total right, left, and total cerebellum; df = 30 for total grey, total right grey, and total left grey.

seen in these high-risk offspring might have implications for cerebellar efficiency.

Of particular relevance to the present findings are results of a study reported by Pujol et al (2004) in which increased cerebellar volume along with reduced amygdala volume in the right hemisphere was seen in OCD patients. An interesting parallel can be noted with the present findings where a trend for increased cerebellar volume was seen in a sample in which reduction in right amygdala volume had been reported (Hill et al 2001). With obvious similarities between compulsive aspects of abusive drinking and other compulsive behaviors seen in OCD, the present results observed in children/adolescents selected for low levels of alcohol consumption and high density of alcohol dependence by family history is especially intriguing.

Developmental changes in cerebellar volume have only infrequently been addressed. Castellanos et al (2002) studied 152 male and female children and adolescents, with some being imaged up to four times. The authors reported a slight increase in cerebellar volume from age 10 to 20, with lesser change seen in late adolescence. Similarly, James et al (2004) obtained MRI scans of 16 schizophrenic patients and 16 control subjects over two points separate by 2–3 years. Participants were approximately 16 years of age at first scan and 18.5 years at the second. Over this period of time, this mixed-gender sample showed a change of $< 1\%$ during late adolescence. These developmental studies of total cerebellar volume indicate rather modest changes in total volume. Grey matter change might be a better indicator of developmental progress than total cerebellar volume. Because the high-risk youngsters came from multiplex families in which genetic transmission of alcohol dependence susceptibility might be expected to be much higher than the population prevalence, any morphological differences seen might provide clues to this genetic susceptibility. Adolescent to young adult age-related changes in grey might serve as a useful indicator of vulnerability to alcohol dependence or other psychiatric illness.

Conclusions from the present study should be considered in the context of some limitations that should be mentioned. Because the scans were acquired with axial proton density and T2-weighted images along with coronal T1 images, segmentation with BRAINS2 required considerable successive approximations to achieve measurements for total grey and white matter. Regions of interest measurements were completed before segmentation was performed. Accordingly, only right, left, and total cerebellar volume was considered reliable, and further analyses by subregion were not undertaken.

Another possible limitation that should be mentioned is the greater frequency of a diagnosable childhood disorder in the high-risk offspring. It could be argued that the volumetric differences might be due to biological variables associated with the particular conditions common to the high-risk group (e.g, 35.3% met DSM-III criteria for ADHD). There are a number of reasons why this could not have explained the present findings. First, findings to date seem to indicate that ADHD children tend to have reduced cerebellar volume rather than increased volume (Castellanos et al 2002). Second, a wide variety of psychopathology was seen among the high-risk cases. Third, DSM-III tends to diagnose a greater frequency of positive cases than does DSM-IV (in the present sample only two cases [11.7%] met criteria for DSM-IV ADHD). Accordingly, it would seem that the particular disorders experienced by the youngsters are less likely to be causative than the increased familial/genetic risk that the high-risk offspring have.

Another limitation of the present report is that inferences regarding age-related changes in total cerebellar volume and the grey matter changes with age are made on the basis of cross-sectional analyses. An ongoing longitudinal study of children in the larger longitudinal study will make it possible to confirm or reject the present findings. Nevertheless, because of the close matching in age, gender, SES, and handedness, we can conclude that cerebellar volume, particularly cerebellar grey, tends to be greater in youngsters with a family history of alcohol dependence.

This cerebellar volume that is larger for age in adolescents/young adults who carry an increased susceptibility for developing alcohol dependence might have been the result of a greater genetic susceptibility arising from membership in a multiplex family (the multiplex families were ascertained through a minimum of two adult alcoholic brothers). Moreover, this morphological difference seems to be present before drinking/drug use began for all but five high-risk participants and reflects a possible neurobiological vulnerability to substance dependence rather than a result of excessive drinking or drug use. With evidence that neural pruning of the cerebellum occurs during development, it seems that the high-risk offspring are not pruning at the same rate as control offspring. These results are consistent with previous results from our laboratory in which growth curves for P300 amplitude for children/adolescents from high-risk families were found to have differing developmental trajectories from those obtained from offspring from normal control families (Hill et al 1999b).

The high-risk offspring in this study have exceptionally high familial/genetic loading for alcohol dependence because of the selection criteria requiring multiplex families. Further work is ongoing with this population, with repeated scans being used with full segmentation potential, to investigate the possibility that high-risk offspring might show developmental delays in the restructuring of brain regions that might be related to their susceptibility.

This research was supported by the National Institute of Alcohol Abuse and Alcoholism (AA 05909 and AA 08082) and an award from the University of Pittsburgh Medical Center, Functional Imaging Research Program, for the cost of the scans. The principal investigator thanks the many families who have participated over the years and especially those who made time for the present study.

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