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Measurement of Cerebral and Cerebellar Volumes in Children with Fragile X Syndrome

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Background and Aim: Fragile X syndrome (FXS) is a genetic disease associated with mental retardation, speech and memory deficits, developmental/learning disabilities, and, frequently, also with neuropsychological findings. It is known that all these abnormal brain functions could be associated with morphological anomalies of various brain regions. We investigated possible brain volume variations in children with FXS who had significant neurobehavioral symptoms and cognitive deficits.

Patients and Methods: This study was performed with 8 male FXS patients who were diagnosed cytogenetically. The Leiter International Performance Scales-Revised, the Stanford-Binet Intelligence Scale, the Vineland Social Maturity Scale, and DSM IV criteria were used for cognitive and behavioral assessment. The Cavalieri method, which is used to analyze the volumetric properties of smooth objects, was used for cranial volume measurements.

Results: The mean age of the patients was 8.8 ± 4.4 years (range: 5-18 years). The mean IQ level was 59.8 ± 10.3 (range: 40-76). The vermis/total cerebellum ratios of the patients decreased with age ($r = -0.851$, $P < 0.01$). There was a correlation between this condition and neurobehavioral symptoms that increased with age.

Conclusions: The study patients had decreased vermis/total cerebellum ratios, indicating the development of vermal atrophy with age. This vermal atrophy may have led to neurophysiological dysfunctions, cognitive deficits, and neurobehavioral phenotypes in the FXS patients.

Key Words: Brain volume, Cavalieri method, fragile X syndrome, magnetic resonance imaging, mental retardation

Frajil X Sendromlu Çocuklarda Serebral ve Serebellar Volümlerin Ölçülmesi

Giriş ve Amaç: Frajil X sendromu (FXS) mental gerilik, dil ve hafıza kusurları, gelişim / öğrenim yetersizlikleri ile ilişkili ve ayrıca nöropsikolojik bulguların da sıkça görüldüğü kalıtımsal bir hastalıktır. Tüm bu anormal beyin işlevlerinin beynin değişik bölgelerinin morfolojik anomalileri ile ilişkili olabileceği bilinmektedir. Bu çalışmada, belirgin nörodavranışsal semptomlar ve bilişsel kusurları olan FXS'li çocukların muhtemel beyin volüm değişikliklerinin incelenmesi amaçlandı.

Hastalar ve Yöntem: Çalışmaya sitogenetik olarak FXS tanısı konulan sekiz erkek hasta alındı. Bilişsel ve davranışsal değerlendirme için Yeniden Düzenlenmiş-Uluslararası Leiter Performans Ölçeği, Stanford Binet Zeka Ölçeği, Vineland Sosyal Gelişim Ölçeği ve DSM IV ölçütleri kullanıldı. Beyin volüm ölçümleri için, düz nesnelerin volümetrik özelliklerini değerlendirmede kullanılan, Cavalieri metodu kullanıldı.

Bulgular: Olguların yaş ortalaması 8,8 ± 4,4 (yaş aralığı 5-18) yıl idi. Ortalama IQ düzeyi 59,8 ± 10,3 (sınır aralığı 40-76) olarak belirlendi. Hastaların vermis / total serebellum oranları yaş ile azalma gösterdi ($r = -0,851$, $P < 0,01$). Yaş ile nörodavranışsal semptomların artması ve bu durum arasında korelasyon vardı.

Sonuç: Çalışma hastalarında, yaşla beraber vermal atrofi geliştiğine işaret eden, azalmış vermiş / total serebellum oranları vardı. Bu vermal atrofi, FXS hastalarda gözlenen nörofizyolojik fonksiyon bozuklukları, bilişsel kusurlar ve nörodavranışsal fenotiplere yol açabilir.

Anahtar Sözcükler: Beyin volümü, Cavalieri metodu, frajil X sendromu, manyetik rezonans görüntüleme, mental gerilik

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Introduction

Recent advances in molecular genetics, human brain imaging, and behavioral studies have started to unravel the complex pathways leading to the cognitive, psychiatric, and physical features that are unique to fragile X syndrome (FXS). It has long been suggested that the decrease or absence of FMR1 protein production associated with FXS may lead to brain abnormalities in affected individuals (1-6). Postmortem microscopic examinations of brain tissue from patients with FXS have revealed that, although the number of neurons falls within the normal range, abnormalities in neuronal dendritic spines exist. These abnormalities are characterized by unusually long and thin, tortuous spines, with very few of the stubby, mushroom-shaped spines found in unaffected controls, and a higher density of spines along the dendrites, suggesting a possible failure of synapse elimination (3,7).

Neuroimaging studies have shown selective changes in brain size in FXS, which include reductions in the posterior cerebellar vermis, age-dependent increases in hippocampal volume, and an enlarged caudate nucleus and thalamus. Structural magnetic resonance imaging (MRI) studies of FXS have further explored the effects of the FMR1 full mutation on neuroanatomy. The first brain abnormality reported in a MRI study was hypoplasia of the cerebellar vermis, the connecting tissue between the right and left hemispheres of the cerebellum (8).

In the present study, we studied the relationship between volumetric changes in the gross structures of the brains of subjects with FXS and neurobehavioral signs, symptoms, and cognitive disorders, which might be observed in FXS patients.

Patients and Methods

This study was performed with 8 FXS patients who were diagnosed cytogenetically by lymphocyte culture in the presence of a folic acid antagonist. For this study, institutional ethical approval and permission from the parents were obtained. MRIs of the patients were evaluated, along with their histories, physical and neurological examinations, and their general laboratory findings.

The Leiter International Performance Scales-Revised, the Stanford-Binet Intelligence Scale, the Vineland Social Maturity Scale, and DSM IV criteria were used by a psychologist for cognitive and behavioral assessment (9-11).

MRI was performed on a 1.5-T open scanner (High Speed Model, General Electric Signa) with 3-mm slice thickness. During cranial analysis, the following sequences were imaged: Spin Echo (SE) T1 (Fov: 240; TR: 500; TE: 20; FA: 90; thickness: 8 mm; interval: 10 mm; matrix: 256 x 256; NSA: 3; multislice: 12; projection: 160); Proton Dansite (PD) (Fov: 240; TR: 4000; TE: 26; FA: 90; Thickness: 8 mm; interval: 10 mm; NSA: 2; matrix: 256 x 256; projection: 192); Fast Spin Echo (FSE) T2 (Fov: 240; TR: 4000; TE: 117; FA: 90; Thickness: 8; interval: 10; multislice: 12; NSA: 4; projection: 160). To evaluate the hippocampus, a 3D coronal spoiled gradient echo (SGE) sequence was added (Fov: 200; TR: 70; TE: 18; FA: 50; Thickness: 3 mm; Interval: 3 mm; projection: 192; NSA: 3; matrix: 256 x 256; multislice: 32). After obtaining the sequences, the brain structures of the subjects were evaluated.

The Cavalieri method, which is used to analyze the volumetric properties of smooth objects, was used for cranial volume measurements (12). According to this method, first the size of the area *(a)* for each MRI plane section was calculated *(cm^2)*. By multiplying the area (a) by the thickness of each MRI section *(t)*, we obtained section volume *($V = ta$)*. This equation was extended according to general mathematical rules and the resulting formula *[$V=t(a_1+a_2+.....+a_n)$ cm^3]* was obtained. The resulting equation was utilized in calculating the volumes. Volumetric measurements of the subjects were carried out by an experienced neuroradiologist and a anatomist. Volumetric measurements were taken of the right and left cerebral hemispheres, total cerebrum, cerebellum, and cerebellar vermis. Total brain volume was calculated by adding the volumes of the cerebrum and cerebellum. Total brain volume did not include the volume of the ventricles or the corpus callosum. We did not have volumetric measurements from normal children to compare with the values obtained from FXS subjects. Consequently, we used values from the literature of age- and sex-matched normal children (13-15).

The data were evaluated in the SPSS 11.0 program. The obtained data are presented with standard deviation. In the evaluation of the relationship between some of the parameters, Spearman's correlation analysis was used.

Results

The average age of the male patients was 8.8 ± 4.4 years (range: 5-18). The most common findings in their physical examinations were large ears (8/8, 100%), elongated face (6/8, 75%), protruding mandible (5/8, 62.5%), and macroorchidism (5/8, 62.5%). In addition to these, in each patient, hypospadias, strabismus, myopia, and macrocephaly were noted (12.5%) as separate cases. All patients had mental retardation (100%). Their average IQ level was 59.8 ± 10.3 (range: 40-76). Moreover, 3 patients had attention deficit hyperactivity disorder (ADHD) (37.5%), one had autism (12.5%), one had stereotyped behavior and speech apraxia, and one had social anxiety (Table).

While MRIs of 6 patients were found to be qualitatively normal, in 2 of the subjects (2/8, 25%), abnormalities were noted in cranial MRIs. These abnormalities were severe cerebellar/vermal hypoplasia (no: 2) and cerebral hemispherical asymmetry (no: 5, right: 699.2 cm^3; left: 533.6 cm^3). The vermis/total cerebellum ratios of the patients decreased with age ($r = -0.851$, $P < 0.01$) (Figure), while neurobehavioral symptoms appeared to increase (Table).

Discussion

The neuroanatomical basis for the cognitive deficits and neurobehavioral symptoms observed in FXS patients has not been satisfactorily established. The possibility that the brain dysfunctions noted in these patients are primarily the result of abnormalities in structural development has not been ruled out as yet (16-20). Abnormal dendritic spine morphology in the cerebral cortex has been observed in neuropathological studies of FXS (3,21-23). However, others (24) found no differences in total cerebral volume in either males or females with FXS. Reiss and colleagues (25,26) found that the posterior cerebellar vermis region was reduced in both males and females with FRX compared to sex-matched controls. Another study by the same researchers, but with a larger sample (27), confirmed the absolute and relative decrease in posterior vermis (lobules VI–X) size in males with FXS (n = 32) when compared with both normal (n = 38) and developmentally delayed (n = 28) controls.

Associations between hypoplasia of the cerebellar vermis and behavioral and cognitive dysfunction in FXS have been reported in 2 studies (28,29). In an investigation of 30 school-age girls with FXS, Mazzocco and colleagues (28) reported a significant negative correlation between the area of lobules VI and VII, and parental ratings of frequency and severity of autistic behaviors, particularly in social communication and stereotypic behaviors. In contrast, measures of anxiety did not correlate with IQ or with the size of the posterior cerebellar vermis, suggesting that anxiety, which is included in the behavioral phenotype of girls with FXS, may have a different neuropathological mechanism that is unrelated to morphology of the posterior cerebellar vermis (1,29). In our study, no correlation between IQ and brain volume was established. This finding suggests that cellular disorders and the abnormalities in transmitter levels previously demonstrated in FXS subjects might have additional negative influence on IQ level (30-33).

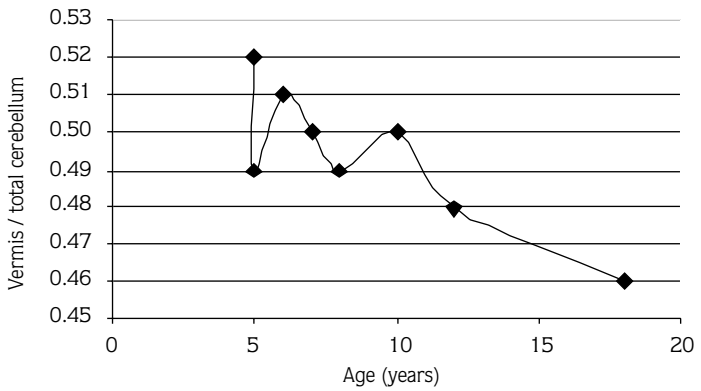

Figure. Decreases in the ratio of vermis/total cerebellum with age ($r = -0.851$; $P < 0.01$).

Table. Cerebral and cerebellar volumes (cm^3) of the patients with Fragile X syndrome.

Patient No	Age (year)	Right cerebral hemisphere	Left cerebral hemisphere	Cerebrum	Cerebellar vermis	Total cerebellum	Vermis / total cerebellum	Total brain volume	IQ	Neuro-behavioral sings and symptoms
1	5	623.7	628.8	1252.4	67.2	129.7	0.52	1382.1	40	MR
2*	5	614.1	624.2	1238.3	45.4	92.1	0.49	1330.3	60	MR / ADHD
3	6	627.9	627.9	1255.8	69.2	135.6	0.51	1391.4	65	MR
4	7	679.1	672.4	1351.6	73.6	144.9	0.50	1496.4	66	MR / ADHD
5*	8	699.2	533.6	1232.7	68.5	137.7	0.49	1370.5	56	MR / autism
6	10	649.8	643.6	1293.4	74.9	148.0	0.50	1442.4	76	MR / ADHD
7	12	666.9	662.9	1329.8	70.3	146.4	0.48	1476.2	60	MR / Sterotyped behavior and speech apraxia
8	18	671.1	671.1	1342.3	68.9	149.2	0.46	1491.4	56	MR / social anxiety
Mean ± SD	¶8.8 ± 4.4	654.0 ± 30.1	633.1 ± 44.8	1287.0 ± 48.7	67.2 ± 9.2	135.6 ± 18.9	¶0.49 ± 0.2	1422.6 ± 62.4	59.8 ± 10.3	
Reference 11	9.8	535.5	507.9			129.6				
Reference 12	10.8							1316.3		
Reference 13	4.4				71.4					

*: patients with cerebral malformations ¶: r=-0.851, P < 0.01 (Spearman's correlation analysis was used)

Reference 13: Hong et al., 2002; Reference 14: Moore et al., 2000; Reference 15: Pierce K et al., 2001.

ADHD: Attention-deficit / hyperactivity disorder

IQ: Intelligence quotient

MR: Mental retardation

SD: Standart deviation

It has been shown that the cerebellar vermis is connected to the frontal lobe and regulates higher cognitive functions (29). The most important finding obtained from our FXS series is that vermis/total cerebellum ratio decreased in these patients as the subjects aged, whereas neurobehavioral symptoms increased. Similar findings have been reported by others who have studied FXS subjects (24,29,32-34). Two brain regions, the cerebellar vermis, which is important for a variety of cognitive tasks and regulation of motor behavior, and the superior temporal gyrus, which is involved in processing complex auditory stimuli, are reported to be reduced in size in FXS patients relative to controls (25-27). The vermis, which is anatomically connected to limbic structures, including the hippocampus and amygdala, has been implicated in the execution and regulation of motor behavior (35), visual saccades (36), auditory processing (37), and some aspects of language (38,39). Abnormalities of the cerebellar vermis may, therefore, be linked to some of the behavioral anomalies associated with FXS, including hyperactivity and repetitive movements, tactile defensiveness, attention deficits, and language dysfunction (1).

Cranial MRI studies performed in our patients indicated that only 2 subjects had qualitative abnormalities, while qualitatively normal MRI findings were obtained in 6. Overall, these findings might be interpreted to mean that in FXS, even though structural brain abnormalities might exist, these changes are not unique to the syndrome.

The mean volumes of the right and left cerebral hemispheres in our FXS series were 654 ± 30.1 and 633.1 ± 44.8 cm^3, respectively. The mean volumes from age- and sex-matched control subjects obtained from the literature were 535.5 and 507.9 cm^3, respectively (13). These findings demonstrated that our subjects had 120 to 125 cm^3 more mean mass on both the right and left hemispheres. Total brain volumes of our subjects were also higher with a mean volume of 1422.6 ± 62.4 vs. 1316.3 cm^3 as published in the literature for the normal 10.8-year-old age group (14). Cerebellar vermis volumes in our series (mean: 67.2 ± 9.2 cm^3) were lower that those reported for the normal 4.4-year-old age group (mean: 71.4 cm^3). However, total cerebellum volumes (mean: 135.6 ± 18.9 cm^3) were similar to those reported (mean: 129.6 cm^3) for the age- and sex-matched control subjects (13,15). These findings show that the FXS series studied in the current report had higher cerebral volume, yet lower cerebellar vermis volume. We are not able to provide a satisfactory explanation for this observation. Contrary to the reported findings (24-26), this discrepancy with the normal values in the literature (13-15) might have been caused by the increased total cerebral volume and decreased cerebellar vermis volume associated with aging and/or by racial or geographical differences existing between our study subjects and those reported in the literature. The lack of local control subjects in our study prevents us from further delineating the origin of this discrepancy.

In conclusion, the observed vermal volumetric changes in FXS patients might be responsible for some of the neurobehavioral signs, symptoms, and cognitive disorders associated with FXS. However, in order for all of these possible relationships to be brought to light, studies with larger patient populations, more sensitive combined research methods (positron emission tomography, diffusion-weighted imaging, proton magnetic resonance spectroscopy, and exciter/inhibitor neurotransmitters and performance tests), and multicenter design are needed.

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