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Cerebellar Atrophy in Human and Murine Succinic Semialdehyde Dehydrogenase Deficiency

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Abstract

Human succinic semialdehyde dehydrogenase deficiency, an autosomal recessive disorder of γ -aminobutyric acid (GABA) catabolism, was modeled by a murine model sharing the phenotype of ataxia and seizures. Magnetic resonance imaging (MRI) with volumetry was obtained on 7 patients versus controls, and MRI with stereology was derived in 3 murine genotypes: null, wild-type, and heterozygous mutants. All patients had T1 hypointensity and T2 hyperintensity in globus pallidus, and 5 also had similar changes in subthalamic and cerebellar dentate nuclei. There was a trend for patients to have a smaller cerebellar vermis. Homozygous null mice had significantly lower total brain and cerebellar volumes than wild-types and heterozygotes. Stereology confirmed cerebellar atrophy and was otherwise normal in multiple regions. Cerebellar volume loss is present in the murine disorder with a trend for cerebellar atrophy in patients. Reduced cerebellar volume can reflect neurodegeneration and may be related to the clinical manifestations.

Keywords

succinic semialdehyde dehydrogenase deficiency; cerebellar atrophy

Succinic semialdehyde dehydrogenase (EC 1.2.1.24) deficiency (γ -hydroxybutyric [GHB] aciduria; OMIM 271980, 610045) is an autosomal recessively inherited neurometabolic disorder of γ -aminobutyric acid (GABA) metabolism. The succinic semialdehyde dehydrogenase gene (aldehyde dehydrogenase 5 family, member A1: ALDH5A1) maps to chromosome 6p22.¹ Enzyme deficiency is demonstrable in blood lymphocytes.² Oxidative conversion of succinic semialdehyde to succinic acid is impaired, which leads to production of GHB.

Succinic semialdehyde dehydrogenase deficiency has considerable phenotypic heterogeneity. Patients usually present with mild to severe developmental delay,

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predominantly involving expressive language. Other typical clinical signs and symptoms include hypotonia, truncal or appendicular ataxia, hyporeflexia, behavior disorders, and seizures. Patients often develop neuropsychiatric symptoms such as inattention, anxiety, hyperkinesia, sleep disturbances, and excessive daytime somnolence.³ Behavior problems including autism spectrum disorder may be associated, which, in combination with hypotonia and ataxia, suggest cerebellar involvement in the pathophysiology.⁴ A minority of patients (approximately 10%) have a progressive course featuring developmental regression and substantial extrapyramidal manifestations, including dystonia, choreoathetosis, and myoclonus.⁵

Reported neuroimaging findings include cerebral atrophy and T2 hyperintensities in globus pallidus, subcortical white matter, cerebellar dentate nucleus, and brainstem.³ Positron emission tomography with fluorodeoxyglucose has shown decreased cerebellar uptake.⁶ Magnetic resonance spectroscopy shows elevated parenchymal GABA in affected subjects, but not obligate heterozygotes.^{7,8}

Hogema and coworkers developed a mouse model of succinic semialdehyde dehydrogenase deficiency, phenotypically expressed as ataxia, failure-to-thrive, and seizures.⁹ Null mice progressively undergo generalized absence seizures, starting at about day 14, followed by convulsive seizures at day 18 to 19, and ultimately lethal tonic-clonic status epilepticus by the third to fourth week of life.¹⁰

We sought to determine if there were brain structural abnormalities in the *Aldh5a1* mouse model, or evidence for regional atrophy, using a combination of *ex vivo* MRI and a set of histologic and stereologic studies in homozygous, heterozygous, and wild-type mice. We also performed MRI on a group of affected subjects, parents (obligate heterozygotes), and healthy volunteers, and measured cerebellar volumes. We sought to determine whether cerebellar abnormalities were present in the human condition on volumetric MRI in parallel with radiographic and histologic studies in the animal model.

Materials and Methods

MRI Studies in an *Ex Vivo* Sample of Succinic Semialdehyde Dehydrogenase Animal Model

A total of 39 mice (14 succinic semialdehyde dehydrogenase ^{-/-}; 14 succinic semialdehyde dehydrogenase ^{+/+}; and 11 succinic semialdehyde dehydrogenase ^{+/-}) were perfused at postnatal day 19 utilizing the transcatheter perfusion technique previously described.¹¹ This time was selected to avoid seizure development in the homozygous mice. Each excised head was kept in formalin until MRI was performed. For imaging, heads were removed from formalin, the surface moisture was minimized, and each one was placed in a 12 mm i.d. glass sample tube and submerged in Fomblin, a proton-free perfluorinated polycarbon oil for magnetic susceptibility matching. The brain was centered in a 13.5 mm i.d. solenoid, transmit/receive radiofrequency coil, in a horizontal 7 Tesla Bruker Avance MRI scanner (Bruker Biospin, Inc, Bellerica, MA). A 3-dimensional volume, encompassing the whole brain, was specified using 3 orthogonal scout images. Fast spin echo 3-D images of the whole brain were acquired (number of echos = 8, repetition time = 3000 ms, echo time = 9 ms, number of averages = 2, total scan time ~ 12 hours) to achieve an isotropic image resolution of 62 μ m.

To calculate volumes, a series of axial slices, interleaved at 500 μ m, were specified from 3-D images. The areas encompassing the whole brain and corresponding cerebellum were evaluated using image display software and tracing the periphery of the regions of interest. These areas were extrapolated over the interleaved gaps to calculate volumes. Subsequently,

brains were removed from the skull after MRI and dry weight was measured. The volumes of the brains were calculated assuming a density of 1g/cm^3 . Investigators were blinded to genotype during volumetry.

Histology and Stereology in Mice

These studies used a combination of the Cavalieri principle and point counting techniques,¹² as reported in previous studies involving the assessment of brain volumes in humans,¹³ nonhuman primates,¹⁴ and rodents.¹⁵ For each brain region, a systematic random sample of 5 to 6 sections was selected and the reference space of interest was outlined using the mouse cursor.¹⁶ The software was automatically positioned on a point-grid randomly over each section within the outlined regions. The distance between points was selected to produce a coefficient of error for individual volume estimates of less than 5%.

Five subregions in a single hemisphere (left) from 15 brains were divided into 3 groups (5 per group): wild-type (controls), heterozygotes, and mutants. The following were reference volumes for the present study: neocortex (V_{ntx}); hippocampus V_{hippo} , which included the 4 subregions of Ammon's horn and the gyrus dentatus; cerebellum (V_{cereb}), including deep cerebellar nuclei (dentate, interpositus [emboliform + globose], and fastigial), white matter tracts in the deep regions, Purkinje, granule, molecular cell layers, white matter, and the cerebellar cortex; and cerebral forebrain (V_{sb}), including caudate, putamen, basal forebrain, amygdala, thalamus, globus pallidus, white matter, and the structures included in V_{ntx} . The fifth subregion, the subcortical volume (V_{sub}), was calculated as the difference between V_{brain} and V_{ntx} .

Human Studies

We studied 7 affected patients (4 male, age 15.7 ± 5.7 years), 10 parents (6 male, age 43.6 ± 5.8), and 8 healthy volunteer controls (4 male, 31.8 ± 10.2 years). Affected patients and family members were screened for inclusion by 1 investigator having extensive clinical experience with succinic semialdehyde dehydrogenase deficiency (Dr Pearl). Inclusion criteria were clinical characteristics consistent with succinic semialdehyde dehydrogenase deficiency, persistent 4-hydroxybutyric aciduria (GHB aciduria), and confirmation of deficient enzymatic activity in lymphocytes.

All subjects underwent MRI using a 1.5-T Horizon scanner (General Electric, Fairfield, CT). For volume measurements, T1-weighted spoiled gradient images (repetition time, 27 ms; minimum echo time; flip angle 20°) were acquired in coronal orientation. MRIs were analyzed using MEDx imaging software (Medical Numerics, Inc, Germantown, MD). Regions of interest were manually traced in each slice in which they appeared by an investigator blinded to the subject's age and diagnosis. The areas of each region of interest were summed and multiplied by the thickness of the total number of slices used. The extent of the cerebellar vermis in the coronal plane was defined according to the method of Press et al.¹⁷ The cerebellar peduncles were not included in the total volume of cerebellar hemispheres. The cerebellar vermis volume was normalized by dividing the measured region of interest by the total volume of both cerebral hemispheres.

Data analysis, including analysis of variation (ANOVA) with post hoc Bonferroni tests, was performed with Systat (Systat Inc, Point Richmond, CA).

Results

Mouse MRI

Morphological changes in internal structures were not identified. The homozygous mutant population had a larger cerebellar-cranial difference than both the wild-type and heterozygous group (Table 1). One-way ANOVA showed significant effects of mutation status on total brain (F ratio, 18.190) and cerebellar volume (F ratio, 26.486). Post hoc pairwise comparisons (Bonferroni) showed that homozygous null mice had significantly lower total brain and cerebellar volumes (Table 1) compared to the wild-type and heterozygous mice. The 2 latter groups did not differ. Extracted total brain volume calculations based on dry weight showed similar results, with smaller average values for homozygous null (0.34 ± 0.02), compared to heterozygous (0.37 ± 0.02) and wild-type mice (0.39 ± 0.03) (F ratio, 11.7; null vs wild-type $P < .001$; null vs heterozygous $P = .06$).

Mouse Stereology

Regional volumes were derived for individual mice ($n = 15$) and group means (SEM) for 3 genotypes (wild-type, heterozygous, homozygous mutant; $n = 5$ per group). Inspection of the mean \pm SEM values for wild-type versus mutant groups indicated possible differences for 2 brain volumes, cerebellum (V_{cereb}) and subcortical structures (V_{sub}). ANOVA results revealed a statistically significant group difference for V_{cereb} ($P < .017$) (Table 1), but nonsignificant differences for V_{sub} ($P < .28$). Comparison of volume data for other regions in wild-type versus mutant groups confirmed a low probability of group differences ($P < .50$). No volume differences were present in any brain region in heterozygotes compared to wild-type controls ($P < .17$ for V_{cereb} ; $P < .20$ for all other regions).

Human Imaging

Five patients had symmetric homogeneous hypointensity on T1-weighted sequences and hyperintensity on T2-weighted sequences in globus pallidus, subthalamic nuclei, and cerebellar dentate nuclei. The other 2 patients had signal abnormality limited to the globus pallidus, which was asymmetrical in 1. There were no cerebral cortical abnormalities.

With group as a main factor and age as a covariate, there was a trend (F ratio, 2.79; $P = .08$) for affected subjects to have a smaller cerebellar vermis, with no effect of age ($P > .20$) (Table 2). Figures 1A and 1B show cerebellar atrophy on structural imaging in 1 patient.

Discussion

Significant cerebral and cerebellar volume loss were found in homozygous succinic semialdehyde dehydrogenase deficient mutant mice compared to wild-type controls and heterozygous animals, on both MRI and stereological analysis. There were no differences in total brain volume or subregions in heterozygous mice versus wild-type controls.

The murine findings parallel MRI measurements in patients who show a trend toward cerebellar vermian atrophy compared to obligate heterozygotes (parents) and healthy control volunteers. Although the natural history of murine succinic semialdehyde dehydrogenase deficiency is fatal demise with status epilepticus, the reduced cerebellar volume in this study was found in animals that had not experienced any observed convulsive seizures. Moreover, the patients studied had relatively mild and well-controlled epilepsy, and had not been exposed to phenytoin, the antiepileptic drug most closely associated with cerebellar atrophy.¹⁸ Thus, both animal and human data suggest the findings are related to the underlying metabolic disorder. The mutant mice do manifest general failure to thrive, although birth weights are comparable to wild-type mice, raising the possibility that the changes reported

in this study are because of an indirect result of failure to thrive with general poor health or decreased feeding. Nevertheless, the end result is loss of cerebellar volume, and some of the symptoms of succinic semialdehyde dehydrogenase deficiency may be because of this cerebellar atrophy.

The MRI signal abnormalities in human images were not identified in mouse brain. This may have been because of the sequence having relatively short repetition times leading to decreased T2 weighting or effects of brain fixation. Alternatively, there may be species-related differences in the expression of the genetic defect. The latter situation has been seen in mouse models in neurofibromatosis type 1.¹⁹ Developmental differences between mouse and man could also affect imaging findings.^{4,20}

Clinical manifestations associated with cerebellar dysfunction, ie, ataxia and hypotonia, are characteristic of human succinic semialdehyde dehydrogenase deficiency. Moreover, cerebellar dysfunction has been closely associated with behavioral and cognitive deficits including hyperactivity, autistic symptoms, and speech disorders, which are all common in succinic semialdehyde dehydrogenase deficiency.^{21,22} GABA alterations have been implicated in the pathophysiology of learning disabilities, autism, behavior problems, and developmental dyslexia.²³⁻²⁵ A recent review²⁶ highlighted the relationships of functional polymorphisms in the succinic semialdehyde dehydrogenase gene and quantitative trait loci, including cognition and dyslexia. Cerebellar volume loss is demonstrated in both human and murine deficiency of succinic semialdehyde dehydrogenase, an enzyme integral to GABA catabolism.

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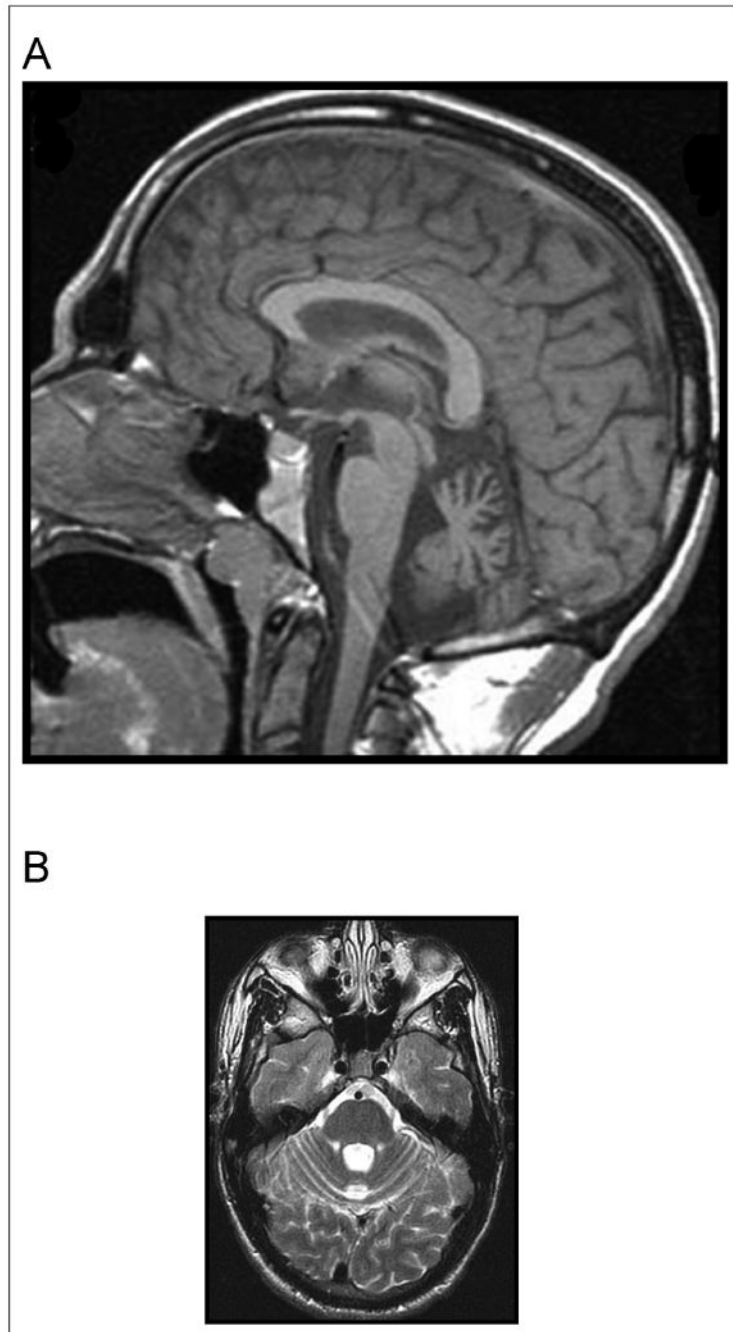


Figure 1. (A) Midsagittal 1.5 T magnetic resonance imaging (MRI) demonstrating cerebellar atrophy predominantly affecting the midline vermis in subject with succinic semialdehyde dehydrogenase deficiency. (B) Axial 1.5 T MRI demonstrating cerebellar atrophy predominantly affecting the midline vermis in subject with succinic semialdehyde dehydrogenase deficiency.

Table 1Mouse Brain Volumetry^a

Group	Total Brain (Mean ± SD) (Radiographic Volumetry)	Cerebellum (Mean ± SD) (Radiographic Volumetry)	Cerebellum (Mean ± SD) (Stereology)
Wild-type	0.407 ± 0.034	0.050 ± 0.005	0.0156 ± 0.00093
Heterozygous mutant	0.399 ± 0.028	0.050 ± 0.006	0.0135 ± 0.00098
Homozygous null	0.340 ± 0.031 ^b	0.038 ± 0.003 ^b	0.0120 ± 0.001 ^c

^a Cubic centimeters.

^b $P < .001$ vs wild-type and heterozygous mutant.

^c $P < .017$ vs wild-type and heterozygous mutant.

Table 2Human Cerebellar Vermis Volumes^a in Succinic Semialdehyde Dehydrogenase Deficiency

Group	Vermis (Mean ± SD)	Vermis Normalized to Total Cerebral Volume (Mean ± SD)
Healthy volunteers (n = 8)	8734 ± 1256	0.065 ± 0.006
Parents (n = 10)	8496 ± 1035	0.060 ± 0.009
Affected subjects (n = 7)	7427 ± 1427	0.056 ± 0.011 ^b

^a Cubic centimeters.

^b *F* ratio 2.79, *P* = .08 indicate a trend for affected subjects to have a smaller cerebellar vermis, with no effect of age (*P* > .20).