Down syndrome: MR quantification of brain structures and comparison with normal control subjects.

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Down Syndrome: MR Quantification of Brain Structures and Comparison with Normal Control Subjects

For quantification of brain structures from MR scans, a novel, powerful stereologic tool known as Cavalieri's principle was applied. This tool enables an objective estimation of volume. The method was applied to detect differences in various brain structures between persons with Down syndrome and control subjects. On the basis of absolute values, smaller volumes for the whole brain, cerebral cortex, white matter, and cerebellum were seen in persons with Down syndrome. Similar results were observed when a normalization procedure, based on the volume of cranial cavity, was used.

Stereologic determinations of the volumes of brain structures from MR images can reliably identify volume differences between persons with Down syndrome and control subjects.

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Morphometry gives reliable, objective, and reproducible data that permit easy comparisons of results derived from brains in different clinical conditions. Stereology, a morphometric discipline, is a body of mathematical methods relating three-dimensional parameters defining the structure to two-dimensional measurements obtainable on sections of the structures [1]. By these means, subtle differences in the structure of the brain as found in various clinical conditions can be assessed easily. The ensuing information is clinically valuable for diagnostic decisions and therapeutic interventions.

Down syndrome (DS) is known to be one of the major conditions related to mental retardation. DS results from meiotic nondisjunction of chromosome 21 in 95% of cases, from translocation of parts of this chromosome in 3% of cases, and from mosaicism in 2% of cases [2, 3]. Neuropathologic features of DS are lower brain weight; smaller frontal lobes, brainstem, and cerebellum; and reduced frontoorbital diameter [4]. These descriptions are based on findings derived from brain autopsies; therefore, they are affected by possible postmortem problems like preterminal events, postmortem delay time, fixation, and weighing. All of the problems mentioned can be avoided with the use of axial CT or MR scans.

Morphometric data derived from the brains of persons with DS and based on stereologic methods are thus far lacking. The only attempts to quantify brains of living persons with DS have been based on CT measurements [5, 6]. However, as resolution and delineation of different brain structures on CT scans are limited, morphometric evaluations based on CT scans seem to be inadequate. In contrast, MR scans give higher resolution and allow proper delineation of gray and white matter as well as of brain nuclei [7, 8].

The present study reports on quantitative data of different structures of brains obtained from MR scans of persons with DS compared with age-matched control subjects. Cavalieri's principle [9], a novel, powerful stereologic tool for estimating the volumes of brain structures, was applied. Furthermore, problems related to normalization of brain volume to body height were considered, and a normalization
procedure based on the volume of the cranial cavity was carried out.

Subjects and Methods

In the investigation group of DS subjects, we studied seven adults, two women and five men 30–45 years old (mean age, 38 years). DS subjects were participants in an interdisciplinary investigation that included medical screening tests as well as psychological assessments [10]. These seven were recruited from their family homes, and all of them showed a trisomy 21 karyotype originating from chromosomal nondisjunction.

The control group comprised seven persons, two women and five men 36–44 years old (mean age, 38 years). Neither neuropathologic nor other clinical symptoms were seen in the control subjects. None were on neuropsychopharmacologic treatment.

Imaging was performed on a 0.5-T superconductivity unit (Philips Gyroscan S5) with a spin-echo (SE) technique and an inversion-recovery (IR) technique. With the SE technique, T2-weighted images, 2000/50/100/2 (TR/TE/excitations), were obtained in axial planes. The IR technique provided T1-weighted images, 1800/400/80/2 (TR/TE/excitations), in axial planes. The images obtained with the IR technique had a slice thickness of 8 mm and a slice gap of 1.6 mm. Thus, the slice thickness plus slice gaps equaled 9.6 mm. The imaging matrix was 256 × 256 and the field of view was 250 mm.

Volume measurements were based on Cavalieri’s principle [9, 11, 12]: The volume of any object may be estimated from randomized and parallel sections separated by a known distance by summing up the areas of all cross sections of the object and multiplying this sum by the known distance. The MR scans were randomized, since the first slice hitting the brain fell randomly; these were followed by systematic sections, with the known fixed interval equal to the slice thickness plus slice gap. The first slice was positioned at a distance less than 9.6 mm below the upper margin of the cortical surface. The MR images were fed with a camera into the image analysis system, IBAS 2000 (Kontron, Eching, Germany). The contours of the different brain structures were manually traced with a cursor by one of the authors. Each brain structure was traced three times. A mean value was calculated and subsequently used for statistical analyses. The accuracy of the measurement was verified by calculating the coefficient of error. The profile area of the brain structures was then measured for each slice. The profile areas of each brain structure were summed up and multiplied by slice thickness.

The volumes of the following brain structures were determined: cerebral cortex, white matter, ventricles, thalamus, caudate nucleus, lentiform nucleus, cerebellum, and brainstem. The measurement of the ventricles included measurements of the lateral, third, and fourth ventricles. Measurement of the lentiform nucleus included measurement of the putamen and globus pallidus. The volume of the whole brain resulted in summing up the volumes of the previously defined structures. In addition, the volume of the intracranial cavity was determined.

The coefficient of error is an indicator for the precision of measurements of individual brain structures. The coefficient of error was calculated for each brain structure. Details regarding the coefficient of error have been published [11].

The following normalization procedure was used: The volume of the different brain structures was related to the volume of cranial cavity. The volume of the cranial cavity was set at 100%. Values for the volume of the different brain structures were calculated relative to the cranial cavity. Furthermore, we related the volumes of different brain structures to the volume of the whole brain. The volume of the whole brain was set at 100%. Values for the volumes of the different brain structures were calculated relative to the whole brain.

For statistical analysis, the one-way analysis of variance (ANOVA) was computed with the software package STATGRAPHICS.

Results

On the MR scans of the DS and control groups, no pronounced signs of atrophic changes were seen on the basis of direct visual examination by an experienced neuroradiologist (Fig. 1). Values for the coefficient of error giving the precision of the measurement were not tabulated. They were less than 5% for the following brain structures: cranial cavity, brain, cerebral cortex, white matter, ventricles, thalamus, cerebellum, and brainstem. They ranged between 5% and 10% for the caudate and lentiform nuclei. Thus, accuracy and precision of the measurement were given corresponding to the rules of the coefficient of error and systematic sampling [11].

The morphometric data of the different brain structures as measured on T1-weighted MR scans for the investigation and control groups are shown in Table 1. A significantly smaller volume of the whole brain was measured in persons with DS as compared with controls. This difference in volume was attributable predominantly to a reduced volume of cerebral cortex and white matter in DS as compared with controls. The volume of the cerebellum in DS also showed significant differences in comparison with the control group. The other brain structures did not show significant differences between persons with DS and control subjects.

Different results were obtained when ratios relating the volume of brain structures either to the volume of the cranial cavity or to the volume of the whole brain were calculated (Table 2). With the use of the proposed normalization procedure based on the volume of the cranial cavity, the ANOVA computations gave approximately the same results as obtained with absolute values (see Table 1). However, when

Fig. 1.—Horizontal MR scan of a person with Down syndrome. The following brain structures displayed on the scan were analyzed: cerebral cortex, white matter, ventricles, caudate nucleus, lentiform nucleus.
TABLE 1: Absolute Values for Volumes of Different Brain Structures Obtained by Morphometry of MR Scans from Persons with Down Syndrome and Control Subjects

<table>
<thead>
<tr>
<th>Structure</th>
<th>Mean Volume in cm³ (SD)</th>
<th>Controls (n = 7)</th>
<th>Down Syndrome (n = 7)</th>
<th>F Ratio</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>1313.1 (146.0)</td>
<td>1081.6 (81.0)</td>
<td>13.5 .003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>632.5 (65.1)</td>
<td>528.8 (40.1)</td>
<td>12.8 .004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White matter</td>
<td>462.4 (56.1)</td>
<td>360.3 (51.1)</td>
<td>12.6 .004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricles</td>
<td>23.3 (9.1)</td>
<td>29.1 (13.2)</td>
<td>0.9 .36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>16.7 (4.9)</td>
<td>14.3 (6.5)</td>
<td>0.6 .46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>8.5 (1.7)</td>
<td>8.6 (2.5)</td>
<td>0.0 .96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td>14.0 (1.5)</td>
<td>14.5 (3.6)</td>
<td>0.1 .76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>149.4 (31.2)</td>
<td>121.3 (12.5)</td>
<td>4.7 .05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>29.9 (6.2)</td>
<td>24.4 (3.4)</td>
<td>4.0 .07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial cavity</td>
<td>1571.2 (231.0)</td>
<td>1443.2 (99.0)</td>
<td>1.6 .20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2: Normalization Procedures of Brain Structures: Ratios in Down Syndrome and Control Subjects

<table>
<thead>
<tr>
<th>Structure</th>
<th>Brain Structure/Whole Brain</th>
<th>Brain Structure/Cranial Cavity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 7)</td>
<td>Down Syndrome</td>
</tr>
<tr>
<td>Brain</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>48.2</td>
<td>48.9</td>
</tr>
<tr>
<td>White matter</td>
<td>35.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Ventricles</td>
<td>1.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td>1.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>11.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Brainstem</td>
<td>2.3</td>
<td>2.2</td>
</tr>
</tbody>
</table>

relating the volume of brain structures to the volume of the whole brain by relying on these relative values, we were unable to observe any difference between the two groups (Table 2).

Discussion

Measurements on CT scans have been performed by different investigators [13–19] in an attempt to quantify changes of brain structures occurring with normal and pathologic aging. In most of these investigations, linear, one-dimensional measurements were used to determine specific parameters related to the ventricular system, skull vault, and external CSF spaces. Since the basic rules of stochastic geometry are not followed, meaningful results based on one-dimensional parameters are limited. Priority has to be given to volume estimation as a three-dimensional parameter rather than to linear, one-dimensional parameters [20].

Objective estimations of the total ventricular volume of the brain in 10 hydrocephalic children and adults have been determined recently by stereologic assessment of CT scans [21]. Values of ventricular volume were compared with values obtained by Evan’s ratio. Evan’s ratio is derived from the widths of the right and left anterior horns divided by maximum width of the internal skull [13, 22]. The authors of the previous study [21] showed that Evan’s ratio is of dubious value.

CT and MR scans have been analyzed by semiautomated image analysis [5, 6, 19, 23–27]. The analysis procedures use the differences in gray level of pixels to differentiate between and delineate brain structures. With some ease, the ventricles and CSF spaces can be resolved on CT. However, the exact boundary of cerebral cortex as well as subcortical nuclei is very difficult to discern from CT scans. Thus, results of morphometric analysis based on CT scans only yield a limited amount of information. MR allows a more exact delineation between gray and white matter, as well as between subcortical nuclei. Recently, semiautomated image analysis procedures have been developed for morphometric analysis of MR brain scans [25–27].

A normalization of brain volume to body height has recently been proposed [28]. These authors rely on data showing that brain size is proportional to body height. Since persons with DS are of smaller stature, the authors performed a normalization of brain volume in the following way: The absolute volume was divided by the subject’s height and then multiplied by 170 cm, the average height of the male coworkers at their laboratory. However, the relationship between brain size and body height remains obscure, and thus its relevance is questionable. On the basis of an analysis of 3406 autopsy brains, no significant correlation was found between brain weight and body height. The correlation coefficient was given for males as r = .28 and for females as r = .30 [29].

Some authors express their data in relative values or as ratios [5, 15, 17, 27]. Such results must be interpreted very carefully, since it should be kept in mind that, when ratios are used, no significant differences in the structures of interest can be detected when the structure of interest as well as the reference structure show equivalent differences. Both arguments lead to the so-called “reference trap,” which has been discussed in detail [30]. As shown in Table 2, the ratio of the volume of the brain structure to the volume of the brain masked differences that were seen when absolute values were used. It shows that the variable of brain volume is correlated to the volumes of the other brain structures, and hence is not a suitable variable for a normalization procedure in this context. For the normalization procedure, therefore, we introduced a reference variable that is independent. In the sample, it could be pointed out that the volume of the cranial cavity showed no significant differences between the DS and control groups. Thus, when using the volume of cranial cavity as the normalization variable relative to other volumes of brain structures, it was demonstrated that the normalized values showed similar differences between the two groups, as those inferred from the use of absolute volume values.

It is interesting that the typical brachycephalic skull form of persons with DS, which was also obvious in our DS subjects, had no influence on the volume of the cranial cavity in comparison with that of the controls (see Table 1). In this study, we could show that the volume of the cranial cavity was an independent variable, as seen in Table 1. However, the volume of the cranial cavity should not be treated as an independent variable per se. Its validity as such a parameter must be determined separately for each study. In another study, the volume of the cranial cavity was measured in a postmortem session by occupying the cranial cavity completely with a lubricated balloon filled with water [31]. It was shown that
the ratio of brain to cranial cavity volume is constant in persons 20–55 years old [31].

The question about the influence of cranial cavity to brain size, and of brain size to cranial cavity, has to be addressed when analyzing changes during developmental stages. In this case each component can have an influence on the other. In the case of hydrocephalus, the overproduction or malabsorption of CSF leads to an accelerated expansion of the brain. Since ossification in this developmental stage is not complete, the bony skull expands in the same way as the brain. The opposite occurs in the case of a premature ossification of the cranial sutures. The bony skull is no longer expanding. The underlying brain is growing but is restricted by a rigid skull. In adults, such cases are not encountered. The sizes of the cranial cavity as well as of the brain have already been determined. The brain is, however, able to regressively change, resulting in brain atrophy. When this occurs, the size of the cranial cavity is not affected. Intracranial space-occupying lesions like tumors, even when they reach a large size, have no expanding influence on the cranial cavity. Considering these points, we are confident that in our study the volume of the cranial cavity was indeed an independent variable.

Absolute values of different brain regions derived from CT or MR scans can be compared with those values obtained from autopsy brains. Thus, the reliability of the measurements performed on CT and MR scans can be evaluated. Major discrepancies in volume estimation are found between investigations based on CT techniques [6, 19, 23] and our own investigation, which was performed on MR scans. Comparing the results of other MR investigations [25, 26] and our own investigation, it can be seen that the values derived from MR scans were very close to those values obtained from analysis of autopsy brains [32].

Until now, only a few quantitative CT investigations have been carried out in persons with DS [5, 6, 28, 33]. In these investigations, it was shown that healthy adults with DS had smaller brains and smaller intracranial volumes than did control subjects [5, 6, 28]. Normalized volumes of CSF, ventricles, and brain parenchyma did not differ between DS and control subjects. Furthermore, both persons with DS and controls showed similar significant age-related incremental increases in volumes of CSF and ventricles [5]. Increased CSF and the third ventricular volumes as well as increased gray and white matter volumes were found in older persons with DS (>45 years), who showed signs of dementia as compared with younger DS adults [6]. The authors concluded that brain atrophy must be present to accompany dementia in older persons with DS, despite the presence of Alzheimer neuropathology in all older subjects with DS. Furthermore, in persons with DS who are over 50 years old, a significant widening of the temporal horns has been demonstrated recently [33]. The decrease in intracranial size seen in the previous studies [5, 6, 28] occurred mainly because the authors used only seven CT scans for their measurements. Thus, they were not able to measure the volume of the whole cranial cavity. This may also explain the discrepancies between our results and those reported previously.

In the present investigation, data derived by morphometric analysis of MR scans showed differences between persons with DS and control subjects in brain volume, cerebral cortex, white matter, and cerebellum. In addition, measurements of the caudate nucleus, lentiform nucleus, and thalamus are reported. No differences in the volume of these latter structures were found between DS and control subjects. Furthermore, the volume of the brainstem was reduced in subjects with DS in comparison with controls, but this difference did not reach statistical significance. The most striking difference between both groups was smaller volumes of whole brain, cerebral cortex, white matter, and cerebellum. However, persons with DS did not show specific atrophic changes in the brain. It is noteworthy that no differences were found in the volumes of the cranial cavity between persons with DS and control subjects.

Despite radiologists’ finely developed ability to interpret morphologic patterns visually and intuitively, there are some things they cannot assess by these means, mainly quantitative differences. Volume differences must be on the order of 30–50% to be discernible [34]. The differences in brain volume in persons with DS amounted to 18%, and thus are within a range that can be reliably detected only by morphometry.

Cavallieri’s principle for volume estimation of structures is a very powerful stereologic tool and, when applied to well-delineated brain structures obtained from MR scans or autopsy slices, provides objective data. The combination of the techniques of morphometry and MR provides the most suitable method for investigating differences and changes in the CNS in a living population for cross-sectional as well as longitudinal studies.

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REFERENCES