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Corpus callosum morphology and ventricular size in chromosome 22q11.2 deletion syndrome

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Abstract

In this paper, novel methods were used to map the corpus callosum morphology of children with chromosome 22q11.2 deletion syndrome in order to further investigate changes to that structure and to examine their possible effects on cognitive function. The callosal profiles were extracted from the centermost MRI midsagittal slice by supervised thresholding and the structure's boundary and midline were computed automatically. Difference analysis was based on non-rigid registration, in which a template image is warped to conform to the shape of each corpus callosum in the sample. Boundaries and midlines were registered to a template and the results used to determine the average callosal shapes for children with the deletion and for controls. Point-wise registration also enabled the detailed evaluation of callosal curvature, width, area and length. Significant differences between the two groups were found in shape, size and bending angle. Results showed group differences that were concentrated in the anterior part of the structure, more specifically in the rostrum, which was larger and longer in the group with the syndrome. Correlation analyses showed that ventricular enlargement does not fully account for callosal morphology differences in children with the deletion. However, areal measurements did reveal important relationships between changes in callosal morphology and cognitive function. These novel findings reveal intricate relationships between genetic and disease-specific factors in the callosal anatomy and the potential impact of those changes on cognitive functions.

Keywords

Children; chromosome 22q11.2 deletion syndrome; cognition; corpus callosum; morphology; image registration

1) INTRODUCTION

Chromosome 22q11.2 deletion syndrome (DS22q11.2) results from a 1.5 - 3Mb microdeletion on the long (q) arm of chromosome 22 (Driscoll, 1992a, 1992b) and encompasses the phenotypes of DiGeorge (DiGeorge, 1965), Velocardiofacial (Shprintzen, 1978) and

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Conotruncal Anomaly Face (Burn, 1993) syndromes, and some cases of Cayler Cardiofacial syndrome (Giannotti, 1994) and Opitz G/BBB syndrome (McDonald-McGinn, 1995). Prevalence is thought to be around 1 in 4000 to 1 in 7000 live births (Burn, 1996). Following characterization of a range of medical manifestations that include cardiac, palatal and immune disorders, recent attention has turned to the neurocognitive implications of the disorder. Children with DS22q11.2 have been shown to exhibit particular impairments in a range of cognitive domains, most of which relate to the competencies measured in the “performance IQ” (PIQ) component of traditional psychometric tests (for reviews of such studies, see Campbell, 2005; Simon, 2005b). This impairment of abilities in the non-verbal and especially spatial domain has been reported for memory Lajiness-O'Neill, 2005; Sobin, 2005) as well as visuospatial attention, numerical and temporal cognition (Debbané, 2005; Simon, 2005a). Related impairments in executive function have also been reported (Bish, 2005; Sobin, 2004; Woodin, 2001). As we have discussed in detail in a previous work (Simon, 2005b), one hypothesis that unifies this pattern of impairments is that they may be the result of changes to the structure and/or function of the frontoparietal attention network upon which all of these competencies have been shown, at least in typical adults, to be somewhat dependent. The primary goal of the study presented here was to assess whether an examination of differences in the structure of the corpus callosum and its relation to at least one task in that set could produce insights into changes in the structure and function of that network.

In recent years several studies have reported regional differences in brain volumes between children and adolescents with DS22q11.2 and controls (Campbell, 2006; Eliez, 2000, 2001, 2002; Kates, 1999; Simon, 2005c), many of which directly affect brain regions that contain components of the canonical frontoparietal attention network. In particular, reductions in gray and white matter seem to be particularly focused in the posterior brain, particularly the parietal lobes, and in the medial cerebellum, which is also now considered part of that network. Two recent studies have also drawn particular attention to changes in the corpus callosum in children with DS22q11.2. A study by Shashi et al. (2004) reported increases in the size of the posterior callosa of affected individuals, most specifically in the isthmus region. Antshel et al. (2005) also reported significant differences in area measurements of the corpus callosum between children with DS22q11.2 and controls.

A more extensive analysis of brain morphology of children with the deletion was carried out by Simon et al. (2005c). The findings linked changes in the corpus callosum to reductions in posterior brain tissue and an associated dilation of the lateral ventricles and raised the possibility of a resultant change in posterior brain connectivity that might be related to the observed cognitive impairments mentioned above. Following the publication of that study, subsequent analyses carried out in our laboratory suggested that different analytical methods may also reveal changes in the anterior aspect of the corpus callosum that were not detected by conservatively thresholded voxel-based morphometry (VBM) analyses. One hypothesis we are currently exploring for why anterior changes were not detected is that the large differences in ventricular shape and size between the children with DS22q11.2 and the controls in our sample may have affected our ability to co-register a set of brains in which this structure differs so widely. If this were to be the case, the ventricular differences would have likely had the biggest effect on comparisons of immediate periventricular tissue, such as the corpus callosum. Therefore, the present study focuses on examining the morphology of the corpus callosum independently of other factors such as ventricular shape and size. By extracting the corpus callosum from the whole brain image for each participant and subjecting that structure to a series of analyses, we were able to generate more direct measures of differences in callosal morphology between the two groups. We then used those new results to independently assess the impact of ventricular size on morphological changes and the effect of those changes on one domain of cognitive function.

In image registration, the points of an image used as a reference are mapped to corresponding points in the images of the participants, so as to establish a common basis for shape comparison. In this study, we were particularly interested in registering the callosal medial axes, based on curvature similarity, so that regional shape and size differences between the groups could be statistically analyzed. The registration of the callosa enabled individual measurements in 85 segments of the structure (Fig. 1a). In order to explore the hypothesized relation between ventricular size and callosal morphology, independently generated measures of the lateral ventricles of all the participants were included in the analysis, so that we could directly evaluate correlations between the two measures.

Finally, we extended the analyses to the investigation of our hypothesis of the relationship between changes in callosal morphology and cognitive dysfunction (Simon, 2005a, 2005c) by carrying out correlations between measures of cognitive function collected in our laboratory and the morphology results generated by the current study. Significant relationships found between these measures would support our proposal that callosal changes in children with DS22q11.2 are a marker for changes in neural connectivity that may contribute to dysfunction involving the frontoparietal attention network. Such dysfunction, we have suggested, is at the root of many of the cognitive impairments observed in children with the deletion.

2) RESULTS

The analyses indicated a significant difference between children with DS22q11.2 (DS) and typically developing controls (TD) in the shape and size of the anterior half of the corpus callosum. The rostrum of the corpus callosum in individuals with the syndrome was larger and longer than in TD. The callosum was also more arched in DS, but this bowing was prominent at the anterior callosum rather than a general vertical displacement of the callosal body. Finally, correlations of callosal morphology with measures of performance on an enumeration task showed that response time, adjusted for accuracy, is inversely correlated to the area of the genu in DS. These findings provide the first direct support for our hypothesis of a relationship between different connective patterns in children with DS22q11.2 and their detrimental affect on cognitive function. Specifically, poorer performance on a numerical task that has been shown, at least in typically developing adults, to depend heavily on the posterior aspects of the frontoparietal attention network (Piazza, 2002; Sathian, 1999) was found to directly relate to measures of the anterior corpus callosum in children with DS22q11.2 but not in controls.

Below we examine separately analyses of callosal curvature, length, width and area differences between the groups. Significance values determined by ANOVA ($df=1,32$) are color-coded in the figures to indicate the level of significance of each segment. The p -values and effect size reported in the text indicate the most significant values found in each region. We then explore the relationship of ventricular size to changes in callosal shape for each group. Finally, we report on correlations between morphology and cognitive performance in order to assess the functional significance of the changes that are described. For the analysis of correlations, significance values determined by t -tests (two-tailed, $df=13$) in each segment are color-coded in the figures. The Pearson correlation coefficient, r , and p -values reported in the text indicate the most significant values found in each region.

Analysis of curvature

The average transformations obtained from the template-to-participant registrations were used to determine the mean callosal shape for each group. Fig. 2 shows the superimposed average shapes for DS (vertical lines) and TD (horizontal lines). It reveals a major difference in the anterior part of the structure where, on average, DS had a more arched anatomy than did TD. The average curvatures at the midline are displayed in Fig. 3a. The figure also shows the template subdivided into seven regions, adapted from the partitioning scheme proposed by

Witelson (1989). In this curved-line version of Witelson's topology, the corpus callosum is divided into the rostrum (segments 1-7), genu (8-18), rostral body (19-33), anterior midbody (34-44), posterior midbody (45-56), isthmus (57-65) and splenium (66-85). Differences in curvature (Fig. 4a) are evident at the genu, where the controls have greater curvature ($ES=1.19$, $F=19.53$, $P=0.0001$), and at the rostral body, where DS has greater curvature ($ES=-0.73$, $F=5.39$, $P=0.0268$).

Analysis of size differences

In order to align the template to the callosum of a given participant, the midline of the template is deformed (with certain segments stretched and others shrunk) to conform to the shape of the participants' midlines. The analysis of this length scaling along the midline is important, as it reveals subtle differences within the overall callosal structure, indicating the segments that differ between the groups, with respect to length. From Fig. 2, it is possible to qualitatively detect the second major difference between the anatomy of the DS and TD groups: the rostrum is longer ($ES=-0.94$, $F=9.92$, $P=0.0035$) (Fig. 3b and 4b) and larger in DS ($ES=-0.95$, $F=10.24$, $P=0.0031$) (Fig. 3c and 4c). In contrast, the callosum is wider at the genu of the TD group when compared to DS ($ES=0.87$, $F=8.10$, $P=0.0077$) (Fig. 3d and 4d).

Analysis of relationship between ventricular size and callosal morphometry

Measurements of ventricular size were computed based on semi-automated contour segmentation and manual delineation. The mean(S.D.) ventricular volumes, corrected for sex and age covariates were $9.31(4.27) \text{ cm}^3$ and $14.41(8.8) \text{ cm}^3$ for the TD and DS groups, respectively. These values were significantly different at the level of $P=0.0341$ ($ES=-0.70$, $F=4.90$, $df=1,32$). This finding is consistent with other indirect and direct measures showing dilated lateral ventricles in children with DS22q11.2 (Campbell, 2006; Simon, 2005c; Sztriha, 2004).

In the current study, we found a significant correlation between ventricular enlargement and curvature along the genu ($r = -0.61$, $p=0.0127$, $t = -2.86$) and anterior midbody ($r = 0.62$, $p=0.0097$, $t=2.99$), for the controls, and at the rostral body ($r = 0.75$, $p=0.0008$, $t=4.23$), posterior midbody ($r = 0.64$, $p=0.0074$, $t=3.13$), isthmus ($r = 0.70$, $p=0.0027$, $t=3.63$) and splenium ($r = -0.54$, $p=0.0312$, $t = -2.39$) of DS group (Fig. 5). However, no significant correlation was found between ventricular size and curvature of the genu and rostrum in the DS group, even though the curvature in these regions was significantly different between the two groups (compare with Fig. 2 and Fig. 4a). Similarly, we found no significant correlation between the ventricular scores and the length or area of the rostrum in the DS group, for any of the partitioning approaches that we applied (Fig. 1), which is consistent with the results reported by Downhill et al. (2000) and Frumin et al. (2002). Therefore, while it appears to be an important factor that influences callosal shape, ventricular dilation alone does not account for all of the changes observed in our sample. Some other mechanism, yet to be described, evidently contributes to the callosal changes seen in our population of children with DS22q11.2.

Analysis of relationship between callosal morphometry and cognitive function

Simon (2005b, 2005c) has hypothesized that underlying the spatial and numerical cognitive impairments in children with DS22q11.2 is a more basic attention system dysfunction that is typically supported by a frontoparietal neural network. If this is the case then significant changes in the morphology of the corpus callosum, which is the major interhemispheric commissure connecting these regions, would be expected to have a significant effect on the ability of this system to function optimally. While the current analyses do not allow us to carry out causal tests of this hypothesis, we can certainly evaluate the correlations between changes in callosal morphology and cognitive functioning in children with the deletion relative to the

typically brain structure and function of the controls. We took cognitive performance data from a task carefully designed to test the nature of visuospatial attention and numerical cognitive processing and correlated these with various pointwise measures of callosal morphology. Since the task is described in detail in a previous work (Simon, 2005a), we shall briefly describe here only the critical features.

Children were presented with displays of one to eight green bars on a red background in displays that subtended less than 2° visual angle so that eye movements could not be used. The child's task was simply to give a speeded vocal response, which was timed using a microphone voice key, for each numerosity displayed. There were 20 instances of each of the eight set sizes or 160 trials. A research assistant, who could not see the visual display, recorded the spoken number using the computer keyboard so that accuracy of responding could also be assessed. Response time was adjusted using the formula: $RT_{adj} = RT / (1 - \% \text{ error})$ (e.g. Bish, 2005), and outlier responses were considered to be those exceeding 2.5 standard deviations above the grand mean for the numerosity concerned.

Our reason for selecting this task to examine first was as follows. We have previously demonstrated (Simon, 2005a, 2005b) selective differences between children with DS22q11.2 and controls on this task that directly support our hypothesis of frontoparietal attention network dysfunction. In the subitizing component of the task, which has been shown in adults to activate brain areas not associated with the frontoparietal attention network, children with the deletion performed at the same speed, rate and accuracy as typically developing controls. Despite an average difference of two standard deviations in global intellectual functioning measures such as Full Scale IQ (32.8 points) and the more relevant Performance IQ (29.6 points), their behavior on this part of the task was indistinguishable from that of the controls. However, in the counting part of the task, where activation of much of the frontoparietal attention network is much of the characteristic neural signal, children with the deletion performed at an overall slower speed and counting rate, and made many more errors than the controls. The question for our correlational exploration was whether systematic relationships between changes in the corpus callosum and enumeration performance might be detected, especially in the counting and not the subitizing range. If so, our inference would be that those differences could be interpreted as, at the very least, consistent with our hypothesis of altered connectivity in the network and would, in turn, generate new hypotheses about the nature and location of that connective change.

In order to ensure that our analyses did not include data from enumeration processes other than the target ones or from guessing (e.g. that any large display consisted of 8 targets), we conservatively defined counting as only including the numerosities four to seven, and subitizing as only including the numerosities one and two. These performance measures produced the following results. For subitizing, mean(S.D.) adjusted response time, corrected for sex and age was 915.4(207.9) msec for TD and 983.12(143.2) msec for DS ($ES = -0.38$, $F(1,30) = 1.25$, $P = 0.2722$). For counting, adjusted response time corrected for sex and age was 1432.18(348.1) msec for TD and 2063.76(426.7) msec for DS ($ES = -1.26$; $F(1,30) = 22.09$, $P = 0.00005$).

The analysis of correlation between adjusted response time for counting 4-7 target items and regional area revealed that reaction time was inversely correlated to the area of the genu ($r = -0.62$, $t = -2.98$, $p = 0.0098$) and positively correlated to the area of the rostral body ($r = 0.70$, $t = 3.67$, $p = 0.0025$), anterior midbody ($r = 0.69$, $t = 3.36$, $p = 0.0028$) and posterior midbody ($r = 0.54$, $t = 2.39$, $p = 0.0314$) in DS, i.e., children with larger genu and small rostral body/midbody produced better performance in terms of faster and more accurate counting (Fig. 6c-d). However, this relationship could not be observed in TD, in which no significant correlation was observed between regional area and response time (Fig. 6a-b).

The correlation between callosal area and response time for subitizing in DS followed a similar pattern in the anterior region of the callosum (Fig. 7c-d). Response time was significantly inversely correlated with the area of the rostrum ($r = -0.61$, $t = -2.88$, $p = 0.0122$), and genu ($r = -0.68$, $t = -3.43$, $p = 0.0041$). In addition, isolated segments at the posterior midbody and splenium presented significant positive correlation with response time (see table 2). The correlation for the TD group, as also observed for the counting task, was not significant, (Fig. 7a-b). The influence of performance IQ differences on the correlation between callosal area and cognitive tests was investigated by adjusting the dataset for PIQ scores. Despite the significant difference between the average PIQ scores of the groups, the adjustment was not sufficient to neutralize the correlation between area differences and cognitive test scores. For subitizing, no significant changes were detected. In the case of counting tasks that naturally involves more intellectual skills, the adjustment only reduced the magnitude of the findings but not their significance at the a priori established threshold level of 0.05. Tables 1 and 2 show the detailed values at each segment, respectively for the counting and subitizing tasks.

So, for counting we see a dissociation pattern between the two groups with better performance being related to the area of the genu for children with the deletion but not controls. This difference is consistent with our hypothesis that different neural networks are being used for each group to complete this task and that likely relates to overall task performance. For subitizing, we have a weaker pattern overall but again a similar dissociation, with response time being negatively correlated to the genu in DS. The pattern for controls was consistent with the already established occipital dependence for subitizing (e.g. Sathian, 1999) while the children with the deletion again showed what appeared to be an atypical and apparently more frontally dependent network. So our results do appear to show, at least correlationally, that changes in the corpus callosum are associated with changes in the performance of a task, part of which is expected to be strongly dependent on the frontoparietal attention network. That part, performance in the counting range, showed the greatest correlation with callosal area and produced a different pattern of relationships between the two groups. This can, at least for the sake of generating a hypothesis to test further, be interpreted as consistent with some evidence of different interhemispheric neural connectivity between the groups. This is especially true because we used the dimension of callosal area, which at least superficially, might be expected to relate in some way to the amount, density or arrangement of fibers represented in that segment of the structure.

3) DISCUSSION

The characterization of callosal morphology is a relatively recent research direction in studies of the chromosome 22q11.2 deletion syndrome and only a few studies have been reported in the literature. Our investigations found several finely characterized differences in the morphology of the corpus callosum between children with DS22q11.2 and controls. Many, but not all, of these are consistent with the few previously published findings. Therefore, in this section we provide a comparative discussion of the results with previously reported findings, regarding the size and shape differences in the callosal anatomy and their relationship to ventricular enlargement.

Size differences

Increased callosal size associated with DS22q11.2 has been consistently reported in the literature (Antshel, 2005; Shashi, 2004; Usiskin, 1999). While we also found increased size of the corpus callosum in children with DS22q11.2 (mean(S.D.)=656.2(58.5) mm² for TD and 688.7(120.3) mm² for DS, $ES = -0.34$), our results on regional differences are less consistent with previous findings, and this may be a consequence of the different sample sizes and partition protocols used in each study. The strategy of subdividing the midsagittal appearance of the corpus callosum into segments for the purpose of studying regional size variation is a

critical component of morphological studies of the structure and has historically followed several different approaches. Since the cross-sectional shape of the corpus callosum is variable and presents few landmarks, investigators have devised schemes to partition the structure into sub-regions that may be roughly considered to connect specific parts of the cerebral hemispheres.

The topological subdivision proposed by Witelson (1989), based on postmortem analysis, is well known. It partitions the callosum into 7 regions, determined as posterior and anterior halves, thirds and fifths of the callosal major axis of orientation (Fig. 1b). The genu and rostrum are defined based on a line, perpendicular to the axis of orientation, which is tangent to the anterior-most point of the ventral boundary. Other approaches that partition the callosum based on the axis of orientation (straight-line methods) (Allen, 1999) divide the structure into fewer sub-regions. An alternative to the straight-line method is the curved-line approach that uses the midline of the callosum as the axis along which perpendicular line segments are extended to demarcate partitions.

These approaches have the drawback of using fixed proportions for the segmentation of all participants in the sample, regardless of individual anatomic differences. The rostrum is a substructure that is particularly susceptible to this problem. Each participant may have the rostrum encompassing different percentages of the callosal length. Moreover, since it is a small substructure, even a small shift in the segmentation may substantially impact the measurement of its area. In our study, morphometric analysis was based on the results of registration, which provides a more flexible and detailed method of partitioning, since the template is deformed to match the anatomy of each participant. The sub-regions of the template are therefore mapped onto the anatomy of the participants, improving the degree of correspondence (Gee, 1999). Few studies on the callosal anatomy have implemented some degree of registration. DeQuardo et al. (1996, 1999) and Tibbo et al. (1998) applied landmark matching to obtain an average configuration used to warp a template. Narr et al. (2000) applied a surface-based anatomical modeling approach to generate parametric representations of the callosal outlines, which were matched with the purpose of generating an average shape. Therefore, the use of more sophisticated methods may account, to a considerable degree, for the difference between our results and others.

Another important factor that may account for a small amount of the variance in the results across these studies is the nature of the study sample. Although ages have generally been similar and measurements have been corrected for covariates, there have been differences in the number of participants, female/male ratio and age range. In recent studies Shashi et al. (2004) analyzed a sample composed of 13 DS, 5 females and 8, (mean(S.D.) age=10.0(4.1) years) and 13 TD children, 5 and 8 males, (age=10.8(4.2) years) ranging in age from 7 to 18 years. Antshel et al. (2006) analyzed a larger sample of 60 DS, 29 females and 31 males, (age=11.1(2.7) years) and 52 TD children 27 females and 25 males, (age=10.8(2.3) years) who ranged in age from 6 to 15 years. Our study included 18 DS 11 females and 7 males, (age=9.9(1.4) years) and 18 TD children 6 females and 12 males, (age=10.4(2.0) years) ranging in age from 7 to 14 years.

Examination of the methods used to partition the corpus callosum is also important to support the comparison of studies based on different approaches. Shashi et al. (2004) measured the area of 5 sub-regions defined after Witelson's topological scheme (Fig. 1b), whereas Antshel et al. (2006) used the curved-line approach to partition the callosum into 5 segments (Fig. 1c). Although our analysis was primarily based on geometrical information from shape transformations on a template, additional studies were performed following the approaches presented by Shashi et al. and Antshel et al., in an attempt to reproduce their findings.

The same experimental procedure used by Shashi et al. was applied to our dataset. The results, shown in Table 3, are consistent for all subregions, except for the isthmus, in which no significant differences were detected. In both studies, the area of the isthmus was larger in the DS group, but the significance values differed: Shashi found a significant difference ($P=0.04$), whereas we could not reproduce this significance ($P=0.432$).

The experimental procedure used by Antshel et al. (2006) was also applied to our dataset. The results of size differences between the groups, obtained with our dataset, are consistent with the values reported by Antshel et al. with respect to the genu (Table 4), although we found no significant differences at the other segments. It is important to note that, based on the partitioning scheme used by Antshel, the genu encompassed a large region of the anterior callosum, including the rostrum, and this may have caused the size difference in the rostrum to be less evident. Using this partitioning scheme, our results also indicated no significant results in the anterior callosum. However, when we applied more refined subdivision, as proposed by Witelson, the larger rostrum in DS became evident (compare with the results in Table 3).

The number of subjects in the sample has direct impact in the statistical power of the analysis (Cohen, 1988). As more subjects are included into the sample, the number of degrees of freedom increases. This may be another reason for some differences between the results found by Antshel and the ones reported in the current study. In the case of the anterior body, for example, we found this region to account for 13.9% and 15.2% of the total callosal area, respectively for DS and TD, while Antshel reported 19.2% and 19.1%. So, even though the difference between the percentages was larger in our dataset, the differences reported by Antshel presented much more significant P -values. Inconsistency in significance values may also be an effect of differences in the overall callosal size. The DS sample studied by Antshel has much larger callosa than TD, so individual regions tend to be also significantly larger. The sample we analyzed was more homogeneous with respect to overall callosal size (difference between DS and TD only at the level of $p=0.246$), so the differences at individual sub-regions were equally reduced.

The registration-based approach used as the primary method for partitioning not only enables the morphological analysis of smaller sub-regions than do other partitioning schemes, but also accounts for individual anatomy. For example, if an individual has a shorter wider splenium, applying the method of segmentation into 5 regions could include part of the isthmus in the posterior-most 20% of the callosal extension, so that the isthmus would contribute to the measurement of the splenial area. The segmentation based on registration tries to accommodate these characteristics, so that a participant's splenium may encompass a larger or smaller percentage of the callosal length, according to its individual anatomy. In our experiments, this approach revealed a significant difference in the rostrum, as depicted in Fig. 3c and 4c. The rostrum in the DS group is not only larger but also longer, i.e. more posteriorly projected. These findings extend the results reported by previous studies and deserve further analysis, since differences in the sample size, average age and partitioning schemes complicate the comparison between studies. Registration has been considered a revolution for the field of morphometric studies and many models are available for this purpose (see Bookstein, 1997; Brown, 1992; Toga, 1999). A caveat to the use of registration algorithms, however, is the requirement of parameter specification: Each model involves a certain degree of parameterization, whose values may be determined experimentally. This fact not only reduces the reproducibility of the experiments, but also demands thorough supervision of the results so that one may get full benefits of this method.

Shape differences

Antshel and colleagues (2006) were the first to report callosal shape differences between DS and TD groups, based on the bending angle measure. The bending angle measure (Schmitt, 2001) is defined as the angle between the line segments that connect the center point of the midline to the tips of rostrum and splenium, which correspond to endpoints of the midline. The average bending angles computed for the sample analyzed in the current study are in accordance with the values reported by Antshel (Table 4). The DS group had a significantly more arched callosum, as shown in Fig. 2. However, the bending angle is very sensitive to the shape of the midline near its center and extremities. Small bending angles, for instance, may be a consequence of an arched callosal body, a more posteriorly projected rostrum, or both. A more detailed shape characterization of the callosum can be achieved by computing the point-wise curvature of the midline, as was done using our methods. In this study, we were able to determine that the arched shape of the callosum in the DS group is specifically associated with the displacement of the anterior portion of the structure. This displacement is not only upward, but in the lateral direction as well, giving the rostrum and genu an accentuated “hook” appearance. A similar displacement was reported by Frumin et al. (2002), while comparing the callosal shape between first-episode schizophrenia patients and controls, based on angular measurements of the callosal midline. A more laterally localized arching effect could be observed in the prototypic shape presented for the patients, resulting in a larger and longer rostrum.

A straightforward conjecture regarding the morphometric differences between TD and DS groups would be to associate the deformation of the callosum with the observed hyperplasia of the ventricles. Our current results found that the latter only partially explained the former, i.e. the size of ventricle in DS is correlated with the curvature of the midbody and rostral body but not with the curvature of the genu and rostrum, at the significance level of 0.05. Ventricular enlargement in children with DS22q11.2 (Campbell, 2006; Simon, 2005c; Sztriha, 2004) and schizophrenia (Okubo, 2001; DeLisi, 2004) has been previously reported. Although Simon et al. (Simon, 2005b) hypothesized a relationship between the two, the influence of ventricular enlargement on the morphology of the corpus callosum in individuals with DS22q11.2 has, to date, not been investigated. Neither has the relationship to ventricular dilation to subsequent onset of psychosis. So, although individuals with DS22q11.2 form one of the highest risk groups for schizophrenia in adulthood, the relationship between ventriculomegaly and psychosis remains speculative at best, especially when discussing a population of nonpsychotic children such as those in our sample.

Areal Measurement differences

Our finding of increased rostral area in the children with DS22q11.2 likely indicates a difference in the tractography of that region of the corpus callosum. The nature of this difference is unclear and, just as Pierpaoli and colleagues (1996) state that fiber characteristics cannot be inferred from diffusion tensor data, we must be clear that we are unable to infer from our data the nature of the difference in tractography that appears to be indicated. However, the likelihood that the volumetric change observed indicates some kind of change in neural connectivity has implications for cognitive function. In our current analyses we have shown how the measures of area in the anterior callosum correlate with performance on enumeration tasks in children with the deletion (who as group perform more poorly than controls on that aspect of counting task) while there is no such correlation for the controls. This dissociation is, to our knowledge, the first demonstration that apparent changes to neural connectivity in children with DS22q11.2 relate directly to performance on a task in the spatial/numerical domain whose typical implementation by a frontoparietal network is well recognized.

In a previous study (Simon, 2005c) we carried out a whole brain analysis and found evidence of relationship between enlarged ventricles, posterior and superior displacement of the corpus callosum and expansion of the splenial section. We hypothesized that these callosal changes may have an effect on cortical connectivity, especially affecting the parietal lobes that are implicated in much of the visuospatial and numerical cognitive dysfunction experienced by children with the deletion. In the current study we analyzed the morphology of the corpus callosum after extracting it from the whole brain image so that changes could be measured independently of other brain changes. Thus our current analyses did not address location of the corpus callosum within the brain. However, the longer rostrum and rostral body in the DS group may be consistent with a posterior displacement of the overall callosum, especially the splenium, as reported in our previous study. Our separate analysis of the effect of ventricular enlargement on callosal morphology using an independently developed measure showed that ventricular dilation did have some effect, especially on the curvature of the midbody and posterior callosum. In general, to the extent that the two sets of findings can be integrated, they suggest that the *global* posterior and superior displacement of the corpus callosum found in the DS group that we previously reported may be complemented by a *local* displacement of the anterior callosum. What the new findings show is that a more sensitive analysis that focused just on factors within the callosum itself indicate that many of the differences between groups are ones affect the shape, size and internal structure of the anterior sections.

The direct functional implications of these changes are, as yet, not entirely clear. However, data from at least one of our tasks does indicate that different patterns of connectivity within the callosum correlate with performance. The fact that the connective pattern in the children with the deletion appears to be atypical and that their performance on the task is significantly impaired further suggest that these changes are functionally important. However, we should still be cautious about over-interpreting the findings. As Thompson et al. (2003) point out, the current understanding is that “heterotopic connections (i.e. between nonequivalent cortical areas in each brain hemisphere) are numerous and widespread even in the genu and splenium where callosal axons are highly segregated” (p. 95) and this contrasts with a long-held view that a simple topographic map of the callosum existed that reflected a pattern of cortical connections to the spatially contiguous cortical regions (i.e. genu to prefrontal, splenium to temporal, parietal and occipital etc). Nevertheless, in a study relating children's brain activations in a spatial working memory task to diffusion tensor imaging data of white matter tracts, Olesen et al. (2003), report correlations between measures of fractional anisotropy in the anterior corpus callosum and neural activation in the inferior parietal lobe on the task, which shares many characteristics to those in which children with the deletion display significant impairments. Our findings may be an illustration that an overdependence on such connections could be contributing to the impairment on the counting task for children with the deletion. Of course, such a hypothesis requires direct investigation and an explanation about how the deletion might lead to such an anomalous connective pattern is also required.

Summary

In this study, we investigated the morphology of the corpus callosum in chromosome 22q11.2 deletion syndrome. The analysis of the callosal anatomy was based on non-rigid registration, in which a template is warped to conform to the shape of each participant. Registration is a theoretically sound method that enables more detailed analysis of size and shape variation than landmark-based warping, straight- or curved-line segmentation. The method made it possible to determine an average shape of the callosum in both DS and TD groups. The results reported in the current study, when combined with the related findings in the literature, suggest that: (a) the corpus callosum in children with DS22q11.2 is significantly different in shape and size from healthy controls; (b) differences are concentrated in the anterior portion of the structure, primarily in the rostrum, which is larger and longer in the DS22q11.2 group; (c) the callosum

is more arched in the DS22q11.2 group, but this bowing is prominent at the anterior callosum rather than a general vertical displacement of the callosal body; (d) ventricular enlargement is not correlated with regional size differences at the rostrum nor with the curvature of the genu; (e) regional area is significantly correlated to cognitive function in enumeration tasks for children with the deletion; adjusted response time is inversely correlated to the size of genu in counting tasks and to the size of genu and rostrum in subitizing for children with DS22q11.2. The findings in (b) through (e) are novel and comprise the main contributions of the present study. These findings deserve further investigation using a larger dataset. Ultimately, studies should be directed at understanding of causal relationships between the many variables, rather than estimating only their correlation. In this way, the mechanisms of anomalous brain development will be better understood along with their implications for the characteristic cognitive impairments and risk for psychopathology that is associated with deletions of chromosome 22q11.2.

4) EXPERIMENTAL PROCEDURES

Participants

Participants in this study were 18 children with chromosome 22q11.2 deletion syndrome, 7 males and 11 females ranging in age from 7.3 to 14.0 years (mean(S.D.)=9.9(1.4) years), mean (S.D.) full scale IQ scores=73.9(10.8), verbal IQ=78.2(13.1) and performance IQ=73.7(9.0). Each was recruited through the “22q and You” Center at The Children's Hospital of Philadelphia and had a diagnosis of chromosome 22q11.2 deletion by virtue of a positive result from the standard fluorescence in-situ hybridization (FISH) test for the deletion. Eighteen typically developing control children, 12 males and 6 females ranging in age from 7.5 to 14.2 years (age=10.4(2.0) years, full scale IQ scores=109.3(13.4), verbal IQ=111.4(15.6) and performance IQ=105.9(13.1)) were recruited through newspaper advertisements. IQ scores were different at the level of $P<0.0001$ ($F=58.0$ for full score and $F=55.3$ for performance score). For the analysis of the correlation between callosal area and cognitive tests, 2 controls were excluded because of incomplete data. Parents of all children signed a consent form in accordance with the Declaration of Helsinki and the Institutional Review Board standards of the Children's Hospital of Philadelphia, and all children signed an assent on the same form (for more details, see Simon, 2005c).

MRI Protocol

Magnetic resonance imaging was performed on a 1.5 Tesla Siemens MAGNETOM Vision scanner (Siemens Medical Solutions, Erlangen, Germany). For each participant, a high-resolution three-dimensional structural MRI was obtained using a T1-weighted magnetization prepared rapid gradient echo (MP-RAGE) sequence with the following parameters: repetition time (TR) = 9.7 ms, echo time (TE) = 4 ms, flip angle = 12°, number of excitations = 1, matrix size = 256×256, slice thickness = 1.0 mm, 160 sagittal slices, in-plane resolution = 1×1 mm. The midsagittal slice of each brain image volume was manually extracted as the best plane spanning the interhemispheric fissure, and on which the anterior and posterior commissures and the cerebral aqueduct were visible. The process, which involves rotating and translating the volume so that the centermost sagittal slice of the repositioned volume directly corresponds to the anatomical midsagittal section, was independently performed by two investigators, for 6 randomly chosen volumes, and the interrater variability measured for the 6 rigid transformation degrees of freedom used to reformat each brain image volume. All correlation coefficients were above 0.92.

Image Analysis Methods

The callosa in the midsagittal images were segmented by manual thresholding and delineation. The process was performed twice by a single rater, who was blind to demographic and clinical

information. The reliability of segmentation was measured by computing the intraclass correlation coefficient, based on the area of the segmented corpus callosum. The intraclass correlation coefficient value for the dataset was 0.96. Measurements of ventricular size were computed with a user-guided semi-automated process (Yushkevich et al., 2006) by a primary rater who was blind to the diagnostic category to which the brain belonged. Hand-traced reliability was evaluated based on the repeated segmentation of 10 subjects, yielding an alpha value of 0.98.

The boundaries of the callosa were automatically determined using the Rosenfeld algorithm for 8-connected contours (Rosenfeld, 1982). The medial axis of the callosa was also extracted. This axis is a curve that splits the corpus callosum into dorsal and ventral regions, such that, at any point along the axis, the two perpendicular line segments emanating from the point connecting the axis to dorsal and ventral points of the boundary have the same length. Medial axis extraction was performed using a variation of the thinning algorithm described in Rosenfeld (1982), with subsequent pruning of spurious branches. The curve representing the medial axis was then extended to terminate at the tips of the rostrum and splenium. The resultant curve was denoted as the *midline* of the callosum. The linear length of the midline was computed as follows: consecutive curve pixels connected by a face were considered 1 mm apart and points connected by a vertex had distance equal to $\sqrt{2}$ mm. The coordinates of the pixels along the midline were interpolated so as to yield an isotropic rotation-invariant representation, in which any two consecutive sampled points were 1 mm apart.

The pointwise curvature of the callosum midline was computed for each participant, using the k -curvature algorithm (Rosenfeld, 1982), where k was empirically chosen to be 10% of the length of the participant curve, so as to provide enough smoothness. Each participant curve was extrapolated at the extremities, based on autoregression, so that the curvature could be computed over the entire extent of the curve.

In the experiments, three different partitioning schemes were implemented: (a) The scheme proposed by Witelson (1989), in which the callosum is divided into 7 sub-regions, allowed for the comparison of our results with the ones reported by Shashi (2004). In this approach, the callosum is partitioned based on segments that are perpendicular to the callosal principal axis of orientation. The *genu* and *rostrum* are defined based on a line, perpendicular to the axis of orientation, which is tangent to the anterior-most point of the ventral boundary. The *rostral body* extends from this line to anterior-most third of the callosum. The *anterior midbody* extends from this point to the half of the callosum. The *posterior midbody* extends from the half to the posterior-most third. The *isthmus* follows the midbody up to the posterior-most fifth from which the *splenium* starts; (b) The partitioning into 5 segments was used to allow for the comparison of our results with the ones reported by Antshel. In this scheme, the midline is divided into 5 equidistant parts. The segments that are perpendicular to the midline at these points are used to partition the callosum; (c) The main partitioning scheme used in the study follows the same approach used by Antshel, but the midline is split into 85 1mm segments, in order to provide detailed description of regional size. This partitioning scheme contributed to detect the differences in the genu and rostrum that had been hidden by the other coarser approaches. It contributes not only to reveal size variation, but also to describe the curvature of the callosum in a more detailed way than was provided by the bending angle.

Shape measurement was based on the sets of vector variables obtained from non-rigidly registering a reference image so as to align its anatomy with the participant anatomy of the sample (Gee, 1999). Registration is an appropriated and sophisticated method for shape analysis, as it enables a point-wise mapping of corresponding points in the individual anatomy of the participants, while taking into account the gross anatomy of the structure. One of the control participants was arbitrarily chosen as the reference. The discretized boundary curves

of the participant callosa were reparameterized to 100 points and registered to within sub-pixel precision to that of the reference, using the approach proposed by Dubb et al. (2003), in which the alignment optimizes correspondence of boundary curvatures under certain topological constraints. The alignment transformations of the reference for each group were averaged to determine their mean callosal shapes. The midline of the reference sampled at 85 equidistant points was registered to the participants' midlines. The result of registration was a mapping from each point in the reference to corresponding points in the participants. The consistency of the registration results was thoroughly verified for each subject.

The morphometric analysis of the corpus callosum was based on the curvature of the structure's midline, width of the callosum, and its point-wise area and midline length scaling with respect to the reference. Fig. 8 shows a schematic of the midline registration in which two neighboring points on the reference curve, R_i and R_{i-1} , are respectively mapped to points S_j and S_k in the participant (k not necessarily equal to $j-1$). Midline length scaling is defined as the ratio m/l , where $m=M_j/\Sigma M$ and $l=L_i/\Sigma L$. Length scaling shows the parts of the structure that are longer or shorter in the participant, compared to the template. In the same figure, the width of the line segment at S_j connecting the dorsal and ventral boundaries of the participant callosum and perpendicular to the midline's tangent, is defined as W_j . The area shown in gray, delimited by the line segments intercepting T_i and T_{i-1} , is denoted as A_i , and the corresponding area is B_j in the participant. Area scaling is defined as the ratio b/a , where $b=B_j/\Sigma B$ and $a=A_i/\Sigma A$, and reveals the parts of the structure that are larger or smaller in the participant, when compared to the reference.

Statistical Methods

The analysis of callosum shape and size differences between children with DS22q11.2 and controls was performed based on multiple analyses of variance (ANOVA), testing the null hypothesis of equal means. Four features were examined: (a) the curvature of the participant's midline at each of the 85 mapped points; (b) the width of the callosa at each mapped point; (c) the midline's length scaling along the midline, i.e., how much stretching or contraction was needed to align the template's midline with the participant's midline; and (d) the area scaling, i.e., the relative amount of local enlargement or reduction that resulted from template-to-participant registration. The analyses of the features were corrected for age and gender, covarying the latter using linear regression. No multiple-comparison correction was performed in this study since we aimed at exploring morphometric differences individually at each region of interest (Bender, 2001).

The analysis of correlation between curvature and ventricular volume and between regional volume and response time, in each group, was performed based on two-tailed t -tests, testing the null hypothesis that the Pearson correlation value was equal to zero (Cohen, 1988). Response time in enumeration tasks was additionally corrected for PIQ scores.

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Abbreviations

DS22q11.2, chromosome 22q11.2 deletion syndrome; DS, children with DS22q11.2; ES, effect size; MRI, magnetic resonance imaging; PIQ, performance intelligence quotient; S.D., standard deviation; TD, typically developing controls.

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Fig. 1. Partitioning schemes used in the study: curved-line-based partition into 85 segments used in registration (a), straight-line-based partition following Witelson's topology, in which the segments are named, from the anterior to posterior part of the callosum, as rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium (b), and curved-line-based partition into 5 segments (c).

Fig. 2.
Superimposed average shapes for children with DS22q11.2 (vertical lines) and controls (horizontal lines).

Fig. 3. Curvature of midline in degrees (a), midline length scaling (b), callosal area scaling (c) and callosal width in millimeters (d) as a function of midline length in millimeters. The averages

for controls and children with DS22q11.2 are shown respectively with solid and dashed curves. The reference used for registration is shown divided into seven regions, with corresponding segments indicated by dotted lines in the plots.

Fig. 4. Statistical significance of the differences between controls and children with DS22q11.2 considering curvature (a), length scaling (b), area scaling (c) and width (d). The segments for which the mean is significantly higher are shown for controls (left) and for children with DS22q11.2 (right).

Fig. 5. Statistical significance of the correlation between the volume of the ventricles and curvature for controls (left) and children with DS22q11.2 (right).

Fig. 6. Statistical significance of the correlation between regional size and adjusted response time in counting tasks: Pearson correlation coefficient (a) and significance (b) for controls; Pearson correlation coefficient (c) and significance (d) for children with DS22q11.2.

Fig. 7.

Statistical significance of the correlation between regional size and adjusted response time in subitizing tasks: Pearson correlation coefficient (a) and significance (b) for controls; Pearson correlation coefficient (c) and significance (d) for children with DS22q11.2.

Fig. 8.

Schematic of the midline registration in which two neighboring points on the reference curve, R_i and R_{i-1} , are respectively mapped to points S_j and S_k in the participant. Midline length scaling is defined as $(M_j/L_i)(\sum L/\sum M)$. The width of the line segment at S_j is defined as W_j . The area shown in gray, delimited by the line segments intercepting R_i and R_{i-1} , is denoted as A_i , and the corresponding area is B_j in the participant. Area scaling is defined as $(B_j/A_i)(\sum A/\sum B)$.

CORRELATION BETWEEN REGIONAL CALLOSAL AREA AND RESPONSE TIME IN COUNTING TASKS FOR CHILDREN WITH DS22q11.2

Table 1

Region	Segment	Values adjusted for sex and age			Values adjusted for sex, age and PIQ		
		r	f ^a	p	r	f ^a	p
GE	10						
	11						
	12						
	13	-0.54	-2.41	0.0305	-0.54	-2.31	0.0379
	14	-0.55	-2.46	0.0275	-0.51	-2.16	0.0498
	15	-0.62	-2.98	0.0098	-0.53	-2.23	0.0442
	16	-0.60	-2.77	0.0149	-0.60	-2.71	0.0180
RB	27	-0.52	-2.28	0.0388	-0.58	-2.54	0.0246
	28						
	29	0.55	2.45	0.0279	0.52	2.17	0.0494
	30	0.66	3.28	0.0054	0.61	2.79	0.0154
	31	0.70	3.67	0.0025	0.64	2.98	0.0107
	32	0.64	3.11	0.0076	0.56	2.42	0.0310
	33	0.58	2.65	0.0189			
AM	34	0.62	2.95	0.0105			
	35	0.63	3.01	0.0093			
	36						
	37	0.66	3.28	0.0055	0.53	2.25	0.0427
	38	0.67	3.32	0.0050			
	39	0.69	3.36	0.0028	0.58	2.60	0.0222
	40	0.62	2.94	0.0108	0.54	2.30	0.0383
PM	46	0.55	2.43	0.0290			
	50	0.54	2.42	0.0298			

Note: GE=genu, RB=rostral body, AM=anterior midbody, PM=posterior midbody.

^a two-tailed *t*-tests, *df*=14. Only segments with significant values are shown (*p*<0.05).

Table 2

CORRELATION BETWEEN REGIONAL CALLOSAL AREA AND RESPONSE TIME IN SUBITIZING TASKS FOR CHILDREN WITH DS22q11.2

Region	Segment	Values adjusted for sex and age			Values adjusted for sex, age and PIQ		
		r	t ^a	p	r	t ^a	p
RT	2	-0.51	-2.19	0.0460			
	3	-0.61	-2.88	0.0122	-0.59	-2.65	0.0202
	4	-0.56	-2.51	0.0249	-0.54	-2.33	0.0363
GE	12	-0.50	-2.15	0.0495	-0.53	-2.25	0.0424
	13	-0.53	-2.37	0.0349	-0.59	-2.64	0.0202
	14	-0.57	-2.62	0.0204	-0.62	-2.83	0.0143
	15	-0.66	-3.27	0.0056	-0.68	-3.33	0.0055
	16	-0.68	-3.43	0.0041	-0.70	-3.54	0.0036
	17	-0.56	-2.50	0.0255	-0.61	-2.81	0.0148
	18				-0.55	-2.39	0.0327
PM	50	0.52	2.29	0.0384			
SP	83	0.57	2.62	0.0200	0.58	2.54	0.0245

Note: RT=rostrum, GE=genu, PM=posterior midbody, SP=splenium.

^a Two-tailed t-tests, df=14. Only segments with significant values are shown ($p<0.05$).

Table 3
COMPARATIVE ANALYSIS OF MEAN AND S.D.s FOR REGIONAL CALLOSAL AREA AND UNIVARIATE ANALYSIS OF VARIANCE

Variable	Current Study			Shashi et al. (2004)		
	DS	TD	$F(1,32)^d$	P	DS	TD
Rostrum	0.42(0.18) ^b 5.9% ^c	0.22 (0.09) 3.3%	17.06	0.0002	N.A. ^d	N.A.
Genu	1.42 (0.47) 20.0%	1.45 (0.24) 22.0%	0.06	0.811	N.A.	N.A.
Rostral body	1.11 (0.31) 15.7%	0.97 (0.19) 14.7%	2.61	0.115	N.A.	N.A.
Rostrum	2.94 (0.81)	2.63 (0.25)	2.38	0.133	2.49 (1.03)	1.99 (0.53)
+rostral body+genu	41.6%	40.0%			37.4%	36.6%
Anterior mid-body	0.78 (0.18)	0.78 (0.10)	0.001	0.970	0.86 (0.81)	0.55 (0.14)
Posterior mid-body	11.0%	11.9%			12.9%	10.1%
	0.74 (0.20)	0.71 (0.07)	0.49	0.490	0.60 (0.16)	0.53 (0.13)
	10.5%	10.8%			9.0%	9.7%
Isthmus	0.66 (0.23)	0.62 (0.09)	0.63	0.432	0.62 (0.20)	0.49 (0.12)
	9.4%	9.4%			9.3%	9.0%
Splenium	1.95 (0.45)	1.82 (0.20)	1.13	0.297	1.78 (0.63)	1.68 (0.41)
	27.5%	27.8%			26.8%	30.9%
Total area	7.07 (1.76)	6.56 (0.53)	1.40	0.246	6.65 (1.69)	5.44 (1.22)

Note: DS=deleted group, TD=typically developing controls.

^a ANOVA, *F*-test scores.

^b Mean (S.D.) area in cm².

^c Percentage of total area.

^d Separate results for the rostrum, genu and rostral body are not available (N.A.) in Shashi's study.

Table 4
COMPARATIVE ANALYSIS OF MEAN AND S.D.s FOR REGIONAL CALLOSAL AREA AND BENDING ANGLE AND UNIVARIATE ANALYSIS OF VARIANCE

Variable	Current Study		Antshel et al. (2005)			
	DS	TD	F(1,32) ^d	P	DS	TD
Genu	2.25 (0.6) ^b	1.93 (0.2)	4.07	0.052	1.7 (0.3)	1.7 (0.2)
	31.9% ^c	29.5%			21.8%	25.0%
Anterior body	0.98 (0.3)	0.99 (0.1)	0.02	0.882	1.5 (0.2)	1.3 (0.2)
	13.9%	15.2%			19.2%	19.1%
Mid-body	0.90 (0.2)	0.88 (0.1)	0.06	0.805	1.4 (0.2)	1.2 (0.2)
	12.7%	13.5%			17.9%	17.6%
Isthmus	0.95 (0.3)	0.88 (0.1)	0.68	0.416	1.3 (0.2)	1.0 (0.2)
	13.5%	13.5%			16.7%	14.7%
Splenium	1.94 (0.5)	1.82 (0.2)	1.09	0.305	2.0 (0.4)	1.7 (0.3)
	27.5%	27.8%			25.6%	25.0%
Total area	7.1 (1.8)	6.5 (0.5)	1.40	0.246	7.8 (1.2)	6.8 (0.9)
Bending angle	92.5 (8.8) ^d	103.4(8.7)	23.13	0.001	94.3 (10.6)	106.3 (10.1)

Note: DS=deleted group, TD=typically developing controls.

^a ANOVA, *F*-test scores.

^b Mean (S.D.) area in cm².

^c Percentage of total area.

^d Mean (S.D.) bending angle in degrees.