Cortical Overgrowth in Fetuses With Isolated Ventriculomegaly

Vanessa Kyriakopoulou1,2, Deniz Vatansever2, Samia Elkommos2, Sarah Dawson2, Amy Mcguinness2, Joanna Allsop1,2, Zoltán Molnár3, Joseph Hajnal1,2 and Mary Rutherford1,2

1Centre for the Developing Brain, Perinatal Imaging & Health, St Thomas’ Hospital, King’s College London, London, UK, 2Centre for the Developing Brain, Hammersmith Hospital, Imperial College London, London, UK and 3Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK

Address correspondence to Mary Rutherford, Centre for the Developing Brain, Perinatal Imaging & Health, King’s College London, 1st Floor South Wing, St Thomas’ Hospital, London SE1 7EH, UK. Email: mary.rutherford@kcl.ac.uk

Mild cerebral ventricular enlargement is associated with schizophrenia, autism, epilepsy, and attention-deficit/hyperactivity disorder. Fetal ventriculomegaly is the most common central nervous system (CNS) abnormality affecting 1% of fetuses and is associated with cognitive, language, and behavioral impairments in childhood. Neurodevelopmental outcome is partially predictable by the 2-dimensional size of the ventricles in the absence of other abnormalities. We hypothesized that isolated fetal ventriculomegaly is a marker of altered brain development characterized by relative overgrowth and aimed to quantify brain growth using volumetric magnetic resonance imaging (MRI) in fetuses with isolated ventriculomegaly. Fetal brain MRI (1.5 T) was performed in 60 normal fetuses and 65 with isolated ventriculomegaly, across a gestational age range of 22–38 weeks. Volumetric analysis of the ventricles and supratentorial brain structures was performed on 3-dimensional reconstructed datasets. Fetuses with isolated ventriculomegaly had increased brain parenchyma volumes when compared with the control cohort (9.6%, P < 0.0001) with enlargement restricted to the cortical gray matter (17.2%, P = 0.002). The extra-cerebral cerebrospinal fluid and third and fourth ventricles were also enlarged. White matter, basal ganglia, and thalamic volumes were not significantly different between cohorts. The presence of relative cortical overgrowth in fetuses with ventriculomegaly may represent the neurobiological substrate for cognitive, language, and behavioral deficits in these children.

Keywords: brain, development, magnetic resonance imaging

Introduction

Mild enlargement of the cerebral ventricles has been associated with schizophrenia (Wright et al. 2000) autism (Palmen et al. 2005), epilepsy (Jackson et al. 2011), and attention-deficit/hyperactivity disorder (Lyoo et al. 1996). In the fetus, ventriculomegaly (Fig. 1) is the most common central nervous system (CNS) abnormality occurring in 1% of the fetal population. It is diagnosed on antenatal ultrasound when the ventricular atrial diameter >10 mm at any gestation (Cardoza et al. 1988; Melchiorre et al. 2009). In normal development, the size of the lateral ventricles remains relatively stable from 14 gestational weeks until birth, with a mean atrial diameter of 6.4 mm (±1.2 mm; Almog et al. 2003). Fifty percent of ventriculomegaly cases have no additional CNS abnormalities, and the etiology of the dilatation remains unknown (isolated ventriculomegaly; Kelly et al. 2001). The neurodevelopmental outcome in children with fetal ventriculomegaly is variable and includes predominantly cognitive, language, and behavior deficits (Sadan et al. 2007; Leitner et al. 2009; Lyall et al. 2012). Classification into mild (10–12 mm), moderate (12.1–15 mm), and severe (>15 mm) is at present the best predictor of outcome (Garel et al. 2003; Wyldes and Watkinson 2004; Mehta and Levine 2005; Gaglioti et al. 2009). The overall risk of neurological abnormality in isolated mild-to-moderate ventriculomegaly is 12% increasing to over 75% in severe cases even when excluding congenital infection and abnormal karyotype (Garel et al. 2003; Mehta and Levine 2005; Gaglioti et al. 2009). Understanding of the neurobiological substrate and the variability of these abnormal outcomes would inform current clinical management and parental counselling.

Neonatal ventriculomegaly is often associated with post hemorrhagic or anatomical obstruction to cerebrospinal fluid (CSF) flow or secondary to white matter atrophy; however, these are uncommon causes in the fetus where the cause for dilation of the lateral ventricles is unknown in the majority (Kelly et al. 2001). Venticulomegaly is associated with variable cerebral overgrowth in a fetal rat model (Eyles et al. 2003), in children with Soto’s syndrome (Palmen et al. 2005; Leventopoulos et al. 2009), hemimegalencephaly (Kalifa et al. 1987), and high-functioning children with autism (Palmen et al. 2005). We hypothesize that fetal ventriculomegaly is a marker of altered brain development characterized by relative brain overgrowth. The aim of this study was therefore to quantify brain tissue volumes in fetuses with isolated ventriculomegaly using antenatal magnetic resonance imaging (MRI).

Materials and Methods

Subjects

Ethical approval was granted by the Hammersmith Hospital Ethics Committee (ethics no. 07/H0707/105) and informed consent obtained from all participants. The study included fetuses undergoing brain MRI at the Robert Steiner MRI Unit in Hammersmith Hospital between November 2007 and August 2011. Normal fetal controls were recruited from women who were either healthy pregnant volunteers, who had had a previous child with a confirmed abnormality or who had had a suspected fetal abnormality on ultrasound excluded on MRI. Subjects were excluded from the normal cohort if there were abnormal findings on fetal MRI, inadequate MR image quality, delivery complications, congenital malformations or infection, chromosomal abnormality, twin pregnancy, premature delivery (<36 weeks gestational age (GA)), abnormal clinical neonatal examination, abnormal findings on neonatal MR examination, and abnormal neurodevelopmental examination at either 1 or 2 years of age. Fetal GA was estimated from a first trimester dating ultrasound scan.

Fetuses presenting with unilateral or bilateral ventricular dilatation (2-dimensional, 2D atrial diameter >10 mm) on the routine clinical anomaly ultrasound performed at around 20 weeks were referred to our department for further assessment of brain development. Three fetuses had a normal routine anomaly ultrasound scan and were diagnosed with ventriculomegaly on later ultrasound scans. Those with additional brain abnormalities, positive infection screening and
normal control fetus aged 31+5 weeks and (Fig. 2) a fetus aged 30 weeks with isolated ventriculomegaly. Fetal ventriculomegaly is diagnosed when the atrial diameter is equal or exceeds 10 mm. The calipers are placed inside the low signal intensity of the inner edge of the ventricular wall and perpendicular to the long axis of the ventricle.

chromosomal abnormalities, intrauterine growth restriction (IUGR), twin pregnancies, maternal drug use, or poor image quality were excluded from this study. Clinical features of atrophy of the surrounding tissue and increased intraventricular pressure were assessed on MR images during the clinical examination. Features of brain tissue atrophy on fetal MRI may include a normal or small occipito-frontal centile, decreased white matter volume, and enlargement of the extracerebral space. Overt lesions or evidence of injury and distortion of the tissue may also be present. Although it is not possible to measure intraventricular pressure in utero, there are specific brain features visible on MR images that would be indicative of increased pressure. Obstruction may be associated with a large or increasing occipito-frontal circumference and brain imaging appearance of ballooned ventricles with a decreased extracerebral space, suggestive of raised pressure within the ventricles and intracranial cavity. Structural obstructions may occasionally be directly visible, such as a large cyst, hemorrhage, or indirectly such as secondary enlargement of the third ventricle due to aqueduct stenosis. In addition, alteration in the signal intensity of the adjacent white matter may be detected resulting from edema due to the increased pressure, presumed secondary to impaired venous drainage.

**Fetal Brain MRI**

Fetal MRI was performed using a 1.5-T MRI System (Philips Achieva; Philips Medical Systems, Best, the Netherlands) with a 32-channel cardiac coil placed around the mother’s abdomen for signal reception. No sedation was used, and the total duration of the MR examination did not exceed 60 min. Clinical imaging was performed using the following sequences: $T_2$-weighted Single-Shot Turbo-Spin Echo (sTSE) was acquired as part of a comprehensive MR examination using the following scanning parameters: time repetition = 15 000 ms, time echo = 160 ms, slice thickness of 2.5 mm, slice overlap of 1.5 mm, flip angle = 90°. Three-dimensional (3D) reconstructed images were constructed using Snapshot MRI with volume reconstruction as previously described (Jiang et al. 2007). In summary, multiple sets of images were acquired in 3 orthogonal planes (4 transverse, 2 coronal, and 2 sagittal acquisitions) with overlapping slices in order to oversample the fetal brain and to ensure that each part of the brain has been sampled even with significant motion. All scans were then reviewed for image quality and the slices corrupted by motion artifacts where the anatomical detail had been lost were removed from the proceeding analysis. Images were registered into a common coordinate space in order to align the acquired slices. The reconstructed 3D volumetric datasets have high resolution, high signal-to-noise ratio, and full brain coverage essential for reliable volumetric analysis. Visual analysis of all acquired images was performed to exclude additional anomalies and to confirm appropriate appearance for gestation.

**Statistical Analysis**

Statistical analysis was performed using SPSS software package version 17 (SPSS, Chicago, IL, USA). Data were log-transformed to achieve normal distribution. Analysis of covariance on log-transformed data was used to compare volumetric results between cohorts when corrected for GA. Correlation between variables was assessed with Spearman’s rank correlation coefficient (r), correlations were not corrected for GA. A confidence level of 0.05 was considered significant. Intra- and inter-rater reliability for the left and right lateral ventricles and left and right hemisphere segmentations were performed between 2 raters (V.K. and D.V.) in 5 fetal datasets (at GA 23.14, 23.57, 24.57, 25.57, and 36.29 weeks). The intra- and interclass correlation coefficients were for both 0.99 ($P<0.0001$). To evaluate the spatial overlap accuracy of the segmentations between raters, the Dice similarity coefficient was measured for the segmentation of the lateral ventricles in 10 cases. The Dice similarity coefficient was calculated at 0.9. For cortical segmentations, both intra- and inter-rater reliability were performed between 2 raters (S.E. and N.M.) by calculating the cortical volume on 5 consecutive transverse slices. This was performed for 5 different fetal datasets (at GA 24.71, 26.29, 28.57, 30.43, and 32.00 weeks). The mean intrarater difference for the cortical volume was 1.2% and mean inter-rater difference was 2.7%.

**Quantitative Volumetric MRI Analysis**

The 3D fetal volumetric brain data were oriented into standard axial, coronal, and sagittal projections, and the voxel size was interpolated (0.2 × 0.2 × 1.0 mm; Fig. 2A) to aid visual display and assist the placement of anatomical bounding surfaces. Volumetric measurements were achieved from semiautomated segmentations performed in a transverse plane using ITK-SNAP (Yushkevich et al. 2006). Total volume of the lateral ventricles was defined as the volume of both left and right lateral ventricles, including the choroid plexus but excluding the third and fourth ventricles, the cavum septum pellucidum (CSP), and vermis (Fig. 2B). Lateral ventricle volume refers to the volume of each lateral ventricle. The third ventricle volume was defined longitudinally by the anterior and posterior commissures, laterally by the thalami, and superiorly by the CSP (Fig. 2C). The fourth ventricle volume was bounded by the superior medullary velum superiorly, the obex inferiorly, the pons anteriorly, the middle cerebellar peduncles laterally, and the vermis posteriorly (Fig. 2D). Extracerebral CSF included all intracranial CSF space surrounding the supratentorial brain tissue and cerebellum (Fig. 2E). The CSP was delimited cranially by the body of the corpus callosum, anteriorly by the genu of the corpus callosum, and caudally by the appearance of the internal cerebral veins, while laterally was delimited by the septal leaves (Fig. 2F). Brain tissue volume was defined as the supratentorial brain tissue, excluding the brainstem, pons, cerebellum, and intracerebral CSF spaces (Fig. 2G). Left and right brain hemispheres (Fig. 2H), lateral, third, and fourth ventricles and CSP were manually segmented. Laterality was established by the position of the fetal heart, stomach, and liver on MR images in a coronal plane (no fetuses had situs inversus or dextrocardia as assessed on antenatal ultrasound). Cortical gray matter, basal ganglia, and thalami were manually segmented in a subgroup of cases (n = 19) due to the extensive work required for manual segmentation (Fig. 2I). The duration of manual segmentation of cortical gray matter in a 28-week fetus was 8 h. The 19 cases were selected to span gestation from 20 to 37 gestational weeks. The substructures of the basal ganglia included the caudate, putamen, globus pallidus, and nucleus accumbens and excluded the anterior and posterior limits of the internal capsule (Fig. 2J). Thalamic segmentation included all the nuclei defined by the tissue contrast of the internal capsule laterally, the atrial region of the lateral ventricles posteriorly, the third ventricle medially, and the hypothalamus anteriorly (Fig. 2J). White matter volumes were calculated by subtracting the cortex, basal ganglia, and thalamic volumes from the total brain tissue volume. Linear measurements were performed using ImageJ. The atrial diameter was measured according to the International Society of Ultrasound in Obstetrics and Gynecology guidelines (ISUOG 2007) at the level of the atrium (Fig. 1).
Results

Subjects

Normal Controls
A total of 89 fetuses were enrolled as normal controls: 31 were excluded in accordance with our exclusion criteria. In the remaining 58 fetuses, MRI was performed at a median age of 28.8 weeks GA (range 22.4–38.9 weeks). Two fetuses were scanned twice during gestation. Fifty-eight mothers of included control fetuses consisted of 41 healthy volunteers and 17 with a previous fetus or infant diagnosed with a CNS or non-CNS abnormality. In summary, 60 MR scans of 58 fetuses were included in the control cohort (30 males/30 females; Fig. 3).

Isolated Ventriculomegaly
A total of 114 fetuses presented with an ultrasound diagnosis of dilatation of the lateral ventricles during the study period and the following were excluded: Additional brain abnormalities (56), IUGR (1), positive infection/chromosomal abnormality (8), and maternal drug abuse (1). None of the included cases had signs consistent with obstruction to CSF flow or atrophy of the surrounding tissue on fetal MRI (as described in Materials and Methods). Forty-eight fetuses (34 males/14 females) were diagnosed with isolated ventriculomegaly with a 2D atrial diameter in the range of 10–17 mm. In 33 of the 48 fetuses, the ventriculomegaly was unilateral. Fifteen fetuses
were scanned twice and one was scanned 3 times during gestation. In summary, 65 MR scans of 48 cases were included in the isolated ventriculomegaly cohort (median GA 30 weeks, range 22–37.3 weeks, 48 males/17 females; Fig. 3). There was no significant difference in GA between cohorts (P = 0.685).

Quantitative Volumetric MRI Analysis

Normal Controls

The lateral ventricles were assessed using the atrial diameter, the volume of each lateral ventricle, and the total lateral ventricular volume. There was a small increase in the above measurements with increasing GA as indicated by a moderate correlation value in the atrial diameter (Spearman’s r = 0.457, P < 0.0001), volume of each lateral ventricle (Spearman’s r = 0.472, P < 0.0001), and total lateral ventricular volume (Spearman’s r = 0.511, P < 0.0001) with GA. The atrial diameter, ventricle volume, and total ventricular volume ranges were 3.5–8.9 mm, 0.56–3.83 cm³, and 1.43–6.7 cm³, respectively (Table 1). Atrial diameter and lateral ventricle volume correlated well (Spearman’s r = 0.754, P < 0.0001). There was no significant difference in atrial diameter (P = 0.519), left (P = 0.317) or right (P = 0.669) lateral ventricle volumes, or total lateral ventricular volume (P = 0.304) between sexes in the control cohort; however, males had a trend to larger ventricular measurements. In males, the left lateral ventricle was significantly larger than the right (P = 0.008), whereas in females this difference did not reach significance (P = 0.08).

The third and fourth ventricles increased in volume with increasing GA (Spearman’s r = 0.927, P < 0.0001; Spearman’s r = 0.733, P < 0.0001, respectively; Table 2). There was a small increase in the volume of the third ventricle at a relative growth rate of 0.098% per week, increasing from 0.074 cm³ at week 22 to 0.359 cm³ at week 38. Similarly, there was a small increase in the volume of the fourth ventricle during GA at a relative growth rate of 0.129% per week, increasing from 0.013 cm³ at week 22 to 0.093 cm³ at week 38. Exracerebral CSF increased with GA at a relative growth rate of 0.06% per week, from 22.68 cm³ at week 22 to 98.52 cm³ at week 38 (Table 2). The volume of the CSP increased between 22 and 30 weeks when it reached a peak and decreased from 30 to 38 weeks. Supratentorial brain tissue increased in volume with increasing GA at a relative growth rate of 11.65% per week from 53.67 cm³ at week 22 to 346.7 cm³ at week 38 (Spearman’s r = 0.976, P < 0.0001; Table 2).

Total cortical gray matter, basal ganglia, thalamic, and white matter volumes were measured in 19 control cases (GA range 22.42–37.17 weeks, mean 29.29 weeks), and results are presented in Table 2. There was no significant difference between the complete cohort (n = 60) and this subgroup in GA, brain volume, and ventricular volume. Between 22 and 37 weeks GA, there was an exponential increase in cortical gray matter volume at a relative growth rate of 13.15% per week. Cortical volume increased from 11.01 cm³ at week 22 to 105.58 cm³ at week 38 (Spearman’s r = 0.873, P < 0.0001). Thalamic volume increased exponentially (Spearman’s r = 0.993, P < 0.0001) at a relative growth rate of 13.13% per week from 0.88 cm³ at week 22 to 7.52 cm³ at week 38. The volume of the basal ganglia increased with increasing GA at a relative growth rate of 11.39% per week from 1.17 cm³ at week 22 to 7.94 cm³ at week 38 (Spearman’s r = 0.975, P < 0.0001). White matter volume increased at a relative growth rate of 12.27% per week from 39.15 cm³ at week 22 to 272.25 cm³ at week 38 (Spearman’s r = 0.986, P < 0.0001).

Correlations With Ventricle Size in the Control Cohort

There was a significant correlation between total lateral ventricular volume and supratentorial brain parenchyma volume (Spearman’s r = 0.54, P < 0.0001), extracerebral CSF volume (Spearman’s r = 0.499, P < 0.0001), third ventricle (Spearman’s r = 0.511, P < 0.0001), and fourth ventricle (Spearman’s r = 0.472, P < 0.0001), respectively; Table 2).

Table 1

<table>
<thead>
<tr>
<th>Ventricular measurement</th>
<th>Range</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrial diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>3.5–8.9</td>
<td>6.58</td>
<td>1.31</td>
</tr>
<tr>
<td>Right</td>
<td>3–8.1</td>
<td>5.91</td>
<td>1.43</td>
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<tr>
<td>Lateral ventricle volume (cm³)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>0.56–3.83</td>
<td>1.81</td>
<td>0.74</td>
</tr>
<tr>
<td>Right</td>
<td>0.57–3.1</td>
<td>1.51</td>
<td>0.68</td>
</tr>
<tr>
<td>Total lateral ventricular volume (cm³)</td>
<td>1.43–6.7</td>
<td>3.32</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Volumetric results for control and ventriculomegaly cohorts</th>
<th>Total lateral ventricles</th>
<th>Total brain tissue</th>
<th>Cortex</th>
<th>Basal ganglia</th>
<th>Thalami</th>
<th>White matter</th>
<th>Third ventricle</th>
<th>Fourth ventricle</th>
<th>Exracerebral CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>Spearman’s r</td>
<td>0.511**</td>
<td>0.976**</td>
<td>0.964**</td>
<td>0.975**</td>
<td>0.993**</td>
<td>0.986**</td>
<td>0.927**</td>
<td>0.733**</td>
</tr>
<tr>
<td>Relative growth rate (%/week)</td>
<td>3.43</td>
<td>11.65</td>
<td>13.15</td>
<td>11.39</td>
<td>12.13</td>
<td>12.27</td>
<td>0.098</td>
<td>0.129</td>
<td>0.06</td>
</tr>
<tr>
<td>Average volume (cm³)</td>
<td>At 22 GW</td>
<td>2.27</td>
<td>53.67</td>
<td>11.01</td>
<td>1.17</td>
<td>0.88</td>
<td>39.15</td>
<td>0.074</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>At 26 GW</td>
<td>2.68</td>
<td>85.56</td>
<td>19.37</td>
<td>1.89</td>
<td>1.51</td>
<td>63.58</td>
<td>0.110</td>
<td>0.022</td>
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<tr>
<td></td>
<td>At 30 GW</td>
<td>3.17</td>
<td>136.41</td>
<td>34.09</td>
<td>3.65</td>
<td>2.97</td>
<td>103.25</td>
<td>0.163</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>At 34 GW</td>
<td>3.75</td>
<td>217.47</td>
<td>60.00</td>
<td>4.93</td>
<td>4.40</td>
<td>167.66</td>
<td>0.242</td>
<td>0.057</td>
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<tr>
<td></td>
<td>At 38 GW</td>
<td>4.43</td>
<td>346.70</td>
<td>105.58</td>
<td>7.94</td>
<td>7.52</td>
<td>272.25</td>
<td>0.359</td>
<td>0.093</td>
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<tr>
<td>Ventriculomegaly</td>
<td>Spearman’s r</td>
<td>0.481**</td>
<td>0.969**</td>
<td>0.971**</td>
<td>0.946**</td>
<td>0.98**</td>
<td>0.963**</td>
<td>0.803**</td>
<td>0.849**</td>
</tr>
<tr>
<td>Relative growth rate (%/week)</td>
<td>5.58</td>
<td>12.35</td>
<td>12.50</td>
<td>9.29</td>
<td>10.61</td>
<td>9.27</td>
<td>0.088</td>
<td>0.119</td>
<td>0.10</td>
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<tr>
<td>Average volume (cm³)</td>
<td>At 22 GW</td>
<td>7.31</td>
<td>61.10</td>
<td>13.84</td>
<td>1.46</td>
<td>0.99</td>
<td>49.41</td>
<td>0.092</td>
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<tr>
<td></td>
<td>At 26 GW</td>
<td>8.44</td>
<td>96.56</td>
<td>23.48</td>
<td>2.16</td>
<td>1.80</td>
<td>73.01</td>
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<tr>
<td></td>
<td>At 30 GW</td>
<td>9.74</td>
<td>152.59</td>
<td>39.85</td>
<td>3.18</td>
<td>2.60</td>
<td>107.88</td>
<td>0.203</td>
<td>0.074</td>
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<tr>
<td></td>
<td>At 34 GW</td>
<td>11.24</td>
<td>241.14</td>
<td>67.62</td>
<td>4.69</td>
<td>4.20</td>
<td>159.40</td>
<td>0.301</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>At 38 GW</td>
<td>12.97</td>
<td>381.07</td>
<td>114.74</td>
<td>6.92</td>
<td>6.79</td>
<td>235.53</td>
<td>0.446</td>
<td>0.192</td>
</tr>
</tbody>
</table>

Note: Volumetric results for control and ventriculomegaly cohorts. The correlation of each intracranial structure and GA is indicated by the Spearman’s r value. Relative growth rate represents the percent volume increase relative to the average volume of the structure.

**P < 0.0001.
Isolated Ventriculomegaly

All fetuses with an ultrasound diagnosis of ventriculomegaly had significantly larger lateral ventricle volumes than controls (295%, $P<0.0001$; Fig. 4A). The ranges of ventricular measurements were 10–17 mm (atrial diameter), 3.6–12.2 cm$^3$ (lateral ventricle volume), and 5.06–21.39 cm$^3$ (total lateral ventricular volume). One control case of a 37$^{+5}$-week-old...
fetus had a normal atrial diameter of 8.3 mm, but a lateral ventricle volume of 3.8 cm³, within the lower ventriculomegaly range as defined in this study. With the exception of this control case, there was no overlap in any lateral ventricle volume. Atrial diameter and lateral ventricle volume correlated well in the ventriculomegaly cohort (Spearman’s r = 0.56, P < 0.0001). Thirty-three of the 48 cases had unilateral ventriculomegaly as classified by an atrial diameter >10 mm in only one ventricle. In 17 of 33 unilateral cases, the volume of the normal sized lateral ventricle (atrial diameter <10 mm) was in the ventriculomegaly range (atrial diameter mean 9.2 mm, range 7.4–9.9 mm; lateral ventricle volume mean 4.4 cm³, range 3.6–5.3 cm³; Fig. 5).

In the ventriculomegaly cohort, the third and fourth ventricles increased in volume with increasing GA (Spearman’s rs = 0.51, P < 0.0001; Spearman’s rs = 0.849, P < 0.0001, respectively) (Table 2). There was a small increase in the volume of the third ventricle at a relative growth rate of 0.088% per week, increasing from 0.092 cm³ at week 22 to 0.446 cm³ at week 38. Similarly, there was a small increase in the volume of the fourth ventricle during GA at a relative growth rate of 0.119% per week, increasing from 0.029 cm³ at week 22 to 0.192 cm³ at week 38. The volume of the third and fourth ventricles was larger in the ventriculomegaly cohort compared with controls by 23% (P < 0.0001) and 75% (P < 0.0001), respectively, at a GA of 29.6 weeks (Fig. 4B,C). Extracerebral CSF volume increased with GA in the ventriculomegaly cohort at a relative growth rate of 0.1% per week from 23.66 cm³ at week 22 to 104.96 cm³ at week 38 (Table 2). The ventriculomegaly cohort had significantly larger extracerebral CSF volumes compared with the control cohort by 14% (P = 0.002) at week 29.6. The volume of the CSF increased between 22 and 30 weeks when it reached a peak and then decreased from 30 to 38 weeks, showing a similar evolution pattern to the control cohort. CSF volume was significantly larger in the ventriculomegaly cohort (P = 0.014; Fig. 4D). Due to the large scatter of the measurements, the data are presented as the mean measurement for each gestational week against gestation age. There was large variation between 34 and 36 weeks due to the limited number of cases.

In the ventriculomegaly cohort, total supratentorial brain parenchyma volume increased exponentially with a relative growth rate of 12.35% per week with increasing GA from 61.1 cm³ at week 22 to 381.07 cm³ at week 38 (Table 2). When comparing between cohorts and correcting for GA, fetuses with isolated ventriculomegaly had significantly larger total supratentorial brain tissue volumes than controls (9.6%, P < 0.0001; Fig. 4E). In unilateral cases, the hemisphere containing the enlarged ventricle was 12.1% larger (P < 0.0001) compared with both the right and left hemispheres in the control cohort. The hemisphere containing the normal sized ventricle was also significantly larger than both right and left hemispheres in the normal control cohort (11.7%, P < 0.0001). There was no significant difference between the 2 hemispheres in fetuses with unilateral ventriculomegaly (P = 0.667). Significance was unchanged when data from repeat scans were excluded and when controlling for sex.

Total cortical gray matter, basal ganglia, and thalamic and white matter volumes were measured in 19 ventriculomegaly cases (GA range 22.14–37.29 weeks, mean 30.16 weeks). There was no significant difference between the complete cohort and this subgroup in GA, brain volume, and ventricular volume in either controls or those with ventriculomegaly. As expected, when comparing between subgroups, brain volumes were significantly larger in the fetuses with ventriculomegaly compared with controls (P = 0.027). Between 22 and 37 weeks GA, there was an exponential increase in cortical gray matter volume at a relative growth rate of 12.5% (not statistically different to controls) from 13.84 cm³ at week 22 to 235.53 cm³ at week 38 (Table 2). The ventriculomegaly cohort had significantly larger cortices than the controls (17.2%, P = 0.002), even when corrected for brain volume (14.6%, P = 0.008; Fig. 4F). There was no significant difference in cortical gray matter volume between the 2 hemispheres in fetuses with unilateral ventriculomegaly (P = 0.998). Basal ganglia and thalamic and white matter volumes increased at a relative growth rate of 9.29%, 10.61% and 9.27% per week, respectively (Table 2). The volume of the basal ganglia increased from 1.46 cm³ at week 22 to 6.92 cm³ at week 38. Thalamic volume increased from 0.99 cm³ at week 22 to 6.79 cm³ at week 38 and white matter volume increased from 49.41 cm³ at week 22 to 235.53 cm³ at week 38. There was no significant difference in the volume of basal ganglia (P = 0.29), thalami (P = 0.656), and white matter (P = 0.228), between cohorts (Fig. 4G–I).

Correlations With Ventricular Size
As mentioned previously, in the normal control cohort, the total ventricular volume correlated with total brain tissue, thalamic, third ventricle, fourth ventricle, and extracerebral CSF volumes, while there was no correlation with cortical, basal ganglia, and white matter volumes. On the contrary, in the ventriculomegaly cohort, the total ventricular volume correlated significantly and positively with all measurements. Results are summarized in Table 3.

Discussion
In this study, using volumetric MRI of the fetal brain, we present the first in vivo MRI evidence, indicating brain overgrowth in fetuses with isolated ventriculomegaly. Fetuses with ventriculomegaly had significantly increased brain tissue volumes when compared with controls with enlargement restricted to the cortical gray matter. The third and fourth
ventricles and extracerebral CSF were also enlarged. White matter, thalamic, and basal ganglia volumes were not significantly different between cohorts.

In normal fetuses, supratentorial brain tissue increased between 22 and 38 weeks GA at a relative growth rate of 11.65% per week. This is comparable with previous fetal MR volumetric studies performed on reconstructed datasets with growth rates ranging from 10.22% to 17% (Clouchoux et al. 2011; Rajagopalan et al. 2011; Scott et al. 2011). Cortical volume in our normal fetuses increased at a relative growth rate of 13.15%, in agreement with the study of Scott et al. (2011) in 34 normal fetuses between 20 and 31 weeks GA. In addition, cortical volume increased at a higher rate than supratentorial brain volume in our control cohort, as previously shown in the fetal (Scott et al. 2011) and in the preterm brain (Kapellou et al. 2006).

Ultrasound studies have shown that the atrial diameter remains relatively stable in the second and third trimesters, although an increase in absolute values is observed (Snijders and Nicolaides 1994; Almog et al. 2005). Our results showed a small but significant increase in absolute ventricle volumetric values from 22 to 38 weeks of gestation. Previous fetal MRI volumetric studies have been inconclusive as to whether the volume of the lateral ventricles increases (Scott et al. 2011), decreases (Clouchoux et al. 2011), or remains stable (Kazan-Tannus et al. 2007) during gestation. This variability may be due to heterogeneous cohorts and inclusion of the third ventricle or the CSP in the measurements. As shown in our study, enlargement of the lateral ventricles was associated with enlargement of the third and fourth ventricles, CSP, and extracerebral CSF.

As expected, fetuses with ventriculomegaly diagnosed on 2D measurement of the atrial diameter (on ultrasound and MRI) had significantly larger lateral ventricle volumes compared with controls. In our cohort, boys were more likely to have ventriculomegaly than girls with a female/male ratio of 1:2.4; consistent with previous studies (Gillmore et al. 2008; Melchiorre et al. 2009). Interestingly, in 1 of 3 of the unilateral ventriculomegaly cases, the normal measuring ventricle (atrium <10 mm) demonstrated a ventricular volume outside the normal range. In addition, while atrial diameter and volume of the lateral ventricles correlated well in both cohorts, data were scattered in the ventriculomegaly cohort. For example, an atrial diameter of 12.3 mm corresponded to volumes ranging from 4.4 to 9.2 cm$^3$. This may explain why bilateral overgrowth was present in unilateral cases. While atrial diameter may be an accurate tool for the diagnosis of ventriculomegaly, it does not appear to be sensitive to intragroup volumetric differences potentially impairing its ability to predict the subsequent clinical neurodevelopmental outcome. Enlargement of the lateral ventricles was associated with enlargement of the interconnected CSF spaces, including the third and fourth ventricles and extracerebral CSF, and is consistent with the absence of intraventricular obstruction.

Fetuses with isolated ventriculomegaly had significantly larger total brain tissue volumes than controls, supporting the hypothesis that ventriculomegaly may be associated with brain overgrowth that is apparent from mid-gestation. Interestingly, in unilateral cases, both hemispheres were enlarged and brain overgrowth was not localized to the hemisphere containing the larger ventricle.

In the control cohort, while total lateral ventricular volume correlated with other ventricles, extracerebral CSF, total brain, and thalamic volume, there was no correlation with the other brain structures. On the contrary, in the ventriculomegaly cohort, total ventricular volume correlated well with all measured intracranial structures, suggesting an altered relationship between the ventricles and developing structures in this cohort.

There have been 4 published studies on MR brain volumetry comparing fetuses with ventriculomegaly to controls (Grossman et al. 2006; Kazan-Tannus et al. 2007; Pier et al. 2011; Scott et al. 2012). Three studies were performed on nonreconstructed $T_2$ MR datasets, not corrected for fetal motion, and these did not find any significant differences in brain parenchyma volume between cohorts (Grossman et al. 2006; Kazan-Tannus et al. 2007; Pier et al. 2011; Scott et al. 2012). Results from these studies are difficult to interpret due to the selection criteria of normal control fetuses, nonhomogeneous ventriculomegaly population, and volumetric analysis on nonreconstructed MR datasets known to be sensitive to fetal motion. These issues have been addressed in our study by the use of registered volumetric datasets, the rigid entry criteria of normal controls, and the follow up of all participants to ensure normal development. In addition, a homogeneous “developmental” ventriculomegaly cohort was selected for analysis by excluding cases with chromosomal abnormalities, infection, brain parenchyma damage, or additional abnormalities. Scott et al. (2012) performed a volumetric comparison of intracranial structures between fetuses with isolated ventriculomegaly ($n=16$) and control subjects ($n=16$) at an age range from 22 to 25.5 weeks. Structures were automatically segmented. They found no significant difference in brain tissue or cortical volume between groups. We repeated our analysis in a subgroup of our cohorts using the same age range (22–25.5 weeks) and found that fetuses with isolated ventriculomegaly had significantly enlarged brain tissue volumes over a similar age range ($P=0.038$; ventriculomegaly $n=14$ and controls $n=18$). Increased accuracy of the manual segmentation method may account for the detection of a volumetric difference.

Our volumetric results including the absolute volumetric ranges and relative growth rates of the white matter and deep gray matter (basal ganglia and thalami) in the control cohort are comparable with the study of Scott et al. (2011) in normal fetuses from 20 to 31 gestational weeks. Interestingly, when comparing between our 2 cohorts, there were no significant differences in the basal ganglia, thalamic, and white matter volumes between control fetuses and those with ventriculomegaly. The brain overgrowth documented in our study was attributed to an increase in cortical volume. Our results are consistent with the study of Gilmore et al. (2008), which used volumetric $T_1$ datasets acquired postnatally and showed that neonates with isolated ventriculomegaly ($n=34$; diagnosed on antenatal ultrasound) had significantly larger cortical gray matter volumes than control subjects ($n=34$) by 10.9%, while there was no difference in absolute white matter volume (decreased when corrected for intracranial volume). In their small follow-up study (ventriculomegaly $n=11$ and controls $n=12$), they showed that ventricular enlargement persisted at the age of 2 years and was then associated with increases in both gray and white matter volumes (Lyall et al. 2012). The authors suggested that increased surface area along the enlarged ventricular wall may result in a larger number of progenitor neurons. Indeed, the human cortical germinal zone
has several cytoarchitectonically distinct zones that contain the various progenitor populations in different proportions (Smart et al. 2002; Hansen et al. 2010). Cortical overgrowth from an increase in progenitor neurons from the germinatal zone would require the presence of ventriculomegaly from early in gestation and would not account for overgrowth documented in fetuses with ventriculomegaly diagnosed later in pregnancy with a normal appearance at 20 weeks. Over migration for these areas after 20 weeks would be more likely due to glial populations as late migrating inhibitory neurons arise from different regions in the ganglionic eminence. In our study, while the majority of fetuses were diagnosed with ventriculomegaly on their anomaly ultrasound scan performed around 20 weeks, 3 fetuses had no brain abnormalities on their anomaly ultrasound scan and developed ventriculomegaly at a later age. Greater brain parenchyma and cortical gray matter volume could also be due to the lack of normal developmental apoptosis in the developing brain (Kuan et al. 2000) rather than an increased number of migrating cells. Early in gestation apoptosis is involved in the development and morphogenesis of the neural tube supporting the role of apoptosis in adjusting the initial progenitor pool size (Kuan et al. 2000). At around 4.5 weeks, apoptotic activity is seen in the ventricular zone, but is very low. By week 6–7, apoptosis in the ventricular zone increases 5-fold and there is evidence of apoptosis in the preplate (Zecevic and Rakic 2001). By week 11, apoptosis has commenced in all cortical layers, although it is most prominent in the proliferative zones. Between 12 and 22 weeks, apoptosis increases significantly throughout the telencephalon. After 32 weeks, apoptosis continues in all cortical layers. Apoptosis is an integral part of cortical development, initially related to the morphogenesis, proliferation, and regulation of progenitor size and during the late fetal and postnatal period to synaptogenesis. Previous studies in preterm and IUGR infants (Peterson et al. 2000; Tolsa et al. 2004) have shown that cortical volume and outcome data correlate well; the cortex mediates cognitive abilities, and a reduction in cortical volume has been associated with neurodevelopmental delay. Interestingly, fetuses with isolated ventriculomegaly also have an increased risk of neurocognitive delay (Sadan et al. 2007; Leitner et al. 2009; Lyall et al. 2012), but according to this study exhibit increased cortical volumes. This implies that it may be the quality of the cortical tissue and its connections not the absolute volume that relates to the neurocognitive outcome. An increase in cortical volume could be due to an increase in cell numbers, synapses, or in the extracellular matrix. Ventriculomegaly has been associated with brain and cortical overgrowth in a rat model of gestational vitamin D deficiency. Cortical overgrowth in the affected rats was associated with an increase in proliferating cells and a decrease in apoptotic cells in the studied cortical regions of the cingulate and dentate gyri (Eyles et al. 2003). However, it is unclear that cells types contributed to the larger brains.

Cortical development and organization is regulated by the basic coordinated morphogenetic processes of proliferation, apoptosis, migration, and differentiation of both neurons and glia (Chan et al. 2002). Apoptosis is prominent in areas that undergo dramatic morphogenetic changes and is essential for the regulation of the proper size and shape of the cortex during development. Several animal knockout studies have shown that alterations in genes regulating developmental apoptosis lead to changes in cortical size and shape (Oppenheim et al. 2001; Chen and Walsh 2002; Depaepe et al. 2005). Cortical overgrowth has been described in children with neurofibromatosis Type 1, and cortical volume has correlated with the degree of learning disability (Moore et al. 2000). The presence of brain overgrowth in autistic children at the ages of 2–4 years is well established (Courchesne, Campbell, et al. 2011). Cortical gray matter and white matter enlargement have been found in MRI volumetric studies in children with autism with enlargement most prominent in the frontal lobe (Courchesne et al. 2001; Carper and Courchesne 2005; Hazlett et al. 2011). An excess of cerebral cortical neurons (67% increase) has been found at autopsy in 7 autistic patients (age median 4 years, range 2–16 years) compared with controls (Courchesne, Mouton, et al. 2011), which could underlie the volume increases in cerebral gray matter reported. In our cases, white matter was not enlarged during fetal life, and it was not possible to analyze the lobes of the brain separately. The group of Lyall et al. (2012) detected increases in white matter volume at 2 years of age, but not in the neonatal period in fetuses with antenataly diagnosed ventriculomegaly.

There are several limitations to the current study. Cortical volume segmentations were performed only in a subset of fetuses due to the extensive work required to perform detailed and accurate manual segmentations. However, we are currently developing and assessing an automatic protocol for cortical segmentation. In addition, developmental data for the fetuses in our study are currently being collected. Detailed neurodevelopmental assessments using the Griffiths Mental Development Scales and Bayley-III Scales of Infant and Toddler Development are performed at 1 and 2 years, respectively, and a battery of autism tests, including the Autism Diagnostic Observation Schedule (ADOS) and Autism Diagnostic Interview-Revised (ADI-R) at 3 years.

In this study, we have demonstrated brain overgrowth, restricted to cortical enlargement, in association with fetal ventriculomegaly. Cortical overgrowth may occur secondary to disruption in the regulation of cell proliferation and apoptosis, and subsequent altered brain connectivity may explain the high risk of later neurodevelopmental delay in these children.

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