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Fractal-dimension analysis detects cerebral changes in preterm infants with and without intrauterine growth restriction

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ABSTRACT

In the search for a useful parameter to detect and quantify subtle brain abnormalities in infants with intrauterine growth restriction (IUGR), we hypothesised that the analysis of the structural complexity of grey matter (GM) and white matter (WM) using the fractal dimension (FD), a measurement of the topological complexity of an object, could be established as a useful tool for quantitative studies of infant brain morphology.

We studied a sample of 18 singleton IUGR premature infants, (12.72 months corrected age (CA), range: 12 months–14 months), 15 preterm infants matched one-to-one for gestational age (GA) at delivery (12.6 months; range: 12 months–14 months), and 15 neonates born at term (12.4 months; range: 11 months–14 months). The neurodevelopmental outcome was assessed in all subjects at 18 months CA according to the Bayley Scale for Infant and Toddler Development – Third edition (BSID-III). For MRI acquisition and processing, the infants were scanned at 12 months CA, in a TIM TRIO 3T scanner, sleeping naturally. Images were pre-processed using the SPM5 toolbox, the GM and WM segmented under the VBM5 toolbox, and the box-counting method was applied for FD calculation of normal and skeletonised segmented images.

The results showed a significant decrease of the FD of the brain GM and WM in the IUGR group when compared to the preterm or at-term controls. We also identified a significant linear tendency of both GM and WM FD from IUGR to preterm and term groups. Finally, multiple linear analyses between the FD of the GM or WM and the neurodevelopmental scales showed a significant regression of the language and motor scales with the FD of the GM.

In conclusion, a decreased FD of the GM and WM in IUGR infants could be a sensitive indicator for the investigation of structural brain abnormalities in the IUGR population at 12 months of age, which can also be related to functional disorders.

Introduction

Preterm newborns are particularly vulnerable to alterations on brain development (Mathur and Inder, 2009). Prematurity is known to be associated with increased risk of brain structural and functional changes (Volpe, 2009) which may present specific features at different stages of brain maturation or in the presence of concomitant perinatal complications. Preterm birth is commonly associated with intrauterine growth restriction (IUGR) due to placental insufficiency, a condition which is associated with foetal exposure to chronic restriction of oxygen and nutrients and which itself carries substantial morbidity and mortality (Tan and Yeo, 2005). Recent evidence suggests that premature newborns presenting IUGR might suffer

Abbreviations: AGA, Appropriate for gestational age; BSID-III, Bayley Scale for Infant Development – Third edition; BW, Birth weight; CA, Corrected age; FD, Fractal dimension; 3DFD, Three-dimensional fractal dimension; GA, Gestational age; GM, Grey matter; ICV, Intracranial volume; IUGR, Intrauterine growth restriction; MRI, Magnetic resonance imaging; TBV, Total brain volume; WM, White matter.

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specific consequences for brain development (Lodygensky et al., 2008; Sizonenko et al., 2006; Tolsa et al., 2004), with an increased prevalence of neurodevelopmental morbidity, especially in terms of cognitive skills and executive functions (Geva et al., 2006a,b; Leitner et al., 2007).

Magnetic resonance imaging (MRI) has been used to demonstrate global and regional brain differences at different ages in subjects born preterm. At term equivalent age, preterm newborns show decreased cortical (Peterson et al., 2000) and subcortical volumes (Srinivasan et al., 2007), impaired growth of the corpus callosum (Anderson et al., 2006) and smaller cerebellar volume (Limperopoulos et al., 2005). Microstructural differences have been described in the centrum semiovale, frontal white matter and genu of the corpus callosum (Anjari et al., 2007), splenium of corpus callosum (Rose et al., 2008) and internal capsule (Cheong et al., 2009). MRI studies during childhood and adolescence have demonstrated abnormal GM and WM distribution in several areas (Soria-Pastor et al., 2009; Nosarti et al., 2008). Similarly to what is observed in the neonatal period microstructural differences appear to cluster in central white matter and its connection regions (Nagy et al., 2009; Constable et al., 2008).

Specific effects of IUGR on brain development have been demonstrated in preterm newborns at birth and at term CA. These include significant volume reductions in GM (Tolsa et al., 2004) and decreased volumes of the hippocampus (Lodygensky et al., 2008). Moreover, a major delay in cortical development, a discordant pattern of gyrification, and a pronounced reduction in cortical expansion have been reported (Dubois et al., 2008). However there is no evidence for the persistence of these effects in older infants.

Fractal dimension (FD) is a measurement of the topological complexity of an object (Fernández and Jelinek, 2001). It has been previously demonstrated than the FD can serve as a surrogate marker of the degree of brain damage, due to its sensitivity not only to changes in normal cerebral aging (Zhang et al., 2007), but also in pathological processes such as multiple sclerosis (Esteban et al., 2009, 2007), schizophrenia (Sandu et al., 2008), attention deficit disorder (Li et al., 2007), cerebellar morphological changes (Wu et al., 2010), stroke, and motor deficits (Zhang et al., 2008).

In the present study, we investigate the morphological complexity of the GM and WM in one-year-old preterm infants with and without growth restriction, with the aim of assessing the usefulness of the FD as a measurement of subtle structural brain differences in these clinical entities. For this, we used a novel computational algorithm for automatically detecting FD in three-dimensional structures (Esteban et al., 2009) and we assessed the correlation of these results with neurodevelopment at 18 months of age. To our best knowledge, this is the first available report using the FD to evaluate differences in the complexity of GM and WM in infants at this age.

Materials and methods

Patients

This study is part of a larger prospective research program on IUGR involving foetal assessment and short- and long-term postnatal follow-up at the Hospital Clinic (Barcelona, Spain). The study design involved prospective recruitment of a consecutive sample of 60 neonates: 20 singleton premature infants with a prenatal diagnosis of severe IUGR diagnosed before 34 weeks of gestation, 20 preterm infants with a birth weight appropriate for their gestational age (AGA), matched one-to-one for gestational age (GA) at delivery (GA +/− 2 weeks) and 20 neonates born full-term. All cases were born between 2006 and 2007. Patients were recruited prenatally in IUGR cases and at birth in the other two groups. Gestational age in all groups was corrected from foetal biometries in the first trimester of gestation (Robinson and Fleming, 1975) and cases where this information was not available were excluded from the study. IUGR was defined as an ultrasound estimated foetal weight below the 10th percentile for GA confirmed at birth, together with abnormal Doppler blood flow in the umbilical artery (pulsatility index>2 standard deviations). The asymmetric/symmetric classification for growth restricted foetuses was not used in this study, since it may lead to false positive and negative diagnosis in comparison with estimated foetal weight and umbilical artery doppler (Marsál, 2009).

Preterm AGA cases were defined as a birth weight (BW) between the 10th and 90th customized centiles according to local reference standards. All cases in preterm and term AGA group were examined in our own Hospital the week before delivery, and foetal well-being was confirmed prenatally by foetal heart rate, the biophysical profile, and Doppler studies. Infants with chromosomal, genetic, or structural defects and signs of intrauterine infection or neonatal early onset sepsis as defined by positive blood culture within the first 72 h of life were excluded from this study. The presence of any neonatal morbidity in at-term births was considered an exclusion criterion. The study protocol was approved by the local Ethics Committee, and written informed consent was obtained from the parents or legal guardians of all participants.

Prenatal and neonatal data were prospectively recorded, including: gestational age, Apgar at 5 min; umbilical artery pH, SNAPP-II (Score for Neonatal Acute Physiology version II), late-onset sepsis, necrotizing enterocolitis, chronic lung disease (defined as oxygen need at 36 weeks post menstrual age), postnatal steroid and neonatal ultrasound findings were recorded. Growth parameters (weight, length, and head circumference) were also recorded. Parental education was recorded as the educational level of the parents (low, intermediate or high).

Neurodevelopmental assessment

The neurodevelopmental outcome was assessed in all subjects at 18 months corrected age (±2 month) with the Bayley Scale for Infant and Toddler Development, Third edition (BSID-III), which evaluates five different scales: cognitive; language scale, consisting of receptive and expressive communication subtest; motor, consisting of fine and gross motor subtests; socio-emotional behaviour; and adaptive behaviour. The scales have scores with a mean of 100 and SD of 15. Sub-tests are evaluated in scalar scores, considering>7 a normal value. All developmental examinations were performed by a single trained psychologist examiner with previous experience with the BSID-III. The examiner was not informed about the infant medical history. All the infants included were testable with the BSID-III.

MRI acquisition and processing

The 3 groups were scanned at 12±2 months corrected age (CA), unsedated, sleeping naturally. Data were obtained using a TIM TRIO 3.0T scanner (Siemens, Germany). A set of high-resolution T1-weighted, 3D images, was acquired using the Magnetization Prepared Rapid Acquisition Gradient Echo sequence (MPRAGE) (TR/TE = 2050 ms/2,41 ms; TI = 1050 ms; FOV = 220×220 mm and 256×256 matrix; scan time = 5: 52 min). The whole-brain data were acquired in a sagittal plane, yielding contiguous slices with isotropic voxel of 0.9×0.9×0.9 mm³. MRI-DTI and spectroscopy were acquired also to be analysed in future works. The total study time was 30 min. For two patients in the IUGR group, five in the AGA group and five in the term group, the exam was not successful because the children did not fall asleep during the exam. Thus, the final study population consisted of 48 subjects: 18 IUGR, 15 AGA and 15 full-term birth infants. There were no infants excluded due to suboptimal images. Structural MRI images were reviewed for the presence of anatomical abnormalities by an experienced neuroradiologist blind to group membership.

Image processing

Initially, all images were checked for artefacts. The data were pre-processed using the SPM5 (Statistical Parametric Mapping, Welcome Trust Centre for Neuroimaging, Institute of Neurology, UCL, London, http://www.fil.ion.ucl.ac.uk/spm) running in Matlab 7.5 (MathWorks, Natick, MA). The two-dimensional DICOM files were organized into volumetric three-dimensional files of each brain using the MRIcron software package (http://www.sph.sc.edu/comd/rorden/mricron/). For the image preparation a single investigator (N.P.) performed the prior manual steps. Line determination of the anterior-posterior commissures and image reorienting were performed.

Brain segmentation

To obtain the grey matter (GM), white matter (WM) and cerebrospinal fluid we used the VBM5 toolbox (http://dbm.neuro.uni-jena.de) replacing the standard priors by the infant brain probability templates of 12 months provided by Altaye et al. (2008). For this purpose, we employed the T1 structural MRI images of the infants studied. All scans were reviewed by an anatomical expert to determine whether the results of the tissue segmentation were accurate (Fig. 1). Global GM and WM volumes, and total brain volume (TBV = GM + WM) were calculated using the native space tissue maps of each subject. A specific value in mm^3 was obtained for each tissue.

3D fractal dimension (3DFD) computation method

The 3DFD of the entire brain was determined from segmented WM and GM in each subject as previously described (Esteban et al., 2009). Briefly, we selected the classical box-counting method (Hou et al., 1990) for computing the 3DFD values because it can evaluate the FD of structures without self-similarity (the brain is not strictly self-similar; Zhang et al., 2006). The corresponding 2D-MR images for each subject were stored in a 3D grid, and the 3D boxes were constructed. All pixels located inside a box having a value greater than or equal to a specified threshold (see below), were classified as BLACK; if the value of the pixels was lower than the specified threshold, then the box was classified as WHITE; otherwise, the box was classified as GREY. Then, for each case study and threshold, we classified boxes with sizes between 1 and 50, where the corresponding BLACK + GREY data constituted the basis for calculating the 3DFD. The 3DFD values were calculated through a log–log linear regression in which the X axis represents the inverse of the size of the boxes and the Y axis represents the box counting for the type of box selected. The final value for the 3DFD corresponds to the slope of this linear regression for the range of box sizes that maximizes the correlation value for the

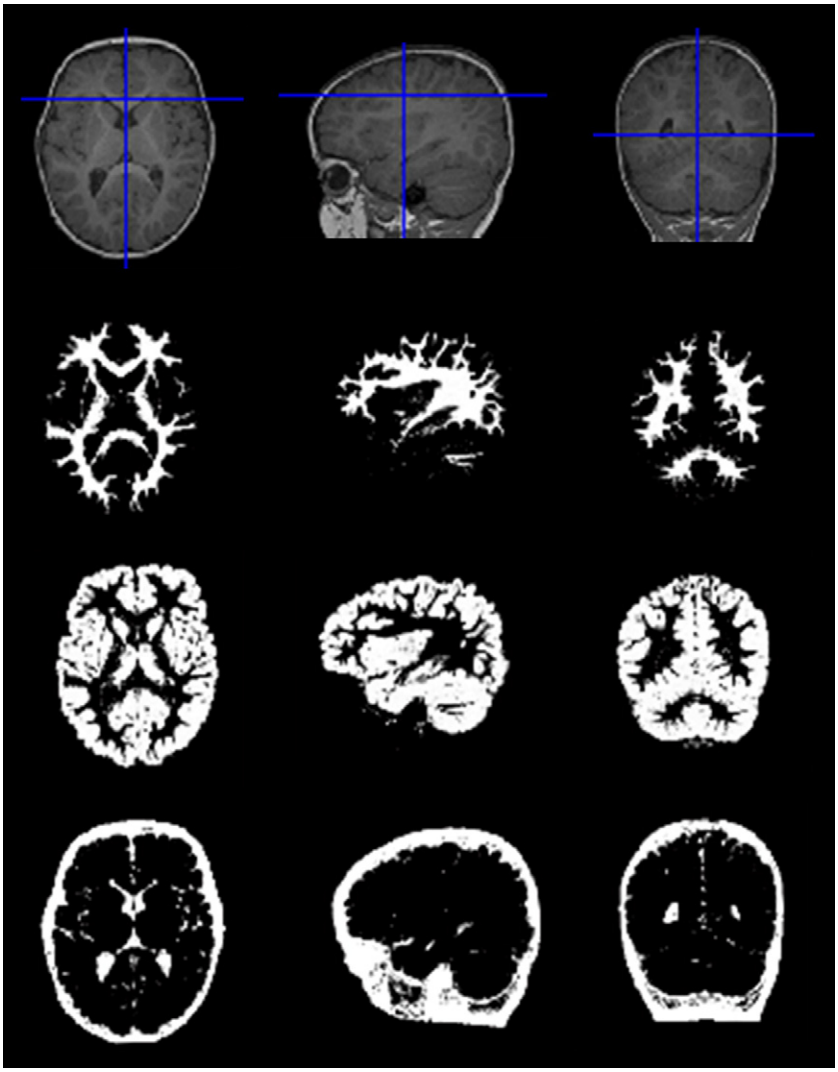

Fig. 1. Axial, sagittal and coronal slices from a single subject representing the T1-weighted image (first row), and tissue segmentation at different levels showing white matter (second row), grey matter (third row) and cerebrospinal fluid (fourth row).

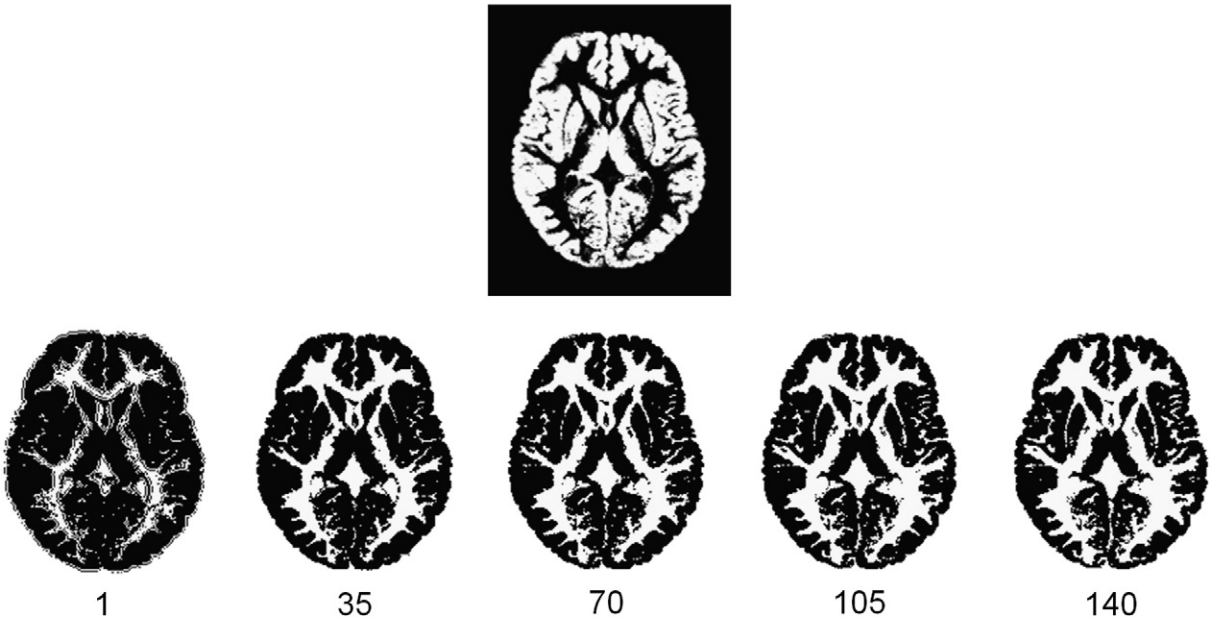

Fig. 2. Grey level segmentation of an axial section (from one random subject included in the study) at the level of the basal ganglia/thalami. Up: original section. Down: segmented section; the number under each image correspond to the grey level threshold. For further FD calculation all the images were segmented using 70 as the threshold value.

given threshold. A previous manual segmentation of the images using ImageJ (http://rsbweb.nih.gov/ij/) showed 70 as the most appropriate threshold value (in a grey scale from 0 to 255; Fig. 2); thus, the 3DFD values finally obtained from each subject for the statistical analysis were those with 70 as the segmentation threshold, and from 2 to 13, or 2 to 8, as the range of box sizes (which maximizes the correlation for this threshold) for WM, or GM, respectively. Skeletonization of the images was carried out implementing the algorithm described elsewhere (Palagyi and Kuba, 1999). The range of box sizes was from 5 to 23, and 9 to 27, for skeletonised GM and WM images, respectively. The accuracy of our method for 3DFD calculation was determined elsewhere (Esteban et al., 2009).

Statistical analysis

Comparisons among groups were performed by analysis of variance (ANOVA), where a polynomial contrast was constructed to test the hypothesis of linear differences between groups. Comparisons between groups were performed by analysis of variance (ANOVA). Categorical variables were analysed by X^2-Pearson and linear differences by linear-to-linear test.

Pearson's or Spearman's correlation tests (parametric and non-parametric, respectively) were applied to assess univariate correlation depending on the distribution of the variables. On the other hand, multiple linear model analysis was then applied trying to detect a relationship between the FD and the neurodevelopmental scale (outcome). The statistical analyses were carried out using SPSS v13.0 (SPSS Inc, Chicago, USA) and STATGRAPHICS Plus 5.1 statistical software. The level of significance for the results was set at $p = 0.05$.

Results

When the preterm groups were compared, the Apgar score at 5 min (9.3 ± 7.6 *vs* 9.1 ± 1.6), umbilical artery pH (7.23 ± 0.08 *vs* 7.27 ± 0.18), and the score of neonatal acute physiology–perinatal extension (SNAPP-II) (19.3 ± 13.8 *vs* 18.4 ± 13.7) detected were similar. In 8/18 (44.4%) IUGR infants and 3/15 (6.7%) AGA preterm infants the HC was less than -2ZS for gestational age and gender. No significant differences between IUGR and AGA preterm groups were found regarding late neonatal sepsis [(2/18 (11.1%) *vs* 2/15 (13.3%), $p = 1.0$]; necrotising enterocolitis (0 vs. 0); chronic lung disease (0 *vs* 0) and postnatal steroid [0 vs. 2/15 (13.3%), $p = 0.19$]. Brain ultrasound

Table 1
Anthropometric and demographic characteristics of the study groups.

Characteristic	IUGR (n = 18)	PTM (n = 15)	TERM (n = 15)	Linear tendency p-value
Gestational age	32.1 (2.05)*	30.7 (2.58)*	39.8 (1.05)	0.000
Gender (M/F)**	7/11	11/7	7/11	0.290
Weight at birth (kg)	1.06 (0.31)*	1.58 (0.47)*	3.34 (0.46)	0.000
Length at birth (cm)	36.78 (3.82)*	39.73 (4.43)*	49.33 (1.87)	0.000
Head circumference at birth (cm)	26.27 (2.19)*	28.63 (2.79)*	34.11 (1.12)	0.000
Weight at 12 months (kg)	8.46 (1.35)*	9.41 (1.33)	9.63 (0.72)	0.000
Height at 12 months (cm)	72.41 (3.75)	75.26 (4.11)	73.10 (2.13)	0.570
Head circumference at 12 months (cm)	45.81 (1.82)	46.60 (1.56)	45.96 (1.17)	0.780
Corrected age at scan (months)	12.7 (0.75)	12.6 (0.73)	12.4 (0.91)	0.250
Corrected age at BSID-III (months)	17.83 (6.04)	19.20 (6.08)	19.67 (3.97)	0.340
Breastfeeding	15/18	12/15	10/15	0.260
Maternal age	32.2 (3.76)	32.3 (5.5)	32.7 (5.1)	0.790
Maternal education less than high school n(%)**	8/18 (44.4)	6/15 (40)	6/15 (40)	0.070

IUGR, intrauterine growth restriction; *P value < 0.05 as compared to term subjects calculated by analysis of variance (ANOVA) with a polynomial contrast. **X^2-Pearson and linear to linear test.

Table 2
Global brain volumes, fractal dimension and neurodevelopmental scores in the study groups.

	IUGR (n = 18)	PTM (n = 15)	TERM (n = 15)	Linear tendency p-value
Grey matter volume (cm^3)	683.71 (64.54)	713.99 (57.03)	702.98 (77.07)	0.410
White matter volume (cm^3)	243.50 (36.89)	240.74 (37.96)	239.70 (32.17)	0.760
Total brain volume (cm^3)	927.21 (98.50)	954.73 (86.62)	942.68 (104.15)	0.650
GM fractal dimension	2.797 (0.007)*	2.802 (0.008)	2.804 (0.009)	0.010
WM fractal dimension	2.476 (0.029)	2.462 (0.036)	2.481 (0.032)	0.680
Skeletonised GM fractal dimension	2.537 (0.017)*	2.545 (0.012)	2.552 (0.026)	0.026
Skeletonised WM fractal dimension	2.408 (0.035)*	2.429 (0.026)	2.454 (0.035)	<0.001
Cognitive score	100.83 (9.27)	105.67 (10.32)	108.67 (12.88)	0.240
Language score	92.72 (12.96)	94.73 (16.57)	95.07 (13.63)	0.780
Receptive communication score	9.22 (3.07)	9.53 (3.44)	9.47 (2.47)	0.980
Expressive communication score	8.50 (2.64)	8.53 (2.80)	8.80 (2.85)	0.850
Motor score	93.72 (17.31)*	102.20 (9.63)	110.0 (17.18)	0.020
Fine motor score	8.83 (3.05)*	10.27 (1.98)	11.93 (2.60)	0.010
Gross motor score	9.28 (3.69)	10.40 (1.76)	11.40 (4.68)	0.330
Social emotional score	116.39 (24.18)	114.47 (24.06)	118.00 (26.30)	0.870
Adaptive behaviour score	94.78 (13.97)*	98.43 (15.25)	107.20 (12.20)	0.020

IUGR, intrauterine growth restriction; *P value < 0.05 as compared to term subjects calculated by analysis of variance (ANOVA) with a polynomial contrast.

at 3 days of life was normal in all cases in both preterm groups. At term equivalent age, 3 preterm IUGR and 1 preterm infant had periventricular leukomalacia grade I. Concerning nutritional management in NICU, preterm neonates had essentially equivalent nutritional intakes. Regarding breastfeeding there were no statistical differences between preterm groups and term group. Anthropometric and demographic characteristics are shown in Table 1. MRI structural evaluation revealed the presence of mild abnormalities (Rademaker et al., 2005) in five of 18 (27.7 %) preterm IUGR – mild ventricular dilation (3), thinning of the corpus callosum (1) and increased cisterna magna (1) – and two of 15 (13.3%) preterm AGA – mild ventricular dilation (1) and thinning of the corpus callosum (1).

The results of the Bayley-III scales showed that the preterm IUGR group performed significantly worse than the term group on motor, fine motor and adaptive behaviour. Comparisons between preterm and term infants demonstrated a non-significant trend to lower scores in fine motor and adaptive behaviour (Table 2).

Regarding the FD of the GM, the results for both the GM and the skeletonised GM (Table 2 and Figs. 3A, B) showed a significant decrease in the FD of the preterm IUGR group, while preterm neonates without growth restriction did not statistically differ with respect to term infants; additionally, a significant linear trend with outcome was found in both analyses (GM and skeletonised GM). Neither statistical differences nor a linear trend of the FD of the WM were found (Table 2 and Fig. 3C); however, the FD of the IUGR skeletonised WM also showed a significant decrease (Table 2 and Fig. 3D), and preterm neonates without growth restriction were not statistically different when related to term infants; as detected for the GM, a significant linear trend with outcome was also found for the FD of the skeletonised WM (Table 2 and Fig. 3D).

Overall, no significant correlations were found between the FD of the GM or WM and any single given neurodevelopmental measurement other than for the FD of the skeletonised GM and the expressive communication ($p = 0.0133$; Table 3). Multiple linear model analysis was then applied trying to detect a relationship between the FD and more than one of the neurodevelopmental scales. The significant ($p < 0.05$) multiple correlations detected were related to the GM and skeletonised GM (Table 3); the multiple linear model with the

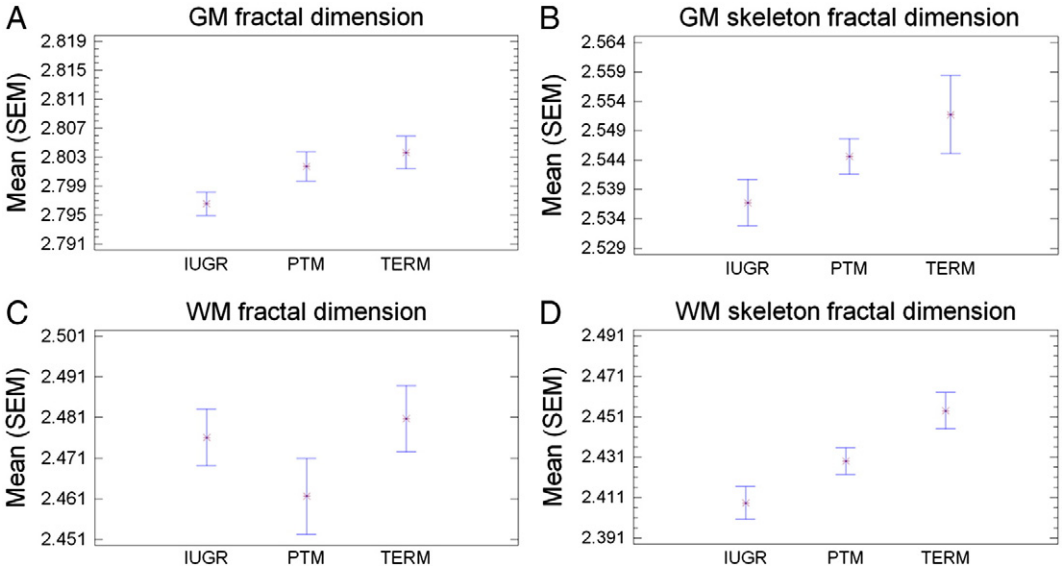

Fig. 3. Plots showing the fractal-dimension of the grey matter (A,B) and white matter (C,D). The FD of the GM and WM was lower in IUGR when compared to term infants with significant differences in the A,B,D plots. All the groups showed a significant upward linear tendency in the A,B,D plots ($P = 0.01$).

Table 3
Multiple regression analysis.

Multiple linear model	p-value	Adjusted R^2
FD_GM = 2.81683 − 0.000559314*lang + 0.00206299*langR + 0.000162014*motor	0.0195	14.47%
FD_GM = 2.81467 − 0.000564943*lang + 0.00205294*langR + 0.000221478*motor − 0.000316046*mGross	0.0385	13.17%
FD_GM = 2.80966 − 0.000406961*lang + 0.00170641*langR − 0.000560379*langE + 0.000172891*motor	0.0386	13.15%
FD_GM = 2.8127 + 0.0000626104*cogn − 0.000568537*lang + 0.00205514*langR + 0.00014735*motor	0.0392	13.08%
FD_GM = 2.81838 − 0.000539796*lang + 0.00201294*langR + 0.000160223*motor − 0.0000233772*social	0.0401	12.97%
FD_GM = 2.81852 − 0.000563535*lang + 0.00208153*langR + 0.000164873*motor − 0.0000176215*adap	0.0434	12.59%
FD_GM = 2.79097 + 0.000515011*langR − 0.00155356*langE + 0.000176874*motor	0.0346	12.02%
FD_skel_GM = 2.56597 − 0.00256489*langE	0.0133	10.69%
FD_skel_GM = 2.54941 − 0.00259571*langE + 0.000168485*adap	0.0315	10.44%
FD_skel_GM = 2.55726 − 0.0023728*langE − 0.000107946*social + 0.000196423*adap	0.0524	10.18%
FD_skel_GM = 2.57457 − 0.00237965*langE − 0.0000876953*social	0.0361	9.88%
FD_skel_GM = 2.55247 + 0.000143805*cogn − 0.00274745*langE	0.0414	9.34%
FD_skel_GM = 2.56739 − 0.0024771*langE − 0.000211534*mGross	0.0467	8.85%
FD_skel_GM = 2.56198 + 0.0000715248*lang − 0.00287802*langE	0.0474	8.79%

lowest p-value include the language, receptive communication and motor scales for the FD of the GM ($p = 0.0195$), and the expressive communication and the adaptative behaviour for the FD of the skeletonised GM ($p = 0.0315$).

Discussion

In the present study, we applied the FD to characterize the morphological complexity of GM and WM in preterm infants with and without growth restriction at 12 months CA. This is the first study available in which the brain morphological complexity in preterm infants with and without growth restriction was assessed using FD. These findings can be related to previous studies on cortical folding, since FD is considered an index of cortical folding complexity (Kalmanti and Maris, 2007; Li et al., 2007). The main finding was that FD decreased in preterm infants with growth restriction, both in the GM (normal and skeletonised) and WM (skeletonised), and that there is an increasing linear tendency of the FD related not only to gestational age (Xue et al., 2007) but also to growth restriction. Our data suggests that structural brain differences previously reported at birth in preterm newborns with growth restriction persist at 12 months CA. It bears noting that no differences were found between preterm infants and term infants. The results of this study support the notion that association of preterm birth with IUGR results in a distinct entity, and that the status of IUGR should be reported in studies evaluating the neurodevelopment of preterm born subjects to allow comparability between studies.

Some reports in preterm neonates have demonstrated that prematurity *per se* might influence processes such as cortical folding, leading to alterations in the cortical surface complexity (Ajayi-Obe et al., 2000) and sulcal morphology (Zubiaurre-Elorza et al., 2009). As the gestational age decreases the structural differences increase (Kapellou et al., 2006). Additionally, when IUGR is added to prematurity, the effects on brain structure seem to be more pronounced than those reported in preterm infants during the neonatal period. Preterm IUGR newborns present a significant reduction in absolute cortical GM volume as compared with normally grown preterm newborns (Tolsa et al., 2004). Dubois et al. (2008) reported that IUGR preterm neonates showed an important delay in cortical development, a discordant pattern of gyrification and decreased cortical thickness. This finding could be supported by previous results in experimental models of foetal growth restriction that have confirmed a constrained growth in dendrites, axons and synaptogenesis (Ress et al., 2008), adjustment in neuronal number (Samuelsen et al., 2007) and differences in glial expression and regulation (Pearce, 2006). Moreover, these changes may result in different patterns of anatomical connectivity reflecting the impaired local cortical architecture caused by IUGR (Ress et al., 2008) as supported also by our results. Regarding connectivity, it has been suggested that modifications in location and shape of sulci are determined by the global minimization over the brain of the visco-elastic tension from WM fibres connecting cortical areas (Van-essen, 1997), which is in line with our results, where a significant trend to decreased FD of the skeletonised WM was found.

The data on FD in infants with IUGR described in this study show noteworthy similarities with the findings reported under other conditions and may open new pathways for investigation. Decreased complexity measured by FD has been reported in functional disorders such as schizophrenia (Ha et al., 2005) and attention deficit hyperactivity (Li et al., 2007), leading us to hypothesise that these conditions are related with impaired cortical convolution. Particularly, the attention-deficit hyperactivity disorder has been associated with a significant cortical thinning in regions important for attention control and executive functions (Makris et al., 2007). Notably, several studies have described difficulties related to schizophrenia (Nilsson et al., 2005), attention deficiency (Strang-Karlsson et al., 2008; Geva et al., 2006a,b), and executive functioning (Leitner et al., 2007) in IUGR children. In addition, significant reductions in the frontal lobe volume (Benavides-Serralde et al., 2009) and cortical folding (Dubois et al., 2008) have been described in perinatal studies on IUGR patients. Thus, in agreement with the findings of the present study, the decreased FD of the GM and WM in IUGR infants could represent a distinctive phenotype in this population at 12 months which might predict functional disorders.

In our study, IUGR infants showed worse performance in the neurodevelopmental assessment. As both preterm groups were comparable in neonatal morbidity, NICU nutritional management (and together to the third group with the breast milk intake), other causes such as nutritional, environmental, genetic (Powers et al., 2008) and post-natal growth factors (Fattal-Valeski et al., 2009) should be considered to explain the developmental differences in the IUGR group. In this regard, we found a characteristic pattern of poor weight, length and HC gain in the first year of life in preterm IUGR infants. This is in line with other studies that have demonstrated growth impairment in IUGR infants (Padilla et al., 2010; Fattal-Valeski et al., 2009) and difficulties in different areas of the neurodevelopment (Leitner et al., 2007).

It bears mentioning that there were significant associations between FD and specific neurodevelopmental scales (domains). Genetic, epigenetic and environmental factors are involved during the ontogenic and developmental processes. Particularly, epigenetic processes can modify brain anatomical phenotypes (Leonard et al., 2006), and hence global and regional differences resulting from external influences in critical periods could be associated with permanent variations in the information-processing efficiency that affects cognitive, social and emotional phenotypes (Lewis and Elman, 2008). The clinical correlates of the neurostructural changes associated with IUGR, as reported in this study at one-year of age and in previous studies on newborns (Dubois et al., 2008; Lodygensky et al., 2008; Tolsa et al., 2004), should be further

evaluated in long-term neurodevelopmental studies. While several well-designed longitudinal studies have demonstrated an increased overall prevalence of neurodevelopmental abnormalities in IUGR (Leitner et al., 2007; Geva et al., 2006a,b), the precise relationship between the severity of the intrauterine condition and the structural as well as functional changes observed later in life remain to be better characterized.

The strengths of this study include a well-characterized growth-restricted group according to prenatal features. Intrauterine growth restriction was diagnosed according to specific criteria for growth restriction, which requires the observation of Doppler measurements indicative of placental insufficiency. In addition, we included three groups, where the preterm groups were similar in GA at delivery and neonatal morbidity, and the term group allowed a better assessment of the differences between groups. The potential bias of environmental influences during the first year of life was also taken into account in the design of the statistical analysis.

However, the relatively small sample size, which may have masked statistical differences in some of the comparisons, can be considered a limitation of this study, where significant differences were not found between groups for several neurodevelopmental scores; on the other hand, the significant differences found in the FD analysis indicated that it could be a useful parameter for detecting differences between groups in small sample size studies.

We do not have information about non formula food in the first year of life, and we have acknowledged that as a limitation of this study and a fact to take into account in the design of future studies. Concerning MRI studies, the tissue segmentation in the 1-year-old brains represents a challenging task due to the poor differentiation between GM and WM related to developmental patterns at this age (Paus et al., 2001). However, to minimize the problem arising from this procedure, we used only T1 images for segmentation and each scan was reviewed by an anatomical expert to determine whether the results of the tissue segmentation were accurate.

In conclusion, FD of the GM, representing cortical-surface complexity, may be a sensitive indicator for the investigation of structural brain abnormalities in preterm IUGR infants at 12 months CA. These findings complement previous evidence in preterm newborns suggesting that the association with IUGR results in specific structural brain and developmental differences. Our results reflect that although prematurity has a high impact on the developing brain, the structural differences in the brain of IUGR infants are more pronounced, suggesting higher vulnerability in this group. We acknowledge that studies involving more subjects are warranted. Likewise, further studies to explore regional FD changes in preterm infants with and without IUGR, as well as clinical implications of the FD including its diagnostic value, should be performed.

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References

Ajayi-Obe, M., Saeed, N., Cown, F.M., Rutherford, M.A., Edwards, A.D., 2000. Reduced development of cerebral cortex in extremely preterm infants. Lancet 356, 1162–1163.

Altaye, M., Holland, S.K., Wilke, M., Gaser, C., 2008. Infant brain probability templates for MRI segmentation and normalization. Neuroimage 43, 721–730.

Anderson, N.G., Laurent, I., Woodward, L.J., Inder, T.E., 2006. Detection of impairment of the corpus callosum in premature infants. Pediatrics 118, 951–960.

Anjari, M., Srinivasan, L., Allsop, J.M., Hajnal, J.V., Rutherford, M.A., Edwards, A.D., Counsell, S.J., 2007. Difusión tensor imaging with tract_based spatial statistics reveals local white matter abnormalities in preterm infants. Neuroimage 35, 1021–1027.

Benavides-Serralde, A., Hernández-Andrade, E., Fernandez-Delgado, J., Plasencia, W., Scheier, M., Crispi, F., Figueras, F., Nicolaides, K.H., Gratacós, E., 2009. Three-dimensional sonographic calculation of the volume of intracranial structures in growth-restricted and appropriate for gestational age fetuses. Ultrasound Obstet. Gynecol. 33, 530–537.

Cheong, J.L.Y., Thompson, D.K., Wang, H.X., Hunt, R.W., Anderson, P.J., Inder, T.E., Doyle, L.W., 2009. Abnormal white matter signal on MR imaging is related to abnormal tissue microstructure. Am. J. Neuroradiol. 30, 623–628.

Constable, R.T., Ment, L.R., Vohr, B.R., Kesler, S.R., Fulbright, R.K., Lacadie, C., Delancy, S., Katz, K.H., Schneider, K.C., Schafer, R.J., Makuch, R.W., Reiss, A.R., 2008. Prematurely born children demonstrate white matter microstructural differences at 12 years of age, relative to term control subjects: an investigation of group gender effects. Pediatrics 121, 306–316.

Dubois, J., Benders, M., Borradori-Tolsa, C., Cachia, A., Lazeyras, F., Leuchter, R.H., Sizonenko, S.V., Warfield, S.K., Mangin, J.F., Hüppi, P.S., 2008. Primary cortical folding in the human newborn: an early marker of later functional development. Brain 131, 2028–2041.

Esteban, F.J., Sepulcre, J., de Mendizábal, N.V., Goñi, J., Navas, J., de Miras, J.R., Bejarano, B., Masdeu, J.C., Villoslada, P., 2007. Fractal dimension and white matter changes in multiple sclerosis. Neuroimage 36, 543–549.

Esteban, F.J., Sepulcre, J., de Miras, J.R., Navas, J., de Mendizábal, N.V., Goñi, J., Quesada, J.M., Bejarano, B., Villoslada, P., 2009. Fractal dimension analysis of grey matter in multiple sclerosis. J. Neurol. Sci. 282, 67–71.

Fattal-Valeski, A., Toledano-Alhadef, H., Leitner, Y., Geva, R., Eshel, R., Harel, S., 2009. Growth patterns in children with intrauterine growth retardation and their correlation to neurocognitive development. J. Child Neurol. 24, 846–851.

Fernández, E., Jelinek, H.F., 2001. Use of fractal theory in neuroscience: methods, advantages, and potential problems. Methods 24, 309–321.

Geva, R., Eshel, R., Leitner, Y., Fatal-Valevski, A.F., Harel, S., 2006a. Neuropsychological outcome of chidren with intrauterine growth restriction: a 9-year prospective study. Pediatrics 118, 91–100.

Geva, R., Eshel, R., Leitner, Y., Fatal-Valevski, A.F., Harel, S., 2006b. Memory functions of children born with asymmetric intrauterine growth restriction. Brain Res. 1117, 186–194.

Ha, T.H., Yoon, U., Lee, K.J., Shin, Y.W., Lee, J.M., Kim, I.Y., Ha, K.S., Kim, S.I., Kwon, J.S., 2005. Fractal dimension of cerebral cortical surface in schizophrenia and obsessive–compulsive disorder. Neurosci. Lett. 384, 172–176.

Hou, X.J., Gilmore, R., Mindlin, G.B., Solari, H.G., 1990. An efficient algorithm for fast O(N*ln(N)) box counting. Phys. Lett. A 151, 43–46.

Kalmanti, E., Maris, T.G., 2007. Fractal dimension as an index of brain cortical changes throughout life. In Vivo 21, 641–646.

Kapellou, O., Counsell, S.J., Kennea, N., Dyet, L., Saeed, N., Stark, J., Maalouf, E., Duggan, P., Ajayi-Obe, M., Hajnal, J., Allsop, J.M., Boardman, J., Rutherford, M.A., Cowan, F., Edwards, A.D., 2006. Abnormal cortical development after premature birth shown by altered allometric scaling of brain growth. PLoS Med. 3, e265.

Leitner, Y., Fattal-Valevski, A., Geva, R., Eshel, R., Toledano-Alhadef, H., Rotstein, M., Bassan, H., Radianu, B., Bitchonsky, O., Jaffa, A.J., Harel, S., 2007. Neurodevelopmental outcome of children with intrauterine growth retardation: a longitudinal, 10-year prospective study. J. Child Neurol. 22, 580–587.

Leonard, C.M., Eckert, M.A., Kuldau, J.M., 2006. Exploiting human anatomical variability as a link between genome and cognome. Genes Brain Behav. 5, 64–77.

Lewis, J.D., Elman, J.L., 2008. Growth-related neural organiation and the autism phenotype: a test of the hypothesis that altered brain growth leads to altered connectivity. Dev. Sci. 11, 135–155.

Li, X., Jiang, J., Zhu, W., Yu, C., Sui, M., Wang, Y., Jiang, T., 2007. Asymmetry ofprefrontal cortical convolution complexity in males with attention-deficit/hyperactivity disorder using fractal information dimension. Brain Dev. 29, 649–655.

Limperopoulos, C., Soul, J.S., Gauvreau, K., Huppi, P.S., Warfield, S.K., Bassan, H., Robertson, R.L., Volpe, J.J., du Plessis, A.J., 2005. Late gestation cerebellar growth is rapid and impeded by premature birth. Pediatrics 115, 688–695.

Lodygensky, G.A., Seghier, M.L., Warfield, S.K., Tolsa, C.B., Sizonenko, S., Lazeyras, F., Hüppi, P.S., 2008. Intrauterine growth restriction affects the preterm infant's hippocampus. Pediatr. Res. 63, 438–443.

Makris, N., Biederman, J., Valera, E.M., Bush, G., Kaiser, J., Kennedy, D.N., Caviness, V.S., Faraone, S.V., Seidman, L.J., 2007. Cortical thinning of the attention and executive function network in adults with attention-deficit/hyperactivity disorder. Cereb. Cortex 17, 1364–1375.

Marsál, K., 2009. Obstetric management of intrauterine growth restriction. Best Pract. Res. Clin. Obstet. Gynaecol. 23, 857–870.

Mathur, A., Inder, T., 2009. Magnetic resonance imaging insights into brain injury and outcomes in premature infants. J. Commun. Disord. 42, 248–255.

Nagy, Z., Ashburner, J., Andersson, J., Jbabdi, S., Draganski, B., Skare, S., Böhm, B., Smedler, A.C., Forssberg, H., Lagercrantz, H., 2009. Structural correlates of preterm birth in the adolescent brain. Pediatrics 124, e964–e972.

Nilsson, E., Stålberg, G., Lichtenstein, P., Cnattingius, S., Olausson, P.O., Hultman, C.M., 2005. Fetal growth restriction and schizophrenia: a Swedish twin study. Twin Res. Hum. Genet. 8, 402–408.

Nosarti, C., Giouroukou, E., Healy, E., Rifkin, L., Walshe, M., Reichenberg, A., Chitnis, X., William, S.C., Murray, R.M., 2008. Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. Brain 131, 205–217.

Padilla, N., Perapoch, J., Carrascosa, A., Acosta-Rojas, R., Botet, F., Gratacós, E., 2010. Twelve-month neurodevelopmental outcome in preterm infants with and without intrauterine growth restriction. Acta Paediatr. 99, 1498–1503.

Palagyi, K., Kuba, A., 1999. A parallel 3D 12-subiteration thinning algorithm. Graphical Mod. Imag. Proces. 61, 199–221.

Paus, T., Collins, D.L., Evans, A.C., Leonard, G., Pike, B., Zijdenbos, A., 2001. Maturation of white matter in the human brain: a review of magnetic resonance studies. Brain Res. Bull. 54, 255–266.

Pearce, W.J., 2006. Hypoxic regulation of the fetal cerebral circulation. J. Appl. Physiol. 100, 731–738.

Peterson, B.S., Vohr, B., Staib, L.H., Cannistraci, C.J., Dolberg, A., Schneider, K.C., Katz, K. H., Westerveld, M., Sparrow, S., Anderson, A.W., Duncan, C.C., Makuch, R.W., Gore, J. C., Ment, L.R., 2000. Regional brain volume abnormalities and long-term cognitive outcome in preterm infants. J. Am. Med. Assoc. 284, 1939–1947.

Powers, G.C., Ramamurthy, R., Schoolfield, J., Kathleen, M., 2008. Postdischarge growth and development in a predominantly Hispanic, very low birth weight population. Pediatrics 122, 1258–1265.

Rademaker, K.J., Uiterwaal, C.S.P.M., Beek, F.J., van Haastert, I.C., Lieftink, A.F., Grenendaal, F., Grobbee, D.E., de Vries, L.S., 2005. Neonatal cranial ultrasound versus MRI and neurodevelopmental outcome at school age in children born preterm. Arch. Dis. Child. Fetal Neonatal Ed. 90, F489–F493.

Ress, A., Harding, R., Walker, D., 2008. An adverse intrauterine environment: implications for injury and altered development of the brain. Int. J. Dev. Neurosci. 26, 3–11.

Robinson, H.P., Fleming, J.E., 1975. A critical evaluation of sonar "crown-rump length" measurements. Br. J. Obstet. Gynaecol. 82, 702–710.

Rose, S.E., Hatzigeorgiou, X., Strudwick, M.W., Durbridge, G., Davies, P.S.W., Colditz, P.B., 2008. Altered white matter diffusion anisotropy in normal and preterm infants at term-equivalent age. Magn. Reson. Med. 60, 761–767.

Samuelsen, G.B., Pakkenberg, B., Bogdanovic, N., Gundersen, H.J.G., Larsen, J.F., Græm, Laursen, H., 2007. Severe cell reduction in the future brain cortex in human growth-restricted fetuses and infants. Am. J. Obstet. Gynecol. 197, 56e1-56e7.

Sandu, A.L., Rasmussen, I.A., Lundervold, A., Kreuder, F., Neckelmann, G., Hugdahl, K., Specht, K., 2008. Fractal dimension analysis of MR images reveals grey matter structure irregularities in schizophrenia. Comput. Med. Imaging Graph. 32, 150–158.

Sizonenko, S.V., Borradori-Tolsa, C., Vauthay, D.M., Lodygensky, G., Lazeyras, F., Hüppi, P.S., 2006. Impact of intrauterine growth restriction and glucocorticoids on brain development: insights using advanced magnetic resonance imaging. Mol. Cell. Endocrinol. 254–255, 163–171.

Soria-Pastor, S., Padilla, N., Zubiaurre-Elorza, L., Ibarretxe-Bilbao, N., Botet, F., Costas-Morangas, C., Falcón, C., Bargalló, N., Mercader, J.M., Junqué, C., 2009. Decreased regional brain volume and cognitive impairment in preterm children at low risk. Pediatrics 124, e1161–1170.

Srinivasan, L., Dutta, R., Counsell, S.J., Allsop, J.M., Boardman, J.P., Rutherford, M.A., Edwards, A.D., 2007. Quantification of deep gray matter in preterm infants at term-equivalent age using manual volumetry of 3-Tesla magnetic resonance images. Pediatrics 119, 759–765.

Strang-Karlsson, S., Räikkönen, K., Pesonen, A., Kajantie, E., Paavonen, E.J., Lahti, J., Hovi, P., Heinonen, K., Järvenpää, A.L., Eriksson, J.G., Andersson, S., 2008. Very low birth weight and behavioural symptoms of attention deficit hyperactivity disorder in young-adulthood: The Helsinki Study of very-low-birth-weight adults. Am. J. Psychiatry 165, 1345–1353.

Tan, T.Y., Yeo, G.S., 2005. Intrauterine growth restriction. Curr. Opin. Obstet. Gynecol. 17, 135–142.

Tolsa, C.B., Zimine, S., Warfield, S., Freschi, M., Sancho Rossignol, A., Lazeyras, F., Hanquinet, S., Pfizenmaier, M., Huppi, P.S., 2004. Early alteration of structural and functional brain development in premature infants born with intrauterine growth restriction. Pediatr. Res. 56, 132–138.

Van-essen, D.C., 1997. A tension-based theory of morphogenesis and compact wiring in the central nervous system. Nature 385, 313–318.

Volpe, J.J., 2009. Brain injury in preterm infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 8, 110–124.

Wu, Y.T., Shyu, K.K., Jao, C.W., Wang, Z.Y., Soong, B.W., Wu, H.M., Wang, P.S., 2010. Fractal dimension analysis for quantifying cerebellar morphological change of multiple system atrophy of the cerebellar type (MSA-C). Neuroimage 49, 539–551.

Xue, H., Srinivasan, L., Jiang, S., Rutherford, M., Edwards, A.D., Rueckert, D., Hajnal, J.J., 2007. Neuroimage 38, 461–477.

Zhang, L., Liu, J.Z., Dean, D., Sahgal, V., Yue, G.H., 2006. A three-dimensionalfractal analysis method for quantifying white matter structure in human brain. J. Neurosci. Methods 150, 242–253.

Zhang, L., Dean, D., Liu, J.Z., Sahgal, V., Wang, X., Yue, G.H., 2007. Quantifying degeneration of white matter in normal aging using fractal dimension. FEBS Lett. 28, 1543–1555.

Zhang, L., Butler, A.J., Sun, Ch.K., Sahgal, V., Wittenberg, G.F., Yue, G.H., 2008. Fractal dimension assessment of brain white matter structural complexity post stroke in relation to upper-extremity motor function. Brain Res. 1228, 229–240.

Zubiaurre-Elorza, L., Soria-Pastor, S., Junqué, C., Vendrell, P., Padilla, N., Rametti, N., Bargalló, N., Botet, F., 2009. Int. J. Dev. Neurosci. 27, 559–565.