Clinical findings and white matter abnormalities seen on diffusion tensor imaging in adolescents with very low birth weight

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Very low birth weight (VLBW) children are at high risk of perinatal white matter injury, which, when subtle, may not be seen using conventional magnetic resonance imaging. The relationship between clinical findings and fractional anisotropy (FA) measurements in white matter of adolescents born prematurely with VLBW was studied in 34 subjects (age = 15 years, birth weight ≤1500 g) and 47 age-matched controls born at term, who were examined both clinically and with diffusion tensor imaging (DTI). Perceptual and cognitive functions were evaluated by visual motor integration (VMI) with supplementary tests and sub-tests from WISC-III, motor function by movement ABC and Grooved Pegboard test and psychiatric symptoms by the schedule for affective disorders and schizophrenia for school-age children semistructured interview, the Autism Spectrum Screening Questionnaire and attention deficit hyperactivity disorder (ADHD) rating scale IV. Overall functioning was scored on the children’s global assessment scale. DTI scans were performed for calculation of FA maps and areas of significant differences in mean FA values between subjects and controls were compared with their clinical data. The VLBW children had reduced FA values in the internal and external capsule, corpus callosum and superior, middle superior and inferior fasciculus. Within this group of children, visual motor and visual perceptual deficits were associated with low FA values in the external capsule, posterior part of the internal capsule and in the inferior fasciculus. Children with low IQ had low FA values in the external capsule and inferior and middle superior fasciculus. Fine motor impairment was related to low FA values in the internal and external capsule and superior fasciculus. Eight VLBW children with inattention symptoms or a diagnosis of ADHD had significantly lower FA values in several areas. Mood social deficits correlated with reduced FA values in the external capsule and superior fasciculus. We conclude that DTI was able to detect differences in FA between VLBW adolescents and controls in several white matter areas at risk of periventricular leucomalacia in VLBW newborns. Our results show that low FA values in these areas were associated with perceptual, cognitive, motor and mental health impairments. These conclusions indicate that perinatal injury of white matter tracts persist with clinical significance in adolescence.

Keywords: VLBW; DTI; adolescents; FA maps

Abbreviations: DTI = diffusion tensor imaging; FA = fractional anisotropy; PVL = periventricular leucomalacia; VLBW = very low birth weight

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Introduction

Very low birth weight (VLBW) children are at substantially increased risk of perinatal brain damage due to haemorrhages and periventricular leucomalacia (PVL) (Volpe, 1997, 2001). The injury may affect both white
DTI and clinical characteristics in VLBW adolescents

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matter tissue and cortical grey matter (Inder et al., 1999; Ajayi-Obe et al., 2000; Woodward et al., 2006). Neuroimarnings and disabilities concerning motor, perceptual and cognitive functioning tend to evolve during childhood and persist into adolescence (Stewart et al., 1999; Rickards et al., 2001; Hack et al., 2002). Numerous articles report abnormal qualitative and quantitative cerebral MR findings that relate to clinical findings in these high-risk children (Skranes et al., 1993; Olsen et al., 1998; Peterson, 2003; Inder et al., 2005). Diffusion tensor imaging (DTI) is a rather novel magnetic resonance imaging (MRI) technique that provides information about white matter microstructure in vivo (Hüppi et al., 1998). The myelin sheath and cell membrane restrict the diffusion of water perpendicular to the direction of the axons in white matter, whereas water diffuses relatively freely parallel to the axons. This directional dependence of diffusion is often quantified as anisotropy, which reflects both size and number of myelinated axons and the coherence of axonal orientation (Neill et al., 1998). Several reports have shown that diffusion anisotropy correlates with normal white matter maturation and with perinatal white matter injury (Hüppi et al., 2001; Mukherjee et al., 2002; Als et al., 2004). Reduced diffusion anisotropy measured as reduced fractional anisotropy (FA) in damaged white matter may be due to a disruption or disorganization of tracts (Werring et al., 2000). Few studies have looked at DTI findings in children beyond the newborn period (Klingberg et al., 1999; Filippi et al., 2003; Nagy et al., 2003). To our knowledge none have looked at DTI findings in low birth weight adolescents and compared FA values with the results of multidisciplinary clinical testing.

Previously we have published regional differences in white matter microstructure evaluated by DTI FA between VLBW children at 15 years of age compared with age-matched controls using voxel-wise statistical analysis (Vangberg et al., 2006). Papers describing cortical thickness differences and conventional MRI findings in the study groups have also been published (Martinussen et al., 2005; Skranes et al., 2005). We found that the VLBW teenagers had higher prevalence of several cortical and white matter abnormalities compared with controls. Among the VLBW adolescents about 80% had dilatation of the lateral ventricles, especially of the posterior horns; while periventricular white matter reduction and corpus callosum (CC) thinning was seen in about half of the children.

The aim of this study was to examine whether there was a relationship between individual FA values in the anatomical areas where the VLBW and the controls differed and the results of extensive perceptual, cognitive and motor assessments. We hypothesized that reduced FA values in specific areas of white matter were correlated with abnormal test results in the VLBW group, and that the location was dependent on the specific test task. We further wanted to examine whether there were more extensive white matter differences between groups seen on DTI than on conventional MRI.

Material and methods

Study design

This study is part of a follow-up study of two groups of adolescents with low birth weight, preterm VLBW and term small for gestational age (SGA), compared with a control group of normal birth weight examined at 15 years of age. The findings in the SGA group will be described in a separate paper.

The VLBW children were admitted to the Department of Neonatal Intensive Care at the University Hospital in Trondheim (the referral hospital) in 1986–88. VLBW children born in 1988 were assessed thoroughly at 1 and 6 years of age (Skranes et al., 1992, 1993, 1997). The control children were born to mothers living in the Trondheim region. They were enrolled before week 20 of pregnancy in a multicentre study between January 1986 and March 1988. A 10% random sample of women (para 1 and 2) was selected for follow-up during pregnancy. At birth, all the children born to mothers in the random sample were included for follow-up. The present study was carried out between November 2000 and November 2003.

Study population

VLBW was defined by a birth weight ≤1500 g. In 1986–88, 121 children were admitted to the NICU at the University Hospital in Trondheim. Of these 33 died, one child with trisomy 21 was excluded and six had moved. Of the remaining 81, 55 (68%) agreed to participate in follow-up and were assessed with cerebral MRI. Owing to unsuccessful sequence acquisition or image artefacts, 21 studies had to be excluded. The main reason for excluding the data was not motion artefacts, but dental braces, which are not uncommon at this age. The braces create large signal dropouts and distortions in the DTI images. For the VLBW group 11 subjects were excluded because of braces, 6 because of missing DTI, 4 for other reasons such as motion, impartial scans or incorrect slice position, resulting in 34 studies suitable for DTI analysis (16 males and 18 females). The control group comprised 120 term-born children with a birth weight ≥10th percentile for gestational age, born to mothers in the 10% random sample. At follow-up 10 had moved and 27 did not consent to participate. Of the remaining 83, 65 underwent MRI scanning. Eighteen MRI scans had to be excluded (3 because of dental braces, 13 because of missing imaging sequences and 2 for other reasons), leaving 47 MRI investigations suitable for DTI analysis (18 males and 29 females). There were no differences in birth weight, gestational age, birth head circumference, anthropometrics at examination, MRI assessment age, mother’s education and social class between the VLBW adolescents with and without DTI performed. Some clinical characteristics of the children in the two study groups are summarized in Table 1.

Non-participants

There were no differences in mothers’ age at childbirth, duration of pregnancy, social class or the infant’s birth weight, body length and head circumference between those who participated in the follow-up study and those who did not consent to participation in any of the groups.
Table I Child characteristics in the study groups

<table>
<thead>
<tr>
<th></th>
<th>VLBW</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g) (SD)</td>
<td>1218 (229)</td>
<td>3670* (439)</td>
</tr>
<tr>
<td>Gestational age (weeks) (SD)</td>
<td>29.3 (2.7)</td>
<td>39.5* (1.1)</td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>29</td>
</tr>
<tr>
<td>MRA assessment age (years)</td>
<td>15.2 (0.7)</td>
<td>15.5 (0.5)</td>
</tr>
</tbody>
</table>

*P < 0.05 controls versus VLBW. VLBW: very low birth weight, SD: standard deviation. Mann–Whitney U-test is used for non-parametric data.

Methods

Clinical tests

All the clinical tests were administered by professionals that were blinded to the neonatal histories and group adherence.

The Developmental Test of Visual–Motor Integration. The Developmental Test of Visual–Motor Integration (VMI–IV) comprises 27 geometric designs in increasing order of difficulty that have to be copied. No time limit was imposed. Visual perception and motor coordination are supplementary tasks requiring the subject to identify depictions of the designs that he/she has copied earlier and to trace the same designs with a pencil without leaving double-lined paths in which the designs were presented. Time limits to complete the tasks are 3 and 5 min. Scores were given according to the manual (Beery, 1997) and raw scores were used. Poor performance was defined as 1 SD below the mean of the control group.

WISC-III. An estimate of the adolescents’ intelligence quotient (IQest) was calculated using four subtests of WISC-III: Arithmetic, Vocabulary, Block design and Picture arrangement (Spreen and Strauss, 1998; Wechsler, 1999). Low IQest was defined as IQest below 2 SD of the mean of the controls. Block design is regarded as a measure of visual spatial organization, while picture arrangement is a measure of more complex perceptual organization. Vocabulary measures language comprehension and general mental ability, and the arithmetic subtest reflects mathematics, but also freedom from distractibility, and includes a verbal factor and working memory function (Lezak, 1995).

The Grooved Pegboard test. The Grooved Pegboard (GP) test (Kleve and Matthews) requires manual dexterity. The adolescent is instructed to insert, successively and as quickly as possible, 25 pegs in the keyhole-shaped holes, ordered in a 5 × 5 matrix and pointing in different directions. The task was performed with the writing hand and thereafter with the non-writing hand. The scores were the times (s) used to complete each task. Adolescents who did not manage to finish the task were assigned a score 3 SD above the mean of the controls. Poor performance was defined as any value 1 SD or more above (i.e. longer than) the mean time of the controls.

Movement ABC test. The Movement Assessment Battery for Children (Movement ABC) (Henderson and Sugden, 1992) was used for motor evaluation. The Movement ABC consists of eight items grouped as three subscores: manual dexterity, ball skills and static/dynamic balance. Each item is scored from 0 (optimal score) to 5 (lowest performance). The highest age band, designed for 11–12-year-old children, was used. According to the manual scores below the fifth percentile is indicative of definite motor problems, and scores between the fifth and the 15th percentile identify children at risk of having motor problems. As the study population was examined at age 14, we used the percentiles derived from the control group. For this study, scores below the fifth percentile were described as motor impairment and scores below the 15th percentile as motor problems.

Psychiatric assessment. Psychiatric disorders and symptoms were diagnosed by using the Schedule for Affective Disorders and Schizophrenia for School-age Children (KSADS) (Kaufman et al., 1997). This semistructured interview was performed separately with the parent and the adolescent. Based on the result of the interview diagnosis (DSM-IV) and symptoms ≥75% level of diagnostic criteria was recorded. In addition, the Autism Spectrum Screening Questionnaire (ASSQ) (Ehlers et al., 1999) was scored during the interview, and Attention Deficit/Hyperactivity Disorder Rating Scale IV (ADHD Rating Scale IV) was reported by mothers (Barkley and Murphy, 1998). Overall mental health functioning among the adolescents was scored on the Children’s Global Assessment Scale (CGAS). This scale ranges from 1 to 100; a score above 80 denotes good functioning (Shaffer et al., 1985).

Diffusion tensor imaging

The scanning was performed on a 1.5 tesla Siemens Magnetom Symphony system with quantum gradients (30 mT/m) and a quadrature head coil. The protocol consisted of high resolution anatomical images acquired with a T1-weighted sagittal 3D MPRAGE sequence (TR 7.1 ms, TE 3.45 ms, TI 1000 ms, and flip angle 7°, FOV 256 mm × 256 mm and slab thickness 170 mm). The acquisition matrix was 256 × 192 × 128, reconstructed to 256 × 256 × 128, giving a reconstructed voxel resolution of 1.0 mm × 1.0 mm × 1.33 mm. The DTI sequence was a single-shot balanced echo EPI sequence with timing parameters of TR 6000 ms and TE 97 ms (Reese et al., 2003). The 20 contiguous transverse slices with a slice thickness of 5 mm were aligned parallel to the anterior commissure and posterior commissure plane and covered all but the topmost part of the brain. The FOV was 228 mm × 228 mm, acquisition matrix 96 × 128, reconstructed to 128 × 128, giving a reconstructed in-plane resolution of 1.78 mm × 1.78 mm. For each slice, one image without diffusion weighting (b = 0 s/mm²), and six images with diffusion gradients (b = 1000 s/mm²) applied along six non-collinear directions were acquired. The six DTI acquisitions for each subject were registered using a mutual information cost function and a 12 parameter affine transformation, with the first b = 0 s/mm² volume as reference. The FLIRT program, part of the FSL library from the Image Analysis Group, FMRIB, Oxford, UK, was used for the image registration. After registration, the six acquisitions were averaged, the diffusion tensor diagonalized, and FA maps were calculated from the eigenvalues. The DTI images underwent motion correction by examining the data visually for motion artefacts and by searching for large values in the motion correction output, before calculating the diffusion tensor. The SPM2 package was used for the postprocessing and statistical analysis (Wellcome Department of Imaging Neuroscience, University College London, UK, http://www.fil.ion.ucl.ac.uk/spm).

To allow for voxel-wise statistical analysis, the images for each subject had to be transformed to a standard space by normalizing the images to a common template. We therefore created a custom template based on the anatomical images from all the 123 subjects.
in the study in a similar manner to the standard SPM2 software templates. A paper containing a more detailed specification of the image acquisition, processing and calculation procedures has already been published (Vangberg et al., 2006).

Statistics
Group comparisons of the FA maps were performed with SPM2, using an absolute threshold of 0.15 for the FA values. Restricting the analysis to only include voxels with a FA value greater than 0.15, essentially confined the data to white matter only. Differences in anisotropy between groups (VLBW and controls) were assessed by analysis of covariance (ANCOVA) with the subject's gender as a covariate. SPSS 13.0 for Windows was used for data analysis of the clinical data. Mean FA values from each of the significant cluster areas where the VLBW children had lower FA values than controls, were then extracted from each VLBW adolescent and compared with the results of the clinical evaluations using the Mann–Whitney test and by non-parametric correlations using Spearman’s ρ.

Ethics
The Regional Committee for Medical Research Ethics approved the study protocol. Written informed consent was obtained from adolescents and parents.

Results
Clinical results
Clinical results from the whole follow-up study population have been reported in several papers (Evensen et al., 2004; Indredavik et al., 2004, 2005; Kulseng et al., 2006). The clinical outcome variables in the VLBW adolescents investigated with and without DTI and in controls are summarized in Table 2. The VLBW adolescents had significantly poorer scores on most of the clinical tests compared with controls. There were no major differences in performance between those with and without DTI in the VLBW group except for the motor coordination test and the ADHD-R hyperactivity score where those without DTI had more adverse scores. In the DTI VLBW group, eight (24%) had ADHD symptoms or diagnosis and seven (21%) had anxiety symptoms or diagnosis based on the KSADS interview. Four (12%) of the VLBW adolescents and none of the controls had cerebral palsy (CP) in forms of walking spastic diplegia. In the non-DTI VLBW group four had ADHD symptoms or diagnosis, three had anxiety symptoms or diagnosis based on the KSADS interview, and two had CP, one spastic diplegia and one hemiplegia. None had any major visual or hearing impairment in any of the groups.

Regions of significant difference in fractional anisotropy between the groups
Group comparison between the VLBW group and controls defined thirteen white matter areas (clusters ≥100 voxels) in seven different anatomical regions where the VLBW group had significantly lower FA values than the control group. The anatomic location and size of these clusters with average FA values for VLBW and controls are shown in Table 3. The areas were in the internal capsule (anterior and posterior part, left and right side), in the external capsule (left and right side), in corpus callosum (CC) (anterior and posterior part), in inferior fascicles (left and right side), in superior fascicles (left and right side) and in middle superior fascicle on the left side (Figs 1–5). There were no cluster areas where the VLBW children had higher FA values than controls. No gender differences in FA values in any anatomical region were found in the VLBW group and in controls. Table 4 shows the differences in FA values in VLBW adolescents with and without CP. The CP children had lower FA values in the anterior part of CC, and in the inferior and superior fascicles left side and the middle superior fascicle left side. There were no areas where
those with CP had higher FA values than the healthy VLBW adolescents.

**Regions of significant difference in FA values and perceptual function**

Anatomical areas where FA values were related with perceptual test scores in the VLBW children are shown in Tables 5 and 6. The VLBW children with scores below −1 SD from the mean score in the control group on the VMI test had significantly lower FA values in external
capsule both sides and in the region of inferior fascicle left side, compared with those with normal VMI scores. Excluding the adolescents with CP did not change these results. On the visual perception test, the VLBW adolescents with low scores had lower FA values in external capsules and in inferior, superior and middle superior fascicles. When the four VLBW adolescents with CP were excluded, lower FA values were found only in external capsule left side of the abovementioned areas. In addition, FA values were lower in the posterior part of the internal capsule on the left side (Table 5). The thirteen VLBW adolescents with low performance on the motor coordination test had lower FA values in superior fascicle right side and middle fascicle left side.

**Regions of significant difference in FA values and cognitive function**

Table 7 shows the relationship between estimated IQ scores and FA values in the VLBW group; the results are similar when CP was included or excluded. The VLBW adolescents with estimated low IQ had lower FA values in external capsule on the left side, in both inferior fascicles and in middle superior fascicle on the left side compared with those with normal estimated IQ.

<table>
<thead>
<tr>
<th>Table 4 Differences in mean FA values in anatomical regions in VLBW adolescents with and without CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical region</td>
</tr>
<tr>
<td>Ant caps int left side</td>
</tr>
<tr>
<td>Ant caps int right side</td>
</tr>
<tr>
<td>Post caps int left side</td>
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<tr>
<td>Post caps int right side</td>
</tr>
<tr>
<td>Caps ext left side</td>
</tr>
<tr>
<td>Caps ext right side</td>
</tr>
<tr>
<td>Corp call post</td>
</tr>
<tr>
<td>Corp call ant</td>
</tr>
<tr>
<td>Inf fasc left side</td>
</tr>
<tr>
<td>Inf fasc right side</td>
</tr>
<tr>
<td>Sup fasc left side</td>
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<tr>
<td>Sup fasc right side</td>
</tr>
<tr>
<td>Middle fasc left side</td>
</tr>
</tbody>
</table>

**P < 0.01, *P < 0.05 (non-CP versus CP) (Mann–Whitney U-test).**

FA: fractional anisotropy; VLBW: very low birth weight; Ant caps int: internal capsule anterior part; Post: posterior; Caps ext: external capsule; Corp call: corpus callosum; Inf fasc: inferior fasciculus; Sup: superior.

<table>
<thead>
<tr>
<th>Table 5 Significant differences in mean FA values in anatomical regions and perceptual function in VLBW adolescents (CP included and non CP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical region</td>
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<tr>
<td>Post caps int left side</td>
</tr>
<tr>
<td>Caps ext left side</td>
</tr>
<tr>
<td>Caps ext right side</td>
</tr>
<tr>
<td>Inf fasc left side</td>
</tr>
<tr>
<td>Sup fasc right side</td>
</tr>
<tr>
<td>Middle fasc left side</td>
</tr>
</tbody>
</table>

**P < 0.01, *P < 0.05 (normal performers versus low performers) (Mann–Whitney U-test).**

FA: fractional anisotropy; VLBW: very low birth weight; VMI: Visual Motor Integration test; Post caps int: posterior part of internal capsule; Caps ext: external capsule; Inf fasc: inferior fasciculus; Sup: superior.

Correlations by Spearman’s rho.

<table>
<thead>
<tr>
<th>Table 6 Correlations between perceptual scores and mean FA values in different anatomical regions in VLBW adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical region</td>
</tr>
<tr>
<td>Post caps int left side</td>
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<tr>
<td>Post caps int right side</td>
</tr>
<tr>
<td>Caps ext left side</td>
</tr>
<tr>
<td>Caps ext right side</td>
</tr>
<tr>
<td>Sup fasc right side</td>
</tr>
<tr>
<td>Middle fasc left side</td>
</tr>
</tbody>
</table>

**P < 0.01, *P < 0.05.** FA: fractional anisotropy; VLBW: very low birth weight; VMI: Visual Motor Integration test; Post caps int: posterior part of internal capsule; Caps ext: external capsule; Sup fasc: superior fasciculus; n.s.: non-significant correlation.
There was a correlation between estimated performance IQ scores and FA values in internal capsule posterior part on the right side \((r = 0.372, P < 0.05)\) and between estimated verbal IQ and FA values in right superior longitudinal fascicle in the VLBW group \((r = 0.363, P < 0.05)\). Looking at the four IQ subtest scores in the VLBW group, only the arithmetic and the block design scores correlated with FA values. The arithmetic score was correlated with FA values in right superior fascicle \((r = 0.526, P < 0.01, \text{Fig. 7})\), the left middle fascicle \((r = 0.447, P < 0.01)\) and the left inferior fascicle \((r = 0.360, P < 0.05)\). The block design score correlated with FA values in right internal capsule anterior \((r = 0.386, P < 0.05)\) and posterior part \((r = 0.408, P < 0.05)\).

### Regions of significant difference in FA values and motor function

Table 8 shows the relationship between FA values and Movement ABC manual dexterity score and Grooved Pegboard score for the VLBW group. On the Movement ABC test only three VLBW adolescents had a manual dexterity score below the fifth percentile. They had low FA values in several white matter areas, but not in internal capsule. Looking at scores below the 15th percentile nine VLBW adolescents with motor problems had low FA values only in superior longitudinal fascicle on the left side compared with those with normal scores (data not shown). Subnormal scores on the ball skill and balance subtests were not related with FA values. On correlation analysis we found no correlation between Movement ABC total and subtest scores and FA values.

The ten VLBW adolescents with low performance on the Grooved Pegboard test (writing hand) had lower FA values in posterior part of the internal capsule on the left side, in external capsules and in superior fascicle right side and middle fascicle left side compared with those with normal performance. Looking at the non-CP VLBW adolescents, low performance was related to low FA values in much the same areas, however also in the anterior part of both internal capsules and in the posterior part on the right side. The differences in FA values in external capsule on the right side and middle fascicle left side did not reach statistical significance when the CP adolescents were excluded.

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Manual dexterity score (CP included)</th>
<th>GP score (CP included)</th>
<th>GP score (non-CP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; fifth percentile</td>
<td>≥ fifth percentile</td>
<td>&lt; −1 SD*</td>
</tr>
<tr>
<td>Ant caps int left side</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ant caps int right side</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Post caps int left side</td>
<td>n.s.</td>
<td>0.4357</td>
<td>0.4681*</td>
</tr>
<tr>
<td>Post caps int right side</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Caps ext left side</td>
<td>0.2822</td>
<td>0.3274*</td>
<td>0.3035</td>
</tr>
<tr>
<td>Caps ext right side</td>
<td>0.2775</td>
<td>0.3367**</td>
<td>0.3164</td>
</tr>
<tr>
<td>Corp call ant</td>
<td>0.3668</td>
<td>0.4489*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Inf fasc left side</td>
<td>0.3328</td>
<td>0.3759*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Inf fasc right side</td>
<td>0.3238</td>
<td>0.3613*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sup fasc right side</td>
<td>0.3108</td>
<td>0.3564*</td>
<td>0.3308</td>
</tr>
<tr>
<td>Middle fasc left side</td>
<td>0.3171</td>
<td>0.3739*</td>
<td>0.3423</td>
</tr>
</tbody>
</table>

*P ≤ 0.01; **P ≤ 0.05 (normal performers versus low performers) (Mann–Whitney U-test). FA: fractional anisotropy; M-ABC: Movement ABC; VLBW: very low birth weight; CP: cerebral palsy; GP: Grooved Pegboard; SD: standard deviation; Ant caps int: internal capsule anterior part; Post: posterior; Caps ext: external capsule; Corp call: corpus callosum; Inf fasc: inferior fasciculus; Sup: superior; n.s.: not significant. *Scores higher or lower than −1 SD from the mean score on Grooved Pegboard (writing hand) in the control group.
Regions of significant difference in FA values and mental health parameters

Anatomical regions where FA values are related to scores for overall functioning as measured by the CGAS, and with ADHD diagnosis and symptoms (≥75% level of diagnostic criteria) based on KSADS in the VLBW adolescents are shown in Table 9. CGAS scores below or equal to 80 were related with low FA values in six different anatomical areas, with strongest associations to external capsule, inferior and middle fascicles on the left side (P < 0.01). Anxiety or other psychiatric diagnoses and symptoms were not related with low FA values in any area. Table 10 shows the significant correlations between FA values and the CGAS, ASSQ and ADHD-R inattention scores. High ASSQ scores were correlated with low FA values in external capsule and superior fascicle on the left side, while the CGAS score correlated with FA values in several areas. The inattention score correlated with FA values in external capsule left side and in right superior and left middle fascicles. The ADHD-R hyperactivity and total score did not correlate with FA values.

Table 9 Mean FA values in different anatomical regions compared with overall mental health functioning (CGAS) and ADHD1 in 34 VLBW adolescents

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>CGAS ≤80 (20)</th>
<th>Above 80 (14)</th>
<th>P</th>
<th>ADHD1 Yes (8)</th>
<th>No (26)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ant caps int right side</td>
<td>0.3669</td>
<td>0.3863</td>
<td>0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post caps int left side</td>
<td>0.4456</td>
<td>0.4756</td>
<td>0.008</td>
<td>0.4295</td>
<td>0.4667</td>
<td>0.013</td>
</tr>
<tr>
<td>Post caps int right side</td>
<td>0.4340</td>
<td>0.4604</td>
<td>0.047</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caps ext left side</td>
<td>0.3129</td>
<td>0.3372</td>
<td>0.006</td>
<td>0.3013</td>
<td>0.3296</td>
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FA: fractional anisotropy; CGAS: Children’s Global Assessment Score; ADHD: attention deficit hyperactivity disorder; VLBW: very low birth weight; Ant caps int: internal capsule anterior part; Post: posterior; Caps ext: external capsule; Corp call: corpus callosum; Inf fasc: inferior fasciculus; Sup: superior . (Mann–Whitney U-test).1ADHD diagnosis (DSM-IV) or symptoms (≥75% level of diagnostic criteria) based on KSADS (Schedule for Affective Disorders and Schizophrenia for School-age Children).

Discussion

We found that low FA values in specific white matter areas; i.e. areas where VLBW children had lower mean FA values than controls, were related with perceptual, cognitive, motor and mental health impairments among the VLBW children. Impairments in more complex functioning like visual motor integration, arithmetic and attention seemed to be related to more extensive disturbance in white matter microstructure based on reduced FA values, and included areas that looked normal on conventional MRI. To our knowledge this is the first study that reports a relationship between clinical findings and regional differences in FA in white matter areas in adolescents with VLBW.

In this study we have used a voxel-wise statistical analysis for comparing the FA values in the whole brain between VLBW and control adolescents, and not only specific areas as in region-of-interest-based methods. However, this approach may create confounders caused by inaccuracies in the spatial normalization process. This is especially important for the VLBW group where MRI showed structural abnormalities in
forms of posterior CC thinning, occipital horn dilation and reduced periventricular occipital and parietal white matter tissue in a large percentage of the adolescents. We have tried to reduce this risk as much as possible by using a custom template based on the anatomical images from all the subjects in the MRI study and a threshold on the FA values (FA > 0.15) that confines the analysis to white matter. We have reported that although differences in white matter density can confound the reduction in FA values, this accounts for only 30% of the areas with low FA values in the VLBW group (Vangberg et al., 2006). By analysis of the eigenvalue maps we found that an increase of the two lowest eigenvalues was the main cause of reduced FA in the VLBW group. This increased radial diffusivity perpendicular to the principal fibre tract direction can be explained by myelin disturbances and reduced axonal density (Song et al., 2005). Both conditions can be seen as results of perinatal brain injury in prematurely born children (Damman et al., 2001). In a recent study Counsell et al. (2006) found elevated radial and axial diffusivity in several white matter areas in premature infants at term-equivalent age suggesting widespread white matter oligodendrocyte and/or axonal abnormalities.

In our study the voxels are obviously anisotropic (1.75 × 1.75 × 5 mm³), which makes the data more susceptible to partial volume effects than data with isotropic voxels. This may at least theoretically affect the final results. However, we are not aware of any study that has looked at the effect of anisotropic voxels. We have not investigated the effect of using an affine only normalization, which would be a considerable undertaking since all FA maps must be re-normalized. It is a relevant question though.

We chose to use a proven non-linear normalization procedure (affine + non-linear) in our study, since we felt that this approach would better correct for local differences such as enlarged ventricles in our study population. Results from other studies indicate that non-linear normalization procedures give generally better results compared to an affine only normalization (Crivello et al., 2002; Robbins et al., 2004).

Very few studies have looked at FA values in prematurely born children beyond the newborn period. Nagy et al. (2003) report lower FA values in the internal capsule and the posterior CC in VLBW children with attention deficits at 11 years of age. They concluded that the functional implications of this deviation in white matter microstructure is unknown and recommend further study to see if it affects neurological functions. Although the relationship between FA values and white matter microstructure is complex and the fact that FA value depends on nerve fibre density, water content, myelination and fibre coherence, several studies have found a relationship between low FA values at term in premature children, perinatal brain injury and later neurological abnormalities (Hüppi et al., 2001; Arzoumanian et al., 2003). We speculate that the low FA values in specific white matter areas in the VLBW adolescents in our study may be caused by perinatal white matter damage that has long term effects on white matter microstructure and connectivity. This may lead to myelin disturbances, disorganization or reduced amount of axons in projectional, commissural and association tracts with poorer connectivity and impairments in abilities that demand cooperation between different brain areas.

**Anatomical implications**

**Occipital white matter**

Occipital white matter is a known predilection site for PVL in VLBW newborns (Volpe, 1997). In our study some of the anatomical areas had low FA values; the occipital part of the inferior and middle superior fascicules belong to this area (Figs 1 and 5). We have already reported the conventional MRI findings in the study group, and we found that dilatation of the occipital horns of the lateral ventricles, and occipital periventricular white matter reduction were common findings in the VLBW group, indicating minor perinatal PVL in this area (Skranes et al., 2005). On DTI the areas of low FA values exceed what is seen as abnormalities on conventional MRI indicating that DTI is a more sensitive method for detecting white matter abnormalities.

**Internal capsule**

The internal capsule contains thalamocortical, corticothalamic and other cortical projection fibres where the anterior limb has connections between the thalamus and prefrontal cortex, and the posterior limb (PLIC) in addition to corticospinal and corticobulbar fibres contains connections between thalamus and motor, somatosensory, and other parietal cortex (Mori et al., 2005). In our study the internal capsule looked normal on conventional MRI in the VLBW adolescents while DTI revealed large clusters of voxels with low FA values in this region, especially in the PLIC (Fig. 1). Several papers have reported PLIC as a predilection site for neuronal damage in hypoxic–ischaemic encephalopathy, also in premature neonates (Rutherford et al., 1998; de Vries et al., 1999).

**Corpus callosum**

On conventional MRI we found that nearly half of the VLBW children had thinning of the posterior part of the CC while the anterior part looked normal. On DTI the abnormalities in white matter microstructure also involved the anterior and middle part of the CC suggesting a more widespread affection than seen on conventional MRI (Fig. 3). Rademaker et al. (2004) found that larger CC size, especially of the posterior part, was associated with better motor performance in VLBW children at 8 years of age. In our study low FA values in CC, as seen in CP and ADHD, were less related to clinical findings than the other areas investigated. Table 3 shows that the clusters defined as CC anterior and posterior part were large compared with the other clusters. It is very difficult based on DTI to limit...
the lateral and dorsal extension of the CC from massive projection fibres (corona radiata) and long association fibres (superior fasciculus, cingulum) located near the CC (Mori et al., 2005). This may have influenced our results when comparing with clinical data.

**External capsule**

The external capsule is a thin layer of white matter lateral to the lentiform nucleus. It contains association fibres such as the superior longitudinal, the inferior fronto-occipital fasciculus and the arcuate fasciculus, the latter connecting the temporal and frontal lobe (Mori et al., 2005). No pathology was noted in this area on conventional MRI while DTI revealed areas in external capsule with low FA values in the VLBW group (Fig. 2).

**Superior and middle longitudinal fascicles**

On DTI reduced FA values in peripheral white matter were detected in areas that looked normal on conventional MRI. These areas were located in the parietal, temporal and frontal lobes and had a more diffuse widespread appearance in the left hemisphere than in the right hemisphere where the clusters were more focal in nature (Fig. 4). The areas affected seem to include the superior longitudinal fasciculus that contains long association fibres connecting all the lobes and the middle superior longitudinal or the superior fronto-occipital fasciculus that connects the occipital and the most frontal regions of the brain (Stuss and Knight, 2002). Low FA values in these tracts may indicate fewer fibre bundles and disturbed fibre organization that cause reduced functioning, for instance of visual spatial processing. Hüppi et al. (2001) have reported fewer fibre bundles in superior longitudinal fasciculus in preterm infants with perinatal brain damage.

**Inferior fascicles**

Inferior fasciculus includes the inferior longitudinal fasciculus, which connects the extrastriatal visual region with the temporal lobe, and the inferior fronto-occipital fasciculus, which originates in the occipitoparietal region. On conventional MRI the VLBW adolescents had reduced white matter in the occipital periventricular area. However, the DTI findings indicated more extensive areas also in the parietal and temporal lobe including the inferior fasciculus with low FA values in the VLBW group (Fig. 5).

**Clinical implications**

Figure 6 gives an overview of the different neuroimpairments and the corresponding strongest related anatomical areas with reduced FA values in the VLBW group.

**Perceptual function**

VLBW adolescents with low scores on the VMI test, indicating problems with visual motor integration, had low FA values in external capsule and inferior fasciculus. This may indicate affection of association tracts containing secondary visual fibres with information for processing in temporal and frontal areas. Disorganization of fibres containing visual information in occipitofrontal tracts may also explain the relationship between low scores on the visual perception test and low FA values in external capsule and the long association tracts. Motor coordination problems also correlated with low FA values in these long association tracts which may indicate compromised connections between visual cortical centres and secondary motor areas.

**Cognitive function**

In the VLBW group low performers on the WISC-III test had low FA values in several white matter areas, all areas containing long association fibres. This is in agreement with a recent report from Schmithorst et al. (2005) who found positive correlations of IQ scores with FA values in white matter association areas, including frontal and occipitoparietal areas in 47 normal children aged 5–18. Charlton et al. (2006) reported that low FA values indicating white matter damage correlated with cognitive decline of working memory function independently of age in 106 healthy middle-aged and elderly adults.

Among VLBW adolescents the arithmetic subtest score was correlated with FA values in superior fasciculus on the right side and middle superior fasciculus on the left side (Fig. 7). Functional studies have shown increased activity in the left hemisphere and in the right frontal lobe during this task performance (Chase et al., 1984). This subtest is a complex measurement that depends on intact memory and verbal and perceptual organization processes. Low scores on the arithmetic test, which is a common finding in cognitive testing of prematurely born children, and learning problems especially related to mathematics, may therefore be caused by a combination of memory and concentration problems and reduced arithmetic skills (Lezak, 1995). We have already reported that the VLBW adolescents in the main follow-up study are at risk of disadvantage in aspects of
Correlation between arithmetic raw score and FA values in Superior long. fasciculus in VLBW adolescents.

**Fig. 7** Scatter diagram that shows the correlation between arithmetic raw score (WISC-III) and fractional anisotropy values in right superior longitudinal fasciculus for the VLBW adolescents.

Attention and/or executive function compared with controls and that they have increased incidence of attention deficit symptoms (Indredavik et al., 2004, 2005; Kulseng et al., 2006). In this study high inattention score based on mother’s report on ADHD Rating Scale correlated with low FA values in right superior and left middle fasciculus, which are exactly the same areas as for the arithmetic subtest.

**Motor function**

Impairments in fine motor function evaluated with the Movement ABC manual dexterity score and the Grooved Pegboard test were related to low FA values in internal capsule including the PLIC, in external capsule and in superior fasciculus. Low FA values in PLIC may reflect reduced amount of fibre tracts and disturbed myelination and connectivity of motor projectional fibres causing motor problems. This is in agreement with other studies which report an important role of PLIC in motor function (Wenzelburger et al., 2005). Abnormalities in PLIC seen on MRI in neonates are an early marker of brain damage, especially causing motor handicaps (Rutherford et al., 1998; de Vries et al., 1999). We speculate that more subtle changes in PLIC not seen on conventional MRI, but on DTI, may cause minor fine motor impairments. The relationship between low scores on the Grooved Pegboard test and reduced FA values in external capsule and superior fasciculus may reflect the fact that this test in addition to manual dexterity depends on speed and the ability of spatial orientation, and the result may indicate disturbed microstructure of long association tracts. There was no relationship between FA values and gross motor function based on the Movement ABC Ball skills and Balance scores in the VLBW group. This was unexpected, but may be due to the fact that the VLBW children that had DTI performed did not have significantly lower scores on these tests than the controls (Table 2). Those with CP had lower FA values than the non-CP VLBW adolescents only in a few areas, including anterior CC and the long fascicles, which are areas that do not contain primary motor fibres, but not in PLIC. However, FA values in PLIC do not necessarily reflect the microstructure of projectional motor fibres since PLIC also contains other types of fibres (corticothalamic and somatosensory tracts) that have other directional orientation (Mori et al., 2005; Wenzelburger et al., 2005).

**Overall and psychiatric functioning**

Low score on the Children’s Global Assessment Scale (CGAS) as a measure of subnormal overall mental health functioning was correlated with low FA values in several white matter areas. Children with ADHD diagnosis or symptoms based on KSADS had lower FA values in most areas compared to those without such symptoms. In addition, high inattention score on the ADHD-R mother report correlated with reduced FA values in some of the same areas, which were external capsule and superior and middle fascicles (Tables 9 and 10). Nagy et al. (2003) investigated with DTI nine VLBW children at 11 years of age selected on the basis of attentional deficits, and found lower FA values in CC and internal capsule. We have already published that about 25% of the VLBW adolescents in the follow-up study had attentional problems without gender differences (Indredavik et al., 2004). We speculate that the relationship between attentional problems and low FA values may indicate disturbed white matter connectivity in rather extensive white matter areas throughout the brain. A higher order cognitive function like attention probably depends on intact communication between several cortical areas through projectional, association and commissural fibres. A higher mean sum score than controls on the autism spectrum screening questionnaire (ASSQ) indicates that the VLBW adolescents had relational problems and deficits in social skills, with difficulties in making adjustments to fit social contexts. High ASSQ score correlated with reduced FA values in external capsule and in superior fasciculus. None has looked at this relationship in VLBW adolescents before, but Barnea-Goraly et al. (2004) used DTI to investigate white matter structure in seven diagnosed with high-functioning autism male children and adolescents with a mean age of 14.6 years. They found reduced FA values in several white matter tracts connecting regions implicated in social cognition and concluded that disruption of these tracts may contribute to the deficits in social information processing that are seen in autism and related disorders. We speculate that VLBW adolescents may exhibit a considerably milder form of this spectrum of social skill deficits that is due to impaired connectivity in long association fibres.
PVL as a cause of changes in white matter microstructure seen on DTI?

Dammann et al. (2001) suggest that white matter damage due to PVL involves both deficits of oligodendroglia, loss of axonal fibres, microgliosis and astroglialosis. DTI seems to be a sensitive method to depict minor structural changes in white matter that are not seen on conventional MRI. This is in agreement with a study from Felderhoff-Mueser et al. (1999) which looked at the relationship between MR imaging and histopathological findings of the brain in preterm infants. MRI was not able to detect subtle histological abnormalities of the diffuse component of periventricular white matter injury that probably is due to moderate perinatal ischaemia. We speculate whether such findings may be seen on DTI as low FA values indicating compromised fibre tract and myelin development in affected areas. There is a dominance of areas with low FA values, both by voxel density and regional findings, in the left hemispheres, and with relation to functional measures. The latter may be explained by deficits in left-sided language functions that affect working memory and thus task performance (Wolke and Meyer, 1999; Fiez, 2001).

Conclusion

DTI was able to detect differences in fractional anisotropy in specific white matter areas between adolescents with very low birth weight and controls. These areas included the occipital periventricular white matter and tringones, which are known predilection sites for PVL in VLBW newborns. In addition, areas such as the internal and external capsule, the CC, and areas corresponding to long association tracts, which may be secondarily affected by PVL due to fibre loss, showed abnormalities in microstructure indicating a distal extension of the white matter injury. Within the VLBW group low FA values in most of these areas were related with perceptual, cognitive, mental health and motor impairments. This may indicate disorganization or mal-development of projectional, commissural and association fibres leading to impairments in abilities that demand cooperation between different brain areas. Compared with conventional MRI, DTI gives additional information about aberrant white matter microstructure that seems to influence brain development and functioning in VLBW adolescents.

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References


