A study of twins born preterm
Functional lateralization, cognition, and brain volumes in twin and single-born children at early school ages

Rebecka Boberg and Sofia Wallström
Abbreviations

APGAR = Appearance, Pulse, Grimace, Activity, Respiration
BPF = Brain Parenchymal Fraction
BW = Birth weight
CSF = Cerebrospinal Fluid
DL = Dichotic Listening Index
DZ = Dizygotic
FSIQ = Full Scale Intelligence Quotient
GA = Gestational Age
GM = Grey Matter
ICV = Intra Cranial Volume
LEA = Right ear advantage
LH = Left handed
MZ = Monozygotic
PRI = Perceptual Reasoning Index
PSI = Processing Speed Index
REA = Right ear advantage
RH = Right handed
SGA = Small for Gestational Age
TBV = Total Brain Volume
VCI = Verbal Comprehension Index
VLBW = Very Low Birth Weight
WMI = Working memory Index
WM = White Matter
A STUDY OF TWINS BORN PRETERM: FUNCTIONAL LATERALIZATION, COGNITION AND BRAIN VOLUMES IN TWIN- AND SINGLE-BORN CHILDREN AT EARLY SCHOOL AGES

Rebecka Boberg & Sofia Wallström

Earlier research has found that preterm birth with low gestational age (GA) and low birth weight (BW) is associated with an increased risk of long-term effects such as atypical lateralization, cognitive deficits and smaller brain volume. Similar consequences have been found in twins. This study compares twins born preterm (n=22, Mean GA=32.1, Mean BW=1781) with GA and BW matched singletons (n=24) and singletons born full term (n=22) on functional laterality, cognition (WISC-IV) and brain volume (SyMRI) at early school ages (M=7.8 years). The result showed that twins had a higher prevalence of left-handedness than both singleton groups. The preterm (PT) singletons show less right ear preference on the Dichotic Listening test than full term (FT) singletons. It was found that the FT-group performed higher than both PT-groups on cognition. Smaller brain volumes were associated with lower performances on WISC-IV in the group of twins. Furthermore it was found that the PT-singletons had smaller Total Brain volume as well as smaller Grey Matter than FT-singletons. No differences were found between the twins and the PT-singletons on intra pair comparisons. Combined with the associations found between GA, BW and cognitive performance and brain volumes the results indicate that low GA and BW are greater risk factors for long-term effects on development than twin-ship per se.

Long term consequences of preterm birth (born before 37 weeks GA) involve general intellectual difficulties (Hoff Esbjørn, Hansen, Greisen & Mortensen, 2006), attention deficits (Månsson, Stjernqvist & Bäckström, 2014), impairments of executive functioning (Aarnoudse-Moens, Smidts, Oosterlaan, Duivenvoorden & Weisglas-Kuperus, 2009), increased Non right hand (NRH) specialization and less well organized movements at 4-8 years (Johansson, Domellöf & Rönnqvist, 2014). Thus, preterm birth is associated with an increased risk of difficulties later on in life, the lower the gestation age (GA) the higher the risk. In Sweden, 42% of multiple pregnancies result in preterm delivery (The National Board of Health and Welfare, 2014). Combined with the tendency of lower birth weight (BW) associated with multiple pregnancies twins are a risk category (Lorenz, 2012). This study aims to compare twins born preterm to singletons matched for GA and BW and additionally to singletons born full term on measures of functional lateralization, cognitive functioning and outcomes of Magnetic Resonance Imaging (MRI).
Preterm birth is strongly correlated with a lower BW (e.g. Stanford Children’s Health, 2015), which is another important predictor of future development. A critical risk factor is a birth weight that in relation to the GA renders the infant to be classified as small for gestational age (SGA). SGA can result in smaller amount of white matter (WM) in brain regions related to attention, language and executive functioning (Tzarouchi et al., 2013), as well as a reduction of total brain volume (TBV) even into early adult age (Østgård et al., 2014). In addition, infants born with a BW less than 1500 g, classified as Very Low BW, have been found to have smaller brain volume (Bjuland, Rimol, Løhaugen & Skranes, 2014). Children born with a BW under 1000 g (Extremely Low BW) have been found to perform lower in cognitive tests (Hoff Esbjørn et al., 2006; van Soelen et al., 2005). Compared to singletons, twins are more frequently born with a low BW, and differences in BW within twin-pairs seems to be an important factor when it comes to cognitive functioning later in life (Lorenz, 2012; Segal, 2012). Twins born discordant in weight (<15-20% difference in BW) are not at any greater risk of cognitive difficulties, whereas the smaller one of twins born discordant (>15-20% difference in BW) are at greater risk of lower Verbal IQ, Performance IQ and Full Scale IQ (Ross, Krauss & Pearlman, 2011). This indicates that the lower BW in the discordant twin is caused by a suboptimal intra-uterine environment that could affect brain development (Ross et al., 2011).

From a recent meta-analyze including 18 studies it was found that children born preterm show a higher frequency (22 %) of NRH (Domellöf, Johansson & Rönqvist, 2011) than the average population, where NRH occurs in approximately 10 % (Ooki, 2014). NRH is also more frequently found in children with different developmental disorders such as Down syndrome and Autism (62%) (Gillberg, 1983). These observations have led to a proposition that NRH could be an indicator of early neurodevelopmental disturbances (Domellöf et al., 2011). In addition left-handedness is shown to be more frequent in twins (13-15 %) than in singletons, possibly caused by prenatal brain damage or intrauterine crowding (Ooki, 2014). When the smaller twin in a pair discordant in BW is left handed, the risk of cognitive impairments increase (Segal, 2012). Contradictorily other research has not found significant differences between left handed and right handed twins regarding verbal fluency or estimated IQ-scores (Gurd, 2013). Left-handedness is also associated with a somewhat higher frequency of atypical right hemisphere language specialization (Perlaki et al., 2013). In accordance, twins discordant for handedness show an increase in discordant language lateralization, i.e. opposite hemisphere language specialization (Ooki, 2014). On the other hand, studies have found a strong genetic influence, since monozygotic twins (MZ) show greater intra-pair similarities than dizygotic (DZ) twins (Morell et al., 2007) on auditory lateralization tests like Dichotic Listening, which can implicate language lateralization (Hugdal, 2002).

Twin studies have found a strong genetic influence not only on auditory lateralization but also on brain structure (Thompson et al., 2001). Further, gray matter (GM) and white matter (WM) volumes have been shown to be heritable (Gilmore, et al., 2010; Peper, et al., 2007; van Leeuwen, et al., 2008). In addition, brain volume is found to correlate with IQ (McDaniel, 2005). Higher TBV, GM and WM have been shown to correlate with higher results on Full Scale IQ (FSIQ), Perceptual Reasoning Index (PRI) and to a lower extent Verbal Comprehension Index (VCI) in children and adolescents (Lange, et al., 2010). This study also found correlations between parental education, family income and the child’s IQ, suggesting both heritability and environment have an
impact on brain development and cognitive functioning. Aside from heritability BW also has influence on brain volumes, for both twins and singletons (Ross et al., 2011). However, only in MZ-twins BW correlated with the cognitive outcomes on Performance IQ and Full Scale IQ.

There are several twin-studies concerning handedness, cerebral asymmetries and cognition (e.g. Ooki, 2014, Gurd, 2013; Lorenz, 2012). In summary, heredity seems to play an important role in lateralization, cognition and brain volumes since twins show greater similarities than singletons and MZ twins show greater intra pair similarities than DZ twins. However the research usually focuses on either pre-term versus full-term or on twins versus singletons, leaving many important factors like preterm birth and low BW unnoticed. There is a need for research considering both preterm birth as well as the specific conditions related to twin-ship (Ooki, 2014). Thus the present study aims to compare twins born preterm to singletons that are matched for GA and BW and additionally to singletons born full term (born between 38 – 42 weeks GA) on outcomes of functional laterality, WISC-IV and brain imaging analysis. Another aim is to investigate possible associations between functional laterality, WISC-IV outcomes and brain imaging analysis outcomes in the two preterm groups and the full term group.

We hypothesized that the group of preterm twin-born children would show significant differences from the group of preterm born singletons (PT-singletons) and full term born singletons (FT-singletons) on outcomes of (a); functional laterality observations (Hand, Foot, and Eye) where twins are expected to show more atypical (NR) lateralization, (b); Dichotic Listening, where twins are expected to show more atypical auditory lateralization, (c); cognitive performance by means of WISC-IV, where twins are expected to show lower performances on all indexes. We also expected both preterm twin- and single-born children to show lower volumes of brain parenchyma on (d); both absolute and relative brain volumes (TBV, WM, GM and Cerebrospinal Fluid) by means of Magnetic Resonance Imaging (MRI) measurements. Further, we expected (e); twin-pairs to show greater intra pair similarities than unrelated singletons on these behavioral and MRI outcomes. Finally, we expected (f); that TBV, WM, and GM are positively associated with result on WISC-IV, functional laterality indices and Dichotic Listening within all the groups.

Method

Participants
A total of 68 children were included in this study. The participants were selected from an ongoing study of long-term effects of preterm birth at the Department of Psychology, Umeå University (PI: Professor L Rönqvist). Participants had to be free of any known developmental or neurological disorders at 7-8 years of age to be included. These criteria resulted in a sample of 11 twin pairs. All 22 twins were born preterm. Due to a lack of time and resources and that the requested background information regarding the twins zygosity from their parents was not fully completed and in one case missing, no diagnosis of zygosity was performed. 22 PT-singletons were selected to match each twin as closely as possible considering GA and BW for intra pair comparisons. Another 2 PT-singletons with MR-data, matched on group level GA and BW, were added for group comparisons. A group of 22 children born full term (FT-singletons) was added to
investigate the effect of prematurity. See Table 1a for more detailed group descriptions. Since this sample is a part of an ongoing study not all participants has completed and analysed MRI data. Only children with completed MRI were included in MRI comparisons, giving a smaller subsample for these analyses. Table 1b includes descriptions of the children from the original group who also participated in the MRI comparisons.

Table 1a. Participants.

<table>
<thead>
<tr>
<th></th>
<th>Twins (n = 22)</th>
<th>PT-singletons (n = 24)</th>
<th>FT-singletons (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD)</td>
<td>Range</td>
<td>M (SD)</td>
</tr>
<tr>
<td>GA</td>
<td>32.1 (3.8)</td>
<td>25.4-34.9</td>
<td>31.8 (3.5)</td>
</tr>
<tr>
<td>BW</td>
<td>1781 (718)</td>
<td>719-2962</td>
<td>1751 (726)</td>
</tr>
<tr>
<td>APGAR 1</td>
<td>7.2 (2.1)*</td>
<td>2-9</td>
<td>7.6 (1.7)</td>
</tr>
<tr>
<td>APGAR 5</td>
<td>8.1 (1.4)*</td>
<td>6-10</td>
<td>8.2 (1.6)</td>
</tr>
<tr>
<td>APGAR 10</td>
<td>8.8 (.8)*</td>
<td>7-10</td>
<td>8.8 (.8)</td>
</tr>
<tr>
<td>Age at testing</td>
<td>7.7 (.5)</td>
<td>7-8.5</td>
<td>7.8 (.8)</td>
</tr>
<tr>
<td>Sex Female (male)</td>
<td>10 (12)</td>
<td></td>
<td>15 (9)</td>
</tr>
</tbody>
</table>

Note: n = number of subjects; M = mean; SD = standard deviation; GA = gestational age; BW = birth weight; * = two subjects missing data.

Table 1b. Participants in the MRI subsample.

<table>
<thead>
<tr>
<th></th>
<th>Twins (n = 10)</th>
<th>PT-singletons (n = 9)</th>
<th>FT-singletons (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD)</td>
<td>Range</td>
<td>M (SD)</td>
</tr>
<tr>
<td>GA</td>
<td>32.9 (2.9)</td>
<td>27.4-34.57</td>
<td>31 (3.6)</td>
</tr>
<tr>
<td>BW</td>
<td>1904 (670)</td>
<td>811.2962</td>
<td>1657 (868)</td>
</tr>
<tr>
<td>Age at MR</td>
<td>8 (.9)</td>
<td>7-9.3</td>
<td>8.2 (1)</td>
</tr>
</tbody>
</table>

Note: n = number of subjects; M = mean; SD = standard deviation; GA = gestational age; BW = birth weight; MR = Magnetic Resonance.

Material

Functional laterality

A laterality index, based on information from hand, foot and eye was evaluated with a modified version of Edinburgh Handedness inventory (Oldfield, 1971) and Coren and Porac’s (1980) laterality questionnaire (Johansson et al., 2014). The instrument consists of different tasks (e.g. kicking a ball, balancing on one foot, writing, using scissors, looking through a kaleidoscope), and the test leader observes which side the child chooses for the different tasks. The instrument includes five tasks for hand, three for foot/leg and one for eye, each repeated five times. The index was calculated (sum of observations (+1 for right side; -1 for left side)/number of series) for each participant, ranging from an extreme right side preference at +1 to an extreme left side preference at -1. Each participant received an index for hand, foot and eye respectively, and one total laterality index based on all observations. For classification of Right- or Non right side preference the cutoff value used was .3, with a value above .3 indicating a right side specialization and a value below a Non right side specialization (Dragovic, 2004). In this
study the index is converted to positive values ranging from 0 for extreme left side preference to 2 for extreme right side preference to better suit statistical analysis. Hence the cut off is set at 1.3 and all values below this cut off indicates for Non right side specialization.

**Dichotic listening**

Dichotic listening (DL) examines functional auditory laterality, which gives an indication of language specialization (Hugdahl, 2002). This study uses the version “Bergen DL-test” (Hugdahl, 2002). Participants are exposed to two similar language based sounds simultaneously, one in each ear. The sounds consist of one short consonant followed by a vowel (e.g. BA and DA). Participants are instructed to repeat the sound that they heard. This test provides information of auditory preference by calculating an index (sum of observations (+1 = right side; -1 = left side)/number of series). Based on the index, participants are classed as having a Right Ear Advantage (REA), Left Ear Advantage (LEA) or no preference (Hugdahl, 2002). REA is associated with a left hemisphere advantage for processing speech like sounds (most common) and LEA a right hemisphere advantage which is deemed atypical. Further, LEA has been associated to brain damage (Hugdahl, 2002). The index was calculated for each participant, ranging from an extreme right side preference at +1 to an extreme left side preference at -1. The index was converted to positive values from 0 to 2 in the same way as for functional laterality. In accordance to the classification described for functional laterality, the cut off value for NR auditory preference is set at 1.3 (Dragovic, 2004).

**WISC-IV**

Cognitive performance was measured with Wechsler Intelligence Scale for Children-Fourth edition (WISC-IV ) a cognitive ability assessment for children from 6-16 years (Wechsler, 2003). The assessment consists of 10 subtests and provides four primary indexes; Verbal Comprehension (VCI), Perceptual Reasoning (PRI), Working Memory (WMI) and Processing Speed (PSI). It also provides a Full Scale Index (FSIQ) that represents the general cognitive ability (Wechsler, 2003). For this study the Swedish version was used (Wechsler, 2007).

**Synthetic MRI**

Brain scans were performed with a 3-Tesla Magnetic Resonance Imaging (MRI). The MRI data was then processed with Synthetic MRI (SyMRI), a semi-automated method of analysis for obtaining milliliter (ml) measures of TBV, GM, WM and cerebrospinal fluid (CSF) as well as pathological tissue (SynteticMR AB, 2015a). The program also estimates the relative volume of TBV, GM, WM, and CSF by calculating their respective percent of intra cranial volume (ICV). Brain Parenchymal Fraction (BPF) is another way to calculate the relative volume. To get the relative percentage of brain parenchymal the CSF volume is excluded and Brain Parenchymal Volume divided by ICV (Vågberg et al., 2012). The software used in this study was SyMRI Brain Studio (SyMRI diagnostics beta 2 release 2). SyMRI has proved to give reliable measures of brain volume in adults (Ambarki et al., 2012).

**Procedure**

The WISC-IV was administered by psychologists with experience of the test and neuropsychological assessments of children. WISC-IV, DL and the assessment of laterality was carried out in a calm undisturbed environment at the Department of
Psychology, Umeå University. Here the participants also had a chance to get accustomed to the MRI-scan, through exposure to a dummy, realistic in both procedure and noise. The actual MRI-scan was performed at Diagnostic Radiology at the Norrland University Hospital.

Statistical analysis
Statistical analyses were performed with IBM SPSS statistics version 22.0. Group differences on behavioral outcomes and brain volumes were analyzed with MANOVA’s. The p-values were corrected in the MANOVA’s by use of Bonferroni correction. The independent variable was Group (Twin, PT-singleton or FT-singleton). The dependent variables were laterality index, DL index, the WISC-IV indexes (VCI, PRI, WMI, PSI), FSIQ and SyMRI outcomes (TBV, WM, GM and CSF in absolute and relative volumes). Intra pair difference for all outcome variables was calculated within each pair. Wilcoxon Signed Ranks test, a mean variance test for non-normal distributed samples was used to analyze mean differences between twin pair’s and their matched PT-singleton pair’s intra pair variance. Pearson’s r was used for correlational analyses. Due to several reasons not all participants have completed all test, hence the analyses are based on the available participants resulting in a variation in n in the different analyses. Laterality Eye was excluded from all analyses due to its non-normal distribution. For all analyses a p-value of .05 was used.

Ethics
Participants in this study were selected from an ongoing study at Umeå University studying the long-term effects of preterm birth, which is approved by the Umeå Regional Ethical Board (Dnr 05-104M, and a supplementary approval related to the MRI/DTI investigations 2009-11-16). The aim of this study lies within the granted research purpose; hence the ethical permission is valid for this study as well. The guardians and the children participating were informed both verbally and in writing about the purpose and content of the study and guardians gave their written consent. Special consideration and concern was also taken when handling all personal identification and results, due to the participants’ young age and the Swedish Personal Data Act (Munck, 2015). Furthermore, all data was stored on an USB-memory stick, which when not in use was stored in a locked cabinet in a locked room in the Department of Psychology, Umeå University. When using the USB-memory stick the computer was never connected to the Internet. All statistical analysis was performed on a de-identified data-file.

Results
Group Differences
Separate MANOVAs were used to analyze group differences for the respective tests included. See Table 2 for description of the dependent variables for each group separately. For functional laterality and DL index the MANOVA demonstrated a significant effect for Group, (Wilks lambda, .76, F(8, 120) = 2.25, p = .03, \( \eta^2_p = .13 \)), indicating a difference in functional laterality and DL index between groups. The Bonferroni post hoc test showed a significant difference in DL index where PT-singletons were less right ear specialized compared with FT-singletons (p = .006). No significant differences regarding laterality were found between twins either PT- or FT-
singletons. For the WISC-IV outcomes scores the MANOVA demonstrated an overall significant effect for group, (Wilks lambda, .62, $F(10, 102) = 2.79$, $p = .004$, $\eta^2_p = .22$). The Bonferroni post hoc test showed a significant difference on the Verbal Comprehension Index, Perceptual Reasoning Index and Full Scale IQ. Twins performed lower than FT-singletons ($p = .008$) on Verbal Comprehension Index. For the Perceptual Reasoning Index both Twins ($p = .017$) and PT-singletons ($p = .032$) performed lower than FT-singletons. For Full Scale IQ both Twins ($p = .005$) and PT-singletons ($p = .03$) performed lower than FT-singletons. No significant differences regarding cognition were found between twins and PT-singletons. For Brain volumes no significant effect of group on

Table 2. Means and standard deviations for outcomes of Functional Laterality, Dichotic Listening, WISC-IV and SyMRI in each group respectively.

<table>
<thead>
<tr>
<th></th>
<th>Twins (n = 22) M (SD)</th>
<th>PT-singletons (n = 23) M (SD)</th>
<th>PT-singletons (n = 22) M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LatHand</td>
<td>1.5 (.6)</td>
<td>1.7 (.4)</td>
<td>1.8 (.2)</td>
</tr>
<tr>
<td>LatFoot</td>
<td>1.2 (.4)</td>
<td>1.2 (.3)</td>
<td>1.2 (.4)</td>
</tr>
<tr>
<td>LatIndex</td>
<td>1.4 (.4)</td>
<td>1.3 (.4)</td>
<td>1.4 (.3)</td>
</tr>
<tr>
<td>DL Index</td>
<td>1.3 (.3)</td>
<td>1.4 (.3)</td>
<td>1.1 (.3)</td>
</tr>
<tr>
<td></td>
<td>Twins (n = 16) M (SD)</td>
<td>PT-singletons (n = 20) M (SD)</td>
<td>PT-singletons (n = 22) M (SD)</td>
</tr>
<tr>
<td>VCI</td>
<td>92.8 (7)</td>
<td>100 (10.6)</td>
<td>101.2 (8.6)</td>
</tr>
<tr>
<td>PRI</td>
<td>99.6 (16.8)</td>
<td>101.2 (10.3)</td>
<td>111.1 (9.4)</td>
</tr>
<tr>
<td>WMI</td>
<td>88.2 (14)</td>
<td>86.3 (10.7)</td>
<td>94.1 (10.5)</td>
</tr>
<tr>
<td>PSI</td>
<td>100 (17.7)</td>
<td>97.3 (11.7)</td>
<td>102.3 (11.9)</td>
</tr>
<tr>
<td>FSIQ</td>
<td>93.9 (14)</td>
<td>96.7 (8)</td>
<td>105.1 (8.6)</td>
</tr>
<tr>
<td></td>
<td>Twins (n = 10) M (SD)</td>
<td>PT-singletons (n = 9) M (SD)</td>
<td>PT-singletons (n = 21) M (SD)</td>
</tr>
<tr>
<td>ICV TBV in ml</td>
<td>1229 (109)</td>
<td>1170 (87)</td>
<td>1268 (84)</td>
</tr>
<tr>
<td>ICV WM in ml</td>
<td>484 (42)</td>
<td>466 (52)</td>
<td>493 (57)</td>
</tr>
<tr>
<td>ICV GM in ml</td>
<td>672 (80)</td>
<td>619 (28)</td>
<td>690 (52)</td>
</tr>
<tr>
<td>ICV CSF in ml</td>
<td>187 (56)</td>
<td>169 (25)</td>
<td>163 (36)</td>
</tr>
<tr>
<td>BPF Total %</td>
<td>87 (3)</td>
<td>87 (2)</td>
<td>87 (2)</td>
</tr>
<tr>
<td>BPF WM %</td>
<td>34 (3)</td>
<td>35 (3)</td>
<td>35 (4)</td>
</tr>
<tr>
<td>BPF GM %</td>
<td>47 (4)</td>
<td>46 (2)</td>
<td>48 (3)</td>
</tr>
<tr>
<td>BPF CSF %</td>
<td>13 (3)</td>
<td>13 (2)</td>
<td>11 (2)</td>
</tr>
</tbody>
</table>

**Note:** Lat Hand = Laterality Hand; Lat Foot = Laterality Foot; Lat Index = Laterality Index; DL = Dichotic Listening Index; VCI = Verbal Comprehension Index; PRI = Perceptual Reasoning Index; WMI = Working memory Index; PSI = Processing Speed Index; FSIQ = Full Scale Intelligence Quotient; ICV = Intra Cranial Volume; TBV = Total Brain Volume; WM = White Matter; GM = Grey Matter; CSF = Cerebrospinal Fluid; BPF = Brain Parenchymal Fraction.

either the ICV ml or BPF measures were detected. However the means and standard deviations (presented in Table 2) indicate a possible difference on ICV in ml outcomes.
Thus a one-way ANOVA was calculated on TBV, GM, WM and CSF. The ANOVA confirmed a significant effect of group on TBV \( (F(2, 37) = 3.73, p = .03, \eta^2_p = .17) \), where the post hoc showed that PT-singletons had a significantly lower TBV than FT-singletons \( (p = .01) \). The ANOVA also demonstrated a significant effect of group on GM \( (F(2, 37) = 4.98, p = .01, \eta^2_p = .21) \), where the post hoc showed that PT-singletons had significantly lower GM volume than FT-singletons \( (p = .01) \). No significant differences were found between twins and either PT- or FT-singletons on brain volumes.

**Functional laterality**

Descriptive data of handedness shows that 82 % of the twins were right handed, 4.5 % showed no preference and 13.5 % were left-handed. In the PT-singleton group 92 % were right handed, 4 % showed no preference. One participant was missing data. In the FT-singleton group 91 % were right handed, 9 % showed no preference; no FT-singleton was left handed. For foot preference 45.5 % of the twins showed a right foot preference, 36.5 % showed no preference and 18 % showed a left foot preference. In the PT-singleton group 50 % showed a right foot preference, 37.5 % showed no preference and 8.5 % showed a left foot preference. One participant was missing data. In the FT-singleton group 50 % showed a right foot preference, 50 % showed no preference. For eye preference 63.5 % of the twins showed a right eye preference, 4.5 % showed no preference and 32 % showed a left eye preference. In the PT-singleton group 50 % showed a right eye preference and 46 % showed a left eye preference. One participant was missing data. In the FT-singleton group 59 % showed a right eye preference and 41 % showed a left eye preference. For auditory preference 32 % of the twins showed a right side ear preference and 68 % showed no preference. In the PT-singleton group 62.5 % showed a right side ear preference, 29 % showed no preference and 4 % showed a left side preference. One participant was missing data. In the FT-singleton group 18 % showed a right side ear preference and 68 % showed no preference and 14 % showed a left side preference.

**Intra pair variance**

A Wilcoxon Signed Rank Test was used to analyze intra pair variance between twin pairs and matched PT-singleton pairs regarding functional laterality and WISC-IV outcomes. The test showed no significant differences between the intra pair variance of twin pairs versus the matched pairs of PT-singletons on either Laterality Hand \( (Z = -.42, p = .68) \), Laterality Foot \( (Z = -.42, p = .68) \), Laterality Eye \( (Z = -1.54, p = .15) \), Laterality Index \( (Z = -1.43, p = .15) \), Dichotic Listening \( (Z = .00, p = 1) \), VCI \( (Z = .98, p = .33) \), PRI \( (Z = -1.33, p = .18) \), WMI \( (Z = -.7, p = .48) \), PSI \( (Z = -.91, p = .36) \) or FSIQ \( (Z = -1.54, p = .12) \). Thus no significant intra pair variances were found.

**Correlations**

**Correlations between brain volumes and behavioral outcomes**

For twins, no significant correlations were found between brain volume and any of the measures of functional laterality or DL. Several correlations were found between SyMRI and WISC-IV outcomes. A larger TBV volume in ml correlated with a higher performance on PRI \( (r = .70, p = .026) \) and FSIQ \( (r = .69, p = .028) \), and a larger volume of WM in ml correlated with a higher performance on PRI \( (r = .68, p = .031) \), WMI \( (r = .67, p = .035) \) and FSIQ \( (r = .71, p = .023) \) in the group of twins. In the group of PT-singletons, a larger TBV volume in ml correlated with a lower DL index \( (r = -.77, p = .027) \), meaning a left side auditory specialization. A larger WM volume in ml correlated with a higher...
performance on PSI ($r = .70, p = .035$). No significant correlations were found between brain volume and the behavioral outcomes in FT-singleton.

Correlations between GA, BW and outcomes
A bivariate correlation including all participants was calculated on GA, BW, the behavioral outcomes and brain volume. For results see Table 3. An increase in GA correlates with higher performance on VCI, PRI, WMI and FSIQ. Increasing GA also correlates with a larger TBV volume, both in ml and BPF %, larger GM volume in ml as well as a lower percentage of CSF in BPF (see Figure 1 A-D). A higher BW correlates with a right hand preference, left side auditory preference and higher performances on VCI, PRI, WMI and FSIQ. A higher BW also correlates with larger TBV and GM volumes in ml.
The primary aim of this study was to compare twins born preterm to singletons matched for GA and BW and to singletons born full term, on functional laterality, cognitive performance and brain volumes at early school age. Based on previous findings we hypothesized that the twin born children would differ from both groups of singletons in the behavioral outcomes and that the two preterm groups would differ from the FT-group regarding brain volumes. The results showed that the group of twins showed a larger percentage of left-handedness than both singleton groups. Statistical analysis showed that PT-singletons were less right ear specialized than FT-singletons. Both twins and PT-singletons performed significantly lower than FT-singleton on WISC-IV indexes. The two groups of children born preterm did not differ on any of the outcomes. Further, PT-singletons were found to have significantly lower TBV and GM volume than FT-singletons. We also hypothesized that twin pairs would show greater intra pair similarities in behavioral outcomes than unrelated PT-singletons, matched for GA and BW. This hypothesis was not confirmed as no significant difference in intra pair variance was found. Our final prediction was that brain volume would correlate positively with behavioral outcomes. This was found to be true for both groups of preterm born children considering their cognitive performance. Additionally, both GA and BW were found to associate with several of the children’s behavioral and MRI outcomes.

**Group Differences**

As expected, differences were found between the FT-singletons and the two groups of children born preterm. These differences are most striking in the cognitive functioning
outcomes, where both preterm groups performed significantly lower on FSIQ. These finding are in line with previous research showing that a preterm birth is associated with risks of long term cognitive impairments (Hoff Esbjørn et al., 2006). Whereas PT-singletons performed lower than FT-singletons on FSIQ; the twin group performed lower than FT-singletons on several of the WISC-IV subscales, indicating that it might be an increased risk of cognitive impairments when born both preterm and as a twin. There are also environmental factors that could have impact on these results, eg the divided attention that comes with parenting twins, which have been used to explain twins lower performance on language tests (Segal, 2012). Most studies reporting differences in cognitive performance have not controlled for GA or BW. Additionally, those who have controlled for GA and BW fail to report any differences (Lorenz, 2012). This could explain why no significant differences between the two preterm born groups, matched for GA and BW, were found. Another explanation could be the somewhat bigger variance in WISC-IV performance within the group of twins, where outliers could have affected the relatively small sample. On the other hand the group of twins could be expected to be more heterogeneous since some of the twin-pairs in the sample are born concordant in BW while other pairs are born discordant in BW (Ross et al., 2011).

Inconsistent with previous findings of NRH being more frequent in preterm born as well as in twins, we found no differences in functional laterality between the preterm groups and the full term group (Domellöf et al., 2011; Ooki, 2014). However, in accordance with previous research the twin sample show a larger percentage of NRH than would be expected in the population (Ooki, 2014). The group of PT-singletons, on the other hand, show a much smaller percentage of NRH than would be expected both in comparisons with preterm samples and the population. A possible difference on handedness between groups, indicated by the demonstrated percentage, should not be rejected, as it is impossible to perform suitable analyses on a sample this size and with an expected non-normal distribution.

Regarding the brain data, no significant differences between groups were found on either absolute volume in ml or the relative BPF volume. Previous research has shown that the brain of children born preterm keeps developing after birth so that they show a normal development of WM at term-equivalent age (Kersbergen et al., 2014) and that children born SGA who catches up weight also catches up in regional brain volume at term-equivalent age (Tzarouchi, 2014). Other studies show that MZ twins have a smaller volume of GM at birth, but that they catch up in the first month of life (Knickmeyer et al., 2011). Further, they were unable to find differences in brain volumes between twins and singletons when controlling for age at MRI. This could explain our lack of results since the groups have a similar range of age at MRI. Further, the possible differences of brain volumes at birth may have diminished with age. Noteworthy however is that other studies have found children born preterm with very low birth weight to have smaller TBV than children born at term, still at the age of 20 (Bjuland et al., 2014).

**Intra pair variance**

Contradictory to previous research (Lopes, Tani, Katzmarzyk, Tomis & Maia, 2014; Morell et al., 2007) and our hypothesis no significant differences in intra-pair variance were found. There are a number of plausible explanations for this result. Earlier studies have shown that MZ twins show greater intra-pair similarities in auditory lateralization (Morell et al., 2007), brain structure (Brun et al., 2009) and purely motor- and adaptive
motor tasks (Lopes et al., 2014) than DZ twins. Since we lack complete information of zygosity and our sample seemingly consist of relatively few MZ twins, a larger heterogeneity could be expected. This assumption is also supported by the amount of twin pairs with opposite sex included in this study. Another factor that could have impact on the results is the intra-pair BW variance. Several studies have found differences in both handedness and cognitive outcomes in twin pairs discordant in BW (Ross et al., 2011; Segal, 2012). If only MZ-twins born concordant in BW were to be included, the results might be affected differently. When it comes to handedness previous research has showed that opposite-handedness occurs in 25 % of twin pairs (Segal, 2012), which means that a greater intra-pair variance of functional laterality could be expected. In summary there are several factors that contributes to the outcome when comparing the intra-pair variance in twin-pairs. A larger sample and more complex analyses are necessary to attempt to differentiate between these factors.

**Correlations between brain volumes and behavioral outcomes**

Several correlations were found between brain volumes and WISC-IV outcomes in the twin group, only one correlation in the PT-singleton group and none in the FT-singleton group. These results contradict earlier studies that showed correlations between brain volumes and IQ in a variety of samples (Lange et al., 2010; McDaniel, 2005). The first study used regional measures of brain volume and the second study, a meta-analysis, did not declare what measures were used. Since this study used overall volume measures possible differences in specific regions are not addressed. Another study, comparing children with Very Low BW with controls, found several correlations between brain volumes and cognitive abilities in the Very Low BW group and none in the control group (Bjuland et al., 2014). Our study did not divide the sample by birthweight, which means the groups of twins and PT-singletons contain children both under and above the cut off for Very Low BW and the FT-singleton group only contains children above. This may explain the lack of correlations in the FT-singleton group. Even though the twins and PT-singletons are very similar in both GA and BW we found several correlations between brain volume and cognition in the group of twins compared to one correlation in the group of PT-singletons. Other studies have found differences between twins and singletons regarding cognitive abilities (Lorenz, 2012). These results indicate that twins are more vulnerable for lower brain volumes affecting the IQ. We also found a negative correlation between DL and TBV in the group of PT-singletons, indicating that the larger the TBV the stronger the atypical left ear specialization, a result difficult to explain. Since our PT-singleton sample consists of only 9 participants in these analyses the result should be interpreted with caution.

**Strengths and Limitations**

There are limitations worth addressing. The small sample size increases the risk of outlier impact. Some of the twin pairs were unable to complete the WISC-IV, giving a smaller sample for analysis. Further, the reason for not completing the test could be associated with a lower level of cognitive functioning, hence there is a possibility of underestimating the risk associated with being born as a twin since those with the greatest difficulties might be those missing in the analyses. Additionally, the choice to only include children without known neurodevelopmental disabilities or delays is worth mentioning since an important part of the population of twins born preterm hence is unrepresented in this sample. In a study with this small sample size however, the exclusion criteria of neurodevelopmental disabilities is rather to be seen as a strength,
giving a more homogenous sample. When including only children with normal development in all three groups it provides better possibilities of explaining results in means of twin-ship and prematurity rather than other pathologies.

Earlier studies have found differences in cognitive functioning between MZ and DZ twins (Lorenz, 2012). In this sample information of zygosity was incomplete and no differentiation between MZ vs. DZ were made. This affects both group and intra pair comparisons since the DZ are expected to have better cognitive functioning and greater intra pair variance.

The MRI analysis program SyMRI has so far only been validated when used on adult brains (Ambarki, et al., 2012), and is not yet validated for children. With this in mind, our results must be interpreted with caution. The age of performing MRI scans range from 7 to 9.5 in all groups. How much the brain develops between these years is not clear and we cannot rule out the risk that the relatively large age range could affect the results. However descriptive statistics shows that that the range is very similar between the groups (see Table 1), and the possible variance is consequently the same for all groups.

Although the number of twins was limited, the inclusion of two comparison groups used can be raised as strength in the present study. Thus, in contrast to many previous studies of twins and functioning we chose to include a group of singletons born preterm in an attempt to control for the important factors GA and BW (Lorenz, 2012; Månsson, Stjernqvist & Bäckström, 2014; van Soelen et al., 2010; Tzarouchi et al., 2013). The inclusion of FT-singletons enables to further differentiate between the risks associated to twin-ship and those associated with, or affected by, a preterm birth and low GA/BW.

Conclusions
Overall, the expected differences between twins and PT-singletons were not found in this sample. Lower BW and GA however, evidently correlated with both lower cognitive performance and smaller brain volumes. Results on group differences also shows that while the two preterm born groups did not differ, they both significantly differ from the FT-group on respectively functional laterality, cognitive performance, and on brain volume outcomes. This is in agreement with previous studies on preterm birth and BW, which often fails to show any significant differences when controlling for these variables (Lorenz, 2012). The result from this study indicates that a preterm birth, accompanying with a low BW may have a greater impact on cognitive and brain development than twin-ship per se.

Future research
Future research should differentiate between MZ and DZ twins, since this might give more detailed information about heredity, intra uterine environment and other environmental factors. A bigger sample to increase generalizability and minimize the effect of outliers is necessary. Also a longitudinal approach could give important knowledge of the developmental trajectories on brain volumes as well as cognition in twins born preterm. Regarding the correlations of brain volumes and cognition, the field of research as well as the result in this study is not consistent. At last we want to stress the importance of either comparing twins with BW and GA matched singletons as well as full term controls or to compare full term twins with full term controls, to better
examine the consequences of twinship and to avoid the risk of results being better explained by the preterm birth.

References


