

The long-term outcome of
childhood leukaemia:
neuropsychological and
neuroimaging investigations

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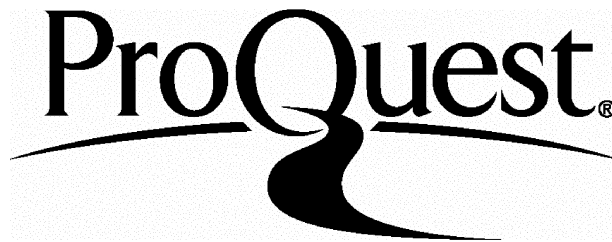
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Abstract

The recent introduction of pre-symptomatic central nervous system (PCNS) therapy in the treatment of childhood acute lymphoblastic leukaemia (ALL) has significantly improved the chances of long-term (LT) survival. However, the LT survival of ALL can be complicated by cognitive and behavioural impairments, and these problems may be related to the administration of PCNS therapy.

This thesis investigated the role of PCNS therapies in determining the LT outcome of childhood ALL. In particular, attempts were made to identify the functional impairments associated with two PCNS therapies currently used in the treatment of standard-risk ALL, and to relate the profile of deficits identified to underlying structural abnormalities. Investigations were conducted in accordance with a proposed model of pathology. This model was based on the biochemical actions of the main component of both PCNS therapies, methotrexate (MTX).

32 LT survivors and 14 siblings participated in this research. Survivors were randomised to receive either (1) HD MTX + IT MTX + FAR (HD group), or (2) IT MTX-only (IT group) as PCNS therapy. Participants completed a battery of neuropsychological tests assessing the functional integrity of the frontal lobes and corpus callosum. In addition, the structural integrity of the brain was evaluated using conventional neuroradiological assessment and voxel-based morphometry (VBM).

The neuropsychological and conventional neuroradiological investigations revealed minimal differences between the survivor and sibling groups. The VBM analyses, however, revealed common, subtle differences in brain structure between the survivors and their siblings. The differences included subtle pathology of the thalamus, caudate nucleus, and corona radiata. The pathology was regionally specific apparently sparing, for example, the pons and cerebellum. The methods used in the current research, however, were unable to establish a clear relationship between the subtle pathology identified and impairments in cognitive function.

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Part 1: Introduction

1 Childhood Leukaemia

Chapter 1 begins with a brief description of acute lymphoblastic leukaemia (ALL) and the treatment of ALL in childhood. This is followed by a review of the current literature describing the long-term outcome of childhood ALL. The limitations of the literature are also presented and the objectives of the present study are listed. The final section proposes a developmental model of pathology and the specific hypotheses to be tested in this thesis.

1.1 General Introduction

1.1.1 Acute lymphoblastic leukaemia

Acute lymphoblastic leukaemia (ALL) is the most common cancer of childhood (Cassano *et al.* 1993). Approximately 400 new cases are diagnosed each year within the United Kingdom (Greaves 2002). Children aged 2-3 years have a relatively high incidence of ALL with an annual rate of 8 per 100,000. This is four times the incidence in children aged 8-10 years and almost 10 times that seen in adolescents (Smith *et al.* 2003). At all ages, ALL is more common in boys than girls (Stiller *et al.* 1995) and in white than black children (Xie *et al.* 2003). There is an increased incidence of ALL in genetic disorders of chromosomal instability (e.g. ataxia telangiectasia) and chromosomal abnormality (e.g. Down's syndrome)(Taylor *et al.* 1996; Rubnitz & Crist 1997; Hasle *et al.* 2000). Pre- or post- natal exposure to high-energy ionising radiation has also been shown to directly increase the incidence of ALL (Greaves 1997).

ALL is a disease characterised by the rapid, uncontrolled proliferation of immature lymphoblasts in bone marrow. These 'blast' cells are unable to function as lymphocytes and infiltrate the lymphoid tissues, such as spleen and lymph nodes, and other organs, such as liver and brain. As a consequence a child is unable to host an effective immune response and is thus at risk of infection. The rapid production of lymphoblasts also utilises resources necessary for the maintenance of other haemopoietic cell lines. A child is therefore vulnerable to haemorrhage as a consequence of a reduced number of platelets, and, anaemia as a result of fewer red blood cells (Pizzo & Poplack 1997). Without treatment the disease is uniformly fatal within 3 to 6 months (Silverman & Sallan 2003).

The most common type of ALL is precursor B-cell or common-ALL (80%)(Stiller & Eatock 1999). Other types classified by immunophenotyping include pre-pre-B-cell, mature B-cell and T-cell (Rubnitz & Pui 1997; Pui 1998).

1.1.2 The treatment of ALL

The aim of treatment for ALL is to restore the blood count to normal and to inhibit further lymphoblast production. These goals can be achieved by gaining control over the disease in an initial remission period and subsequently administering treatment to prevent relapse. Initial remission is achieved in the majority (>95%) of children with ALL. However, the prevention of relapse is more difficult (Rubnitz & Pui 1997; Chessells 2000). Prevention of relapse is a particular challenge in the treatment of ALL as many conventional systemic treatments are unable to penetrate the blood brain barrier and enter the central nervous system (CNS). Conventional treatments, therefore, allow blast cells to survive within the CNS and cause relapse (Franks & Teich 1998).

1.1.3 Survival rates in ALL

The chance of disease-free, long-term survival for children diagnosed with ALL has significantly increased over the past 30 years. In the early 1970's, approximately 4% of children survived more than five-years post-diagnosis and poor survival was often associated with CNS relapse (Aur *et al.* 1972; Stiller & Draper 1998). Estimates indicate that more than 80% of children diagnosed with ALL can now be expected to survive five years without relapse and are considered 'cured' of their disease (Stiller & Eatock 1999). 95% of these survivors will remain in remission for a further five years (Maloney *et al.* 2000).

Current prognostic factors associated with favourable outcome include young age (i.e. 1-9 year olds (not infants)), female sex, early diagnosis (i.e. minimal evolution and incorporation of additional mutations), high cell chromosome number (hyperdiploidy), and the absence of translocations involving specific chromosomes at diagnosis (e.g. t(9;22); t(4;11))(Stiller 1994; Friedmann & Weinstein 2000; Colby-Graham & Chordas 2003). The response to treatment is also an important factor with a rapid rate of remission (e.g. achieved within 14 days) associated with a lower chance of relapse (Eden *et al.* 2000; Rubnitz & Pui 2003).

1.1.4 PCNS therapy

The current high, disease-free, long-term survival rate reflects the recent introduction of pre-symptomatic central nervous system (PCNS) therapy in the treatment of ALL (Pui 1995). PCNS therapy specifically targets the CNS to prevent relapse at this site. PCNS therapy therefore, affords higher chances of sustained remission and hence long-term survival.

Current PCNS therapies employed in the treatment of ALL involve the administration of the chemotherapeutic agent methotrexate (MTX). This can be administered in one of four ways: (1) intrathecally (IT MTX), (2) as a part of triple intrathecal therapy (MTX + cytarabine + hydrocortisone; TIT), (3) in high intravenous doses followed by folinic acid rescue (HD MTX + FAR), or (4) intrathecally in combination with cranial irradiation (MTX + CI). While PCNS therapies are essential for the long-term survival of ALL, methotrexate, cytarabine, and cranial irradiation are neurotoxic¹ (Filley 1999).

The administration of methotrexate alone, or in combination with cytarabine or cranial irradiation, in the treatment of ALL can be associated with acute, sub-acute, and delayed clinical syndromes (see Appendix A)(Pizzo *et al.* 1979; Keime-Guibert *et al.* 1998). Fortunately, the development of clinical neurotoxic syndromes associated with the administration of MTX-based PCNS therapies is rare. Despite this, the long-term survival of ALL is complicated by subtle behavioural and cognitive impairments.

1.1.5 The long-term outcome

The long-term (LT) survival of ALL has been associated with subtle deficits in intelligence (Moss *et al.* 1981; Rowland *et al.* 1984; Jankovic *et al.* 1994; Kingma *et al.* 2001; Precourt *et al.* 2002), memory and learning (Pfefferbaum-Levine *et al.* 1984; Mulhern *et al.* 1988), visuo-spatial processing (Moore *et al.* 1992), fine motor control (Copeland *et al.* 1996), problem solving (Brown *et al.* 1992), attention and concentration (Appleton *et al.* 1990; Brown *et al.* 1992; Moore *et al.* 1992; Butler *et al.* 1994; Kingma *et al.* 2002). In addition, these impairments emerge with time and may first be detected several years after diagnosis (Rubenstein *et al.* 1990; Brown *et al.* 1992; Jankovic *et al.* 1994).

¹ Children treated with either IT MTX or HD MTX + FAR are the focus of this study. CI and cytarabine are associated with their own neurotoxicities. Therefore, a distinction will be made between children receiving MTX only (i.e. IT MTX or HD MTX + FAR) and children receiving combined therapies (i.e. MTX + CI or TIT) throughout the remainder of this thesis.

Age-at-diagnosis and sex have also been identified as important predictors of LT sequelae. For example, children receiving treatment at a young age (<5-8 years) show more LT deficits than children treated at an older age (Eiser & Lansdown 1977; Eiser 1980; Moss *et al.* 1981; Jannoun 1983; Lansky *et al.* 1984; Bleyer *et al.* 1990; Jankovic *et al.* 1994; Copeland *et al.* 1996; Smibert *et al.* 1996; Anderson *et al.* 1997; Waber *et al.* 2001; von der Weid *et al.* 2003)(but see also Ivnik *et al.* 1981; Rowland *et al.* 1984; Mulhern *et al.* 1988; Mulhern *et al.* 1991). Reports also suggest that girls may be more vulnerable than boys (Schlieper *et al.* 1989; Waber *et al.* 1990; Mulhern *et al.* 1991; Waber *et al.* 1992; Christie *et al.* 1994; Waber *et al.* 1995)(but see also Rowland *et al.* 1984; Copeland *et al.* 1996; Smibert *et al.* 1996).

Whilst the impairments may appear mild in nature, they cause academic underachievement and poor social skill development in later years (Appleton *et al.* 1990; Williams *et al.* 1991; Christie *et al.* 1994; Brown *et al.* 1996; Mackie *et al.* 2000)(but see also Noll *et al.* 1990). For example, LT survivors show a higher rate of referral for special education and are less likely to enter programmes for gifted students than their siblings or age-matched peers (Mitby *et al.* 2003). In addition, long-term survivors accomplish lower levels of secondary education (Katz *et al.* 1977; Taylor *et al.* 1987; Peckham *et al.* 1988; Zelter *et al.* 1997; Langeveld *et al.* 2003) especially when children are treated at a young age (Haupt *et al.* 1994; Kingma *et al.* 2000).

It is likely that these LT difficulties are related to the use of PCNS therapy since children treated without PCNS therapy for ALL (Moss *et al.* 1981) or for other cancers (e.g. solid tumours) do not develop these problems (Copeland *et al.* 1988; Giralt *et al.* 1992; Brown *et al.* 1996). In addition, the range and severity of symptoms developing in the longer-term has been related to the type of PCNS therapy administered. For example, PCNS treatment with combined cranial irradiation and chemotherapy has been associated with greater impairments (e.g. reductions in intelligence and poor achievement in reading, spelling and maths) than chemotherapy-only options (Pavlovsky *et al.* 1983; Lansky *et al.* 1984; Pfefferbaum-Levine *et al.* 1984; Schlieper *et al.* 1989; Dowell *et al.* 1991; Moore *et al.* 1992; Jankovic *et al.* 1994; Smibert *et al.* 1996; Precourt *et al.* 2002). However, differences have not always been reported between children treated with alternate PCNS therapies (Ivnik *et al.* 1981; Whitt *et al.* 1984; Mulhern *et al.* 1991; Waber *et al.* 1995; Copeland *et al.* 1996) and the exact relationship between the profile of deficits and PCNS treatment is unclear.

1.1.6 Limitations of the LT outcome literature

To date much of the literature concerning the LT outcome of children treated for ALL has focused on the detrimental effects of combined cranial irradiation and chemotherapy PCNS therapy (for review see (Roman & Sperduto 1995)). As a consequence of this work, cranial irradiation is now avoided in favour of chemotherapy-only options in the treatment of standard-risk children with ALL, and, few studies have been conducted to describe the LT cognitive outcome for standard-risk children treated with chemotherapy-only.

In addition, the studies describing the LT outcome for children treated with chemotherapy-only have been limited by numerous methodological problems. For example, children were not randomised for treatment (Eiser 1978; Tamaroff *et al.* 1982; Dowell *et al.* 1991; Butler *et al.* 1994; Jankovic *et al.* 1994; Copeland *et al.* 1996; Kingma *et al.* 2002) or were treated with heterogeneous protocols (Appleton *et al.* 1990; Mulhern *et al.* 1991; Smibert *et al.* 1996; Kaleita *et al.* 1999; Sawyer *et al.* 2000; Precourt *et al.* 2002). Studies have also included children treated for relapse (Pfefferbaum-Levine *et al.* 1984; Appleton *et al.* 1990); children developing neurotoxic syndromes (Meadows & Evans 1976; Chen *et al.* 1996); and children with CNS disease at diagnosis (Meadows & Evans 1976; Dowell *et al.* 1991; Waber *et al.* 1995; Kaleita *et al.* 1999); or have combined children treated for ALL with children treated for other cancers (Meadows & Evans 1976; Dowell *et al.* 1991; Moore *et al.* 1992; Copeland *et al.* 1996; Sawyer *et al.* 2000; Smibert *et al.* 1996). In addition, outcome studies have pooled results from newly diagnosed children with children several years post-diagnosis (Lansky *et al.* 1984; Butler *et al.* 1994), and often do not account for pre-diagnosis ability (Giralt *et al.* 1992; Moore *et al.* 1992; Uberall *et al.* 1996; Ueberall *et al.* 1997; Lamothe *et al.* 1998). Moreover, studies have assessed a limited range of functions. For example, intelligence only (Eiser 1978; Jankovic *et al.* 1994), or intelligence and academic achievement (Lansky *et al.* 1984; Brown *et al.* 1996; Smibert *et al.* 1996). It is possible that these tests may not be sensitive enough to capture the more subtle difficulties experienced by this population (Eiser 1991). Furthermore, many studies fail to compare survivors to a non-ALL control group (Eiser 1978; Ivnik *et al.* 1981; Pavlovsky *et al.* 1983; Pfefferbaum-Levine *et al.* 1984; Rowland *et al.* 1984; Whitt *et al.* 1984; Mulhern *et al.* 1988; Appleton *et al.* 1990; Dowell *et al.* 1991; Mulhern *et al.* 1991; Brown *et al.* 1992; Moore *et al.* 1992; Jankovic *et al.* 1994; Waber *et al.* 1995; Ueberall *et al.* 1997; Kingma *et al.* 2002)(but see comparisons with children treated for other cancers Tamaroff *et al.* 1982; Copeland *et al.* 1988; Butler *et al.* 1994; Brown *et al.* 1996; Copeland *et al.* 1996 von der Weid *et al.* 2003, siblings Lansky *et al.*

1984; Brown *et al.* 1992; Giralt *et al.* 1992, or healthy controls Smibert *et al.* 1996; Uberall *et al.* 1996; Lamothe *et al.* 1998; Sawyer *et al.* 2000; Precourt *et al.* 2002).

Finally, few studies have attempted to identify the pathological substrate and radiological expression of the LT deficits observed in ALL survivors. Moreover, these studies have failed to identify common abnormalities within this population and have been unable to consistently relate the abnormalities identified to cognitive performance (Phillips *et al.* 1991; Asato *et al.* 1992; Harila-Saari *et al.* 1997; Kahkonen *et al.* 1999; Kahkonen *et al.* 2000; Paakko *et al.* 2000). For example, abnormalities including atrophy, white matter changes, and calcifications have been identified infrequently in the LT survivors of ALL using conventional magnetic resonance imaging (MRI) techniques. In addition, abnormalities as revealed by MRI, have been identified in LT survivors with no obvious cognitive impairment, while no apparent MRI abnormalities have been identified in LT survivors with poor cognitive outcome (Harila-Saari *et al.* 1997; Kahkonen *et al.* 1999).

On the basis of existing literature, therefore, descriptions of the LT cognitive outcome for a new generation of children treated with current chemotherapy-only protocols are inadequate. Furthermore, the pathology associated with the LT outcome requires identification.

1.2 Study aims

The focus of the present study was to characterise the cognitive deficits and reveal the underlying pathology in the LT survivors of ALL. In particular, the study aimed to describe the LT outcome for children treated for standard-risk ALL with current MTX-based PCNS therapies (i.e. no cranial irradiation). Importantly, attempts were made to describe the LT outcome for the average child (i.e. a child who does not develop complications of treatment nor relapse). Furthermore, the relationship between the types of MTX-based PCNS therapy administered and the LT difficulties experienced was explored.

In comparison to previous work the sensitivity of the current investigation was increased by

- (1) *a priori* defining a model of pathology based on the biochemistry of the main component of current PCNS therapies used across institutions (i.e. MTX),

- (2) Selecting survivors based on stringent criteria. For example, all survivors had been
 - a. Diagnosed with standard-risk ALL (i.e. without CNS involvement or increased risk of relapse)
 - b. Randomised to receive MTX-based PCNS therapies according to one protocol (i.e. either (1) HD MTX + IT MTX + FAR, or (2) IT MTX-only)
 - c. Treated without complications (e.g. no clinical neurotoxic syndromes)
 - d. In first continuous remission (i.e. no relapses) and more than six years post-diagnosis
- (3) Comparing survivors to a sibling control group,
- (4) Accounting for cognitive development prior to diagnosis and during the early years post-diagnosis (i.e. baseline neuropsychological data),
- (5) Employing a tailored neuropsychological approach to identify specific LT impairments,
- (6) Exploiting new MRI techniques to quantify brain integrity on a group level, and
- (7) Directly exploring brain and behaviour relationships.

1.3 Structure of thesis

The remainder of this chapter proposes a model of pathology based on the biochemistry of MTX and lists the hypotheses to be tested in the current research. This is followed in Chapter 2 by a description of the study participants and the baseline neuropsychological data.

Part 2 (Chapters 3-4), reviews the specific neuropsychological investigations exploring executive functions and bimanual coordination, while Part 3 (Chapters 5-6) presents conventional and experimental neuroimaging studies conducted to assess brain integrity. Section 3 also discusses the relationship between the neuropsychological and neuroimaging results. Finally, Part 4 highlights the main findings and limitations of the current research. Directions for future research are also presented.

1.4 Model of pathology

As discussed in the previous section the use of PCNS therapies to prevent CNS relapse in children diagnosed with ALL has significantly improved the chances of LT survival. However, PCNS therapies are neurotoxic and have occasionally been associated with clinical neurotoxic syndromes (see Appendix A). More commonly LT survivors suffer subtle cognitive and behavioural impairments and these problems may also be related to PCNS therapy. Nevertheless, the exact nature of the deficits and underlying pathology giving rise to the impairments has not been identified.

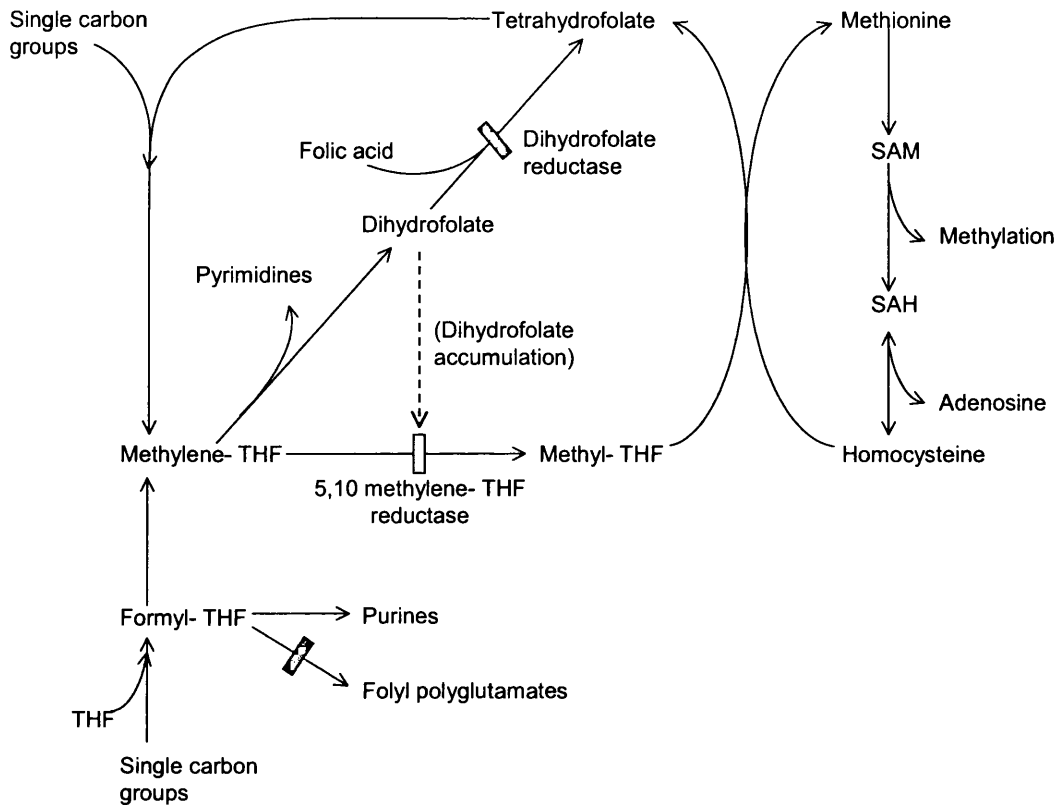
This section reviews the biochemical actions of the chemotherapeutic agent included in current PCNS therapies, methotrexate (MTX). It is proposed that within the CNS MTX not only prevents relapse but also causes demyelination. This secondary action results in pathology that may be responsible for some of the cognitive and behavioural impairments observed in the LT survivors of ALL. Furthermore, in children treated for ALL this process predominately affects the maturation of the frontal lobes and corpus callosum.

1.4.1 Methotrexate and single carbon metabolism

Methotrexate (MTX) is a structural analogue of folic acid (Serman & Schaumburg 2000). The principal action of MTX in the treatment of ALL is to inhibit blast cell production by depleting the intracellular pool of metabolically active folates. MTX achieves this by (1) binding with dihydrofolate reductase (DHFR) to inhibit tetrahydrofolate (THF) production and recycling; and (2) competing with folates in the synthesis of folyl-polyglutamates (Schornagel & McVie 1983)(see Figure 1.1).

While the primary action of MTX is to prevent cell replication in blast cells, the administration of MTX also leads to a reduction of intracellular folate stores in normal tissue. For example, rhesus monkeys receiving intramuscular MTX 2 mg/kg weekly for one year, a treatment thought to equal that of a child's standard protocol for ALL, show a 90% decrease in total folate within the brain (Winick, Kamen, *et al.* 1987). Furthermore, the inhibition of DHFR activity in normal cells results in the accumulation of the oxidised folate, dihydrofolate (DHF)(Abelson 1978)(see Figure 1.1).

Figure 1-1 Single carbon metabolism in the CNS



SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; THF, tetrahydrofolate. Solid blocks show the main sites of inhibition by methotrexate, and the hatched block, secondary inhibition.

The accumulation of oxidised folates has been shown to impair the activity of the enzyme methylene-THF reductase (MTHFR)(Matthews & Haywood 1979). MTHFR is essential for the conversion of methylene-THF to methyl-THF and the physiological purpose of this reaction is to maintain the supply of S-adenosylmethionine (SAM), rather than to regenerate THF (Chanarin 1979).

1.4.2 MTHFR deficiency

The long-term inhibition of MTHFR activity in childhood, for example in MTHFR deficiency, is characterised by distinct neurological, radiological, and CSF profiles.

For example, MTHFR deficiency during childhood and adolescence is associated with progressive neurological deterioration (e.g. psychomotor slowing, retardation, and delay)(Kanwar *et al.* 1976; Hyland *et al.* 1988; Ogier *et al.* 1998), developmental regression (e.g. stopped walking, speaking, crawling, or smiling)(Clayton *et al.* 1986) and seizures (Wong *et al.* 1977; Haworth *et al.* 1993; Walk *et al.* 1994). In addition,

MTHFR deficiency can be accompanied by mental deterioration (Kanwar *et al.* 1976; Wong *et al.* 1977; Visy *et al.* 1991), poor coordination and hand-writing (Haworth *et al.* 1993), poor visual acuity (Haworth *et al.* 1993), gait imbalance (Walk *et al.* 1994), tremors (Clayton *et al.* 1986), hypotonia and ataxia (Hyland *et al.* 1988), spastic paresis (Kanwar *et al.* 1976), and pyramidal signs in four limbs (Visy *et al.* 1991). The presentation of MTFHR deficiency in adolescence or adulthood can also be characterised by psychiatric symptoms (e.g. schizophrenia)(Freeman *et al.* 1975; Pasquier *et al.* 1994) or may be asymptomatic (Haworth *et al.* 1993).

Typical radiological findings in MTHFR deficiency include bilateral, symmetrical, hyper-intense areas of demyelination (on T2 WI) with moderate gliosis and neuronal loss (Visy *et al.* 1991). This pathology preferentially involves white matter of the CNS including the periventricular regions (Walk *et al.* 1994), centrum semiovale, and/or spinal cord (Clayton *et al.* 1986). It may also be accompanied by cerebral atrophy (Visy *et al.* 1991; Haworth *et al.* 1993) and brain stem atrophy (Surtees *et al.* 1991)(Surtees 1998). The pathology associated with the symptoms in MTHFR deficiency has been related to demyelination, or to ischemic and vascular changes (Kanwar *et al.* 1976). Furthermore, demyelination can be unaccompanied by vascular changes (Clayton *et al.* 1986).

The cerebrospinal fluid (CSF) profile associated with MTHFR deficiency is characterised by low concentrations of methyl-THF, methionine and SAM. While the exact mechanism is unclear, it has been demonstrated that decreases in CSF levels of SAM (rather than methyl-THF or methionine) are related to the extent of CNS demyelination (Hyland *et al.* 1988; Surtees *et al.* 1991). In addition, treatment to restore SAM results in apparent remyelination (Surtees *et al.* 1991; Engelbrecht *et al.* 1997).

1.4.3 Demyelination during treatment with MTX

While the severity of neurological deterioration observed in MTHFR deficiency is not typically seen in children treated for ALL, imaging studies have highlighted the vulnerability of white matter to MTX treatment. Furthermore, there is evidence from CSF studies to indicate that children treated with MTX experience a period of demyelination and the level of demyelination can be related to the type of MTX-based PCNS therapy received. Finally, experimental studies support the sensitivity of white matter to the MTX treatment.

White matter changes during treatment with MTX

Conventional radiological studies have inconsistently documented white matter changes during the treatment phase in symptomatic and asymptomatic children treated with MTX (Ochs *et al.* 1983; Wilson *et al.* 1991; Asato *et al.* 1992; Paakko *et al.* 1996; Mahoney *et al.* 1998)(see Chapter 5 for review). These changes often involve periventricular regions and the centrum semiovale (Asato *et al.* 1992; Kingma *et al.* 1993). In addition, the appearance of white matter changes has been linked to MTX administration. For example, white matter changes appear on MRI after at least one trial of IT MTX (8-12 mg/m²) + IV MTX (1-5 g/m²) + FAR in ALL. Furthermore, the size of the white matter lesion (but not number) increases with continuing MTX treatment. Alternatively, interrupting further IT MTX treatment improves the abnormalities observed (Asato *et al.* 1992). Similarly, white matter abnormalities apparently resolve with time following completion of treatment (Wilson *et al.* 1991; Asato *et al.* 1992; Paakko *et al.* 2000).

CSF studies during treatment with MTX

A recent CSF study provides further evidence for demyelination associated with the administration of MTX (Surtees *et al.* 1998). In this study, children diagnosed with ALL were randomised to receive either HD MTX + IT MTX + FAR or IT MTX-only as PCNS therapy. In addition, none of the children reported complications of treatment (e.g. clinical encephalopathy). Concentrations of methyl-THF, methionine, and SAM were measured at six time points throughout the two years of treatment including the PCNS phase. Results were similar for both treatment groups in that concentrations of all metabolites fell during the PCNS phases of treatment and recovered to pre-treatment values during the later stages of treatment. Furthermore, the concentration of methionine and SAM were lowest during the PCNS phase and were significantly reduced when compared to a reference population. Levels of SAM observed during the PCNS and late intensification phases were also similar to values seen in MTHFR deficiency prior to treatment (Surtees 1998) and to the level reported in a second study of asymptomatic children treated with MTX for ALL (Kishi *et al.* 2000).

As part of the CSF study, concentrations of myelin basic protein (MBP) a CSF marker of CNS demyelination (Gangji *et al.* 1980; Davies *et al.* 1987) were also collected and showed a related inverse pattern. Specifically, MBP levels increased over the course of treatment to peak during the PCNS and late consolidation phase before recovering at the end of treatment (Surtees *et al.* 1998). This is consistent with results of a second

study in which increases in MBP were observed during the treatment phase in asymptomatic children treated with MTX for ALL (Kishi *et al.* 2000).

The degree of metabolite change in CSF was also related to the type of PCNS therapy received. In particular, children receiving IT MTX-only displayed a significantly greater decrease in SAM and a greater increase in MBP during the PCNS and late intensification stages of treatment than children treated with HD MTX + IT MTX + FAR.

Together these results indicate that during the PCNS and late intensification phases of therapy, children treated for ALL with MTX have a CSF profile consistent with impaired MTHFR activity and subclinical CNS demyelination. Furthermore, the type of PCNS therapy determines the degree of abnormality in that children treated with IT MTX-only were more affected than children treated with HD MTX + IT MTX + FAR.

Experimental studies of MTX

Animal models have also demonstrated that astrocytes are a primary target for MTX toxicity (Bruce-Gregorios *et al.* 1991a; Bruce-Gregorios *et al.* 1991b; Bruce-Gregorios *et al.* 1991c; Gregorios & Soucy 1990). In particular, the transport of 5-formyltetrahydrofolate into primary cultured rat astrocytes is blocked by MTX (Serrano & Schimke 1990; Cai & Horne 2003). Furthermore, gliosis (astrocytic proliferation) has been identified at autopsy in the brains of children treated with MTX (Ebner *et al.* 1989).

These studies provide additional evidence to indicate that white matter is sensitive to the effects of MTX.

1.4.4 LT outcome following a discrete period of demyelination

A single period of CNS demyelination during childhood can result in LT pathology and deficits of cognitive function. For example, during recovery from acute disseminated encephalomyelitis (ADEM)², many demyelinating lesions identified at presentation resolve with time (Hynson *et al.* 2001). However, 2/3 will show only partial lesion resolution as revealed by MRI at 2 months to 9 years post-diagnosis (Dale *et al.* 2000)(Go & Imai 2000). Histologically, these findings are consistent with gliosis and

² ADEM is a monophasic demyelinating disease that primarily affects white matter of the CNS. The disease is more commonly diagnosed in children than adults and generally follows antecedent infection or vaccination. The disease is characterised by myelin loss with relative preservation of axons (Dale *et al.* 2000; Nasr *et al.* 2000). Imaging studies have revealed preferential involvement of the white matter of frontal lobes, parietal lobes, corpus callosum, and subcortical white matter with relative sparing of periventricular white matter (Dale *et al.* 2000; Hynson *et al.* 2001). Deep grey matter may also be involved (Hynson *et al.* 2001).

demyelination (Kesselring *et al.* 1990). Furthermore, children suffering ADEM experience LT impairments of function. For example, reductions in attention, processing speed, and academic achievement have been observed in children up to 6 years post-diagnosis when compared to age-matched controls (Neale *et al.* 2001). LT reductions in intelligence and behavioural problems (Dale *et al.* 2000; Neale *et al.* 2001) have also been reported in this population. In addition, the level of LT impairment has been associated with the severity of the disease as indicated by the area of white matter involved (Neale *et al.* 2001).

These studies indicate that a period of demyelination sustained during childhood has LT consequences on brain structure and function. In addition, the level of functional impairment is related to the degree of white matter involved. Furthermore, MRI and neuropsychological techniques are sensitive to these LT changes.

1.4.5 Areas of vulnerability

MTX is likely to have the greatest impact on brain areas undergoing development at the time of treatment (Schwartz & Goldman-Rakic 1990; Vander-Knapp & Valk 1995). During early to late childhood (a period associated with a high diagnosis rate of ALL) these areas include the frontal lobes and corpus callosum.

In the frontal lobes there is a dramatic change in the neuronal density between the ages of 2 and 7 years. In particular, neuronal density begins at 55% above adult levels and declines to approximately 10% above adult levels over this period (Huttenlocher 1990). In addition, dendritic arborisation continues in the frontal lobes (middle frontal gyrus) up to age 7 years (Huttenlocher 1996). There is also a significant decrease in synaptic density over this period that continues until approximately 16 years of age (lateral prefrontal cortex)(Huttenlocher 1979; Huttenlocher & Dabholkar 1997). Myelination of prefrontal regions is also protracted and continues into early adolescence (Yakovlev & Lecours 1967; Reiss *et al.* 1996; Steen *et al.* 1997; Cummings 1998; Klingberg *et al.* 1999; Paus *et al.* 1999; Giedd *et al.* 1999; Paus *et al.* 2001). Furthermore, metabolic activity of the prefrontal cortex continues to change throughout childhood (Chugani 1998).

Similarly, the development of the corpus callosum continues into adolescence (Yakovlev & Lecours 1967; Barkovich & Maroldo 1993; Pujol *et al.* 1993; Giedd *et al.* 1996; Rajapakse *et al.* 1996; Giedd *et al.* 1999; Thompson *et al.* 2000; Keshavan *et al.* 2002). Specifically, increases in corpus callosum volume have been identified in children between the ages of 5 and 18 years (Giedd *et al.* 1999). In addition,

experiments with rhesus monkeys indicate that the increases in volume over this period are most likely related to an increase in myelin rather than an increase in the number of corpus callosum axons (LaMantia & Rakic 1990). Furthermore, myelination of the corpus callosum occurs along a rostral-caudal gradient where greater changes are observed in anterior sections during the ages of 3 to 6 years. In contrast, greater changes in myelination are seen in posterior sections between the ages of 6 and 15 years (Thompson *et al.* 2000).

1.5 Hypotheses

In summary, a secondary consequence of the administration of MTX is impaired MTHFR activity. This can be associated with CNS demyelination and the degree of demyelination is related to the type of PCNS treatment administered. During childhood, a single period of demyelination can result in LT deficits of function and brain pathology. The level of LT impairment is related to the extent of white matter involved. Brain areas developing at the highest rate at the time of insult are likely to show the greatest impact of MTX treatment. For children treated with MTX in early to late childhood these areas include the frontal lobes and corpus callosum.

Based on these findings, the hypotheses to be tested in this thesis are as follows -

1. Survivors will show reduced cognitive function in comparison to their siblings on the tasks dependent on the integrity of the frontal lobes (e.g. executive functions) and the corpus callosum (e.g. bimanual coordination).
2. Among the survivors, significant differences in performance will be observed such that children treated with IT MTX-only will have a poorer functional outcome than children treated with HD MTX + IT MTX + FAR.
3. Neuroimaging investigations will reveal structural brain changes in the survivors of ALL when compared to their siblings. The areas affected will include the frontal lobes and corpus callosum, and will be more extensive in children treated with IT MTX-only than HD MTX + IT MTX + FAR.
4. The structural abnormalities identified will correlate with indices of cognitive function.

2 Long-term survivors and their siblings

The long-term survivors enrolled in the current study received treatment as part of a nationwide medical trial. Survivors and their siblings had also participated in a related neuropsychological study. Chapter 2 describes the UKALL XI Medical trial and the related UKALL XI Neuropsychological study. This is followed by a description of the selection criteria and characteristics of all children included in the current research. Finally, statistical considerations and the results of the retrospective analyses are presented. These analyses focus specifically on the cognitive development of all children involved in this thesis over the first five years post-diagnosis.

2.1 Introduction

All survivors participating in the current research were involved in a medical trial for the treatment of ALL. This medical trial, UKALL XI, was a nationwide study enrolling more than 90% of all children diagnosed with ALL between 1990 and 1997 within the UK (Eden *et al.* 2000). One of the main aims of the trial was to assess the efficacy of several types of pre-symptomatic central nervous system (PCNS) therapy. The results of the trial indicated that the 8-year disease-free survival rate was 60% and was similar between PCNS therapies (Eden *et al.* 2000).

In addition, many children treated with UKALL XI protocols were also enrolled in a nationwide neuropsychological study. The UKALL XI Neuropsychological study aimed to assess the implications of PCNS treatment on cognitive and behavioural function in children diagnosed with ALL. This study involved the neuropsychological assessment of children within the first six months post-diagnosis, and subsequently at 3-, and 5-years post-diagnosis. At each assessment an age-appropriate battery of tests designed to tap general functioning, language, memory and learning, visual perceptual abilities, fine motor skills, and academic achievement was administered. The UKALL XI Neuropsychological study ran from October 1992 to March 2000.

Participants in the research detailed in this thesis were selected from the London based cohort of subjects studied as part of the UKALL XI Neuropsychological study.

2.2 Selection criteria

2.2.1 Survivors

32 LT survivors of ALL participated in this research. All children had been diagnosed with standard-risk ALL between 1991 and 1995, and received treatment in accordance with UKALL XI protocols. As part of the UKALL XI trial all children received four-weeks of induction therapy consisting of prednisolone, vincristine, asparaginase, daurubicin, and a single intrathecal MTX injection. Providing remission was achieved children were then randomised to receive one of two therapies during the PCNS treatment phase (Tables 2.1-2.2). PCNS therapy was either -

- (1) 3 doses of intrathecal methotrexate (IT MTX) + 3 doses of high dose intravenous methotrexate (HD MTX) + 3 doses of folinic acid rescue (FAR) (HD group) or
- (2) 3 doses of intrathecal methotrexate only (IT MTX) (IT group)

PCNS therapy was followed by further treatment during the continuing and late intensification phases. All children received a total of 16 doses of IT MTX and cranial irradiation was not administered to any child.

In addition, children had no evidence of CNS involvement at diagnosis (i.e. blast cells not detected in CSF) and were treated without complications (e.g. no signs of encephalopathy). All children were in their first continuous remission (i.e. no relapses) and at least 6 years post-diagnosis. Finally, all children had been monitored as part of the UKALL XI Neuropsychological study having completed at least two of three follow-up assessments (i.e. at diagnosis and at 3 years post-diagnosis, at diagnosis and at 5 years post-diagnosis, at 3 years and at 5 years post-diagnosis, or at diagnosis, at 3- and at 5- years post-diagnosis) in a London testing centre (e.g. Great Ormond Street Hospital for Children, St Bartholomew's Hospital, the Royal Marsden Hospital, or the Norfolk and Norwich Hospital).

Table 2-1 UKALL XI protocol for children in the HD group (Weeks 1 – 29 of 100)

Weeks	Induction			PCNS			Continuing										Late		Continuing*											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
Treatment																														
HD MTX**						X		X		X																				
FAR						X		X		X																				
IT MTX***	X	X			X	X		X		X		X											X						X	
L-asparaginase	X	X	X																											
Vincristine	X	X	X	X	X						X					X					X		X						X	
Daunorubicin	X																						X							
Prednisolone	X	X	X	X							X					X					X		X						X	
Etoposide																							X							
Cytarabine																							X							
Thioguanine																							X							
Mercaptopurine					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					X	X	X	X
Oral MTX												X	X	X	X	X	X	X	X	X	X	X					X	X	X	X
Cotrimoxazole											X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Late = late intensification

(*) Continuing therapy (a repeating cycle involving the administration of IT MTX every 12 weeks to week 100)

(**) HD MTX dose by age: 1-4 years = 8 g/m²; 4-15 years = 6 g/m².

(***) IT MTX dose by age: 1 year = 7.5 mg; 2 years = 10 mg; 3-15 years = 12.5 mg.

Table 2-2 UKALL XI protocol for children in the IT group (Weeks 1 – 26 of 100)

Weeks	Induction			PCNS				Continuing											Late			Continuing*					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	
Treatment																											
IT MTX**	X	X			X	X	X	X	X											X				X			
Asparaginase	X	X	X																								
Vincristine	X	X	X	X	X				X			X					X			X			X				
Daunorubicin	X																			X							
Prednisolone	X	X	X	X					X			X					X			X			X				
Etoposide																				X							
Cytarabine																				X							
Thioguanine																				X							
Mercaptopurine					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					X	X	X	X
Oral MTX									X	X	X	X	X	X	X	X	X	X					X	X	X	X	
Cotrimoxazole					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Late = late intensification

(*) Continuing therapy (a repeating cycle involving the administration of IT MTX every 12 weeks to week 100)

(**) IT MTX dose by age: 1 year = 7.5 mg; 2 years = 10 mg; 3-15 years = 12.5 mg.

85 children were identified from the UKALL XI database matching these criteria. Initially, letters of invitation were sent to parents of the group of children assessed longitudinally as part of the UKALL XI Neuropsychological study (N=37). 57% (21/37) of these families agreed to participate in the current research. To increase the number of participants letters were sent to the remaining 48 families. From the second round of letters a further 11 families agreed to participate. The previous neuropsychological assessments completed by each of the participants included in the current study are shown in Table 2.3.

The main reasons given for declining to participate were not wishing for the child to miss any further days of school, the family wanting to move forward from the child's illness, and travel constraints.

The 32 survivors included in the current study represented 2% (32/1531) of all of the children diagnosed with standard-risk ALL and treated in accordance to UKALL protocols. The sample also represented 8% (32/419) of all standard-risk children assessed on at least one occasion at any testing centre in the UK as part of the UKALL XI Neuropsychological study.

Table 2-3 Number of children completing at least two assessments as part of the UKALL XI Neuropsychological study

	Assessments completed at diagnosis and at 3 years post-diagnosis	Assessments completed at diagnosis and at 5 years post-diagnosis	Assessments completed at 3 years and at 5 years post-diagnosis	Assessments completed at diagnosis, at 3 years and at 5 years post-diagnosis
Complete London cohort				
HD	11	1	18	19
IT	5	1	12	18
Participants in the current study				
HD	2	0	3	11
IT	2	0	4	10

2.2.2 Siblings

To account for the role of genetic and environmental factors (e.g. associated family stress) on brain and cognitive development a control group of siblings was included in the current study. In addition, previous studies have noted that children treated for ALL have high pre-morbid IQ's (i.e Full-scale IQ > 100)(Moss *et al.* 1981; Jannoun 1983; Giralt *et al.* 1992). It was therefore more appropriate to compare children to their siblings³ than test norms or other groups (e.g. children treated for other cancers or age-, sex- matched controls).

It was necessary for each sibling to have been assessed at least once as part of the UKALL XI Neuropsychological study to allow comparisons between groups over the early years post-diagnosis. 17 survivors had siblings meeting these criteria and 83% (14/17) participated in this study. 7 children were siblings of survivors in the HD group and 7 were siblings of survivors in the IT group. 13/14 had been assessed as part of the 5 years post-diagnosis follow-up (Table 2.4)

Table 2-4 Number of assessments each sibling completed as part of the UKALL XI Neuropsychological study

Group	Diagnosis only	3 years only	5 years only	Diagnosis + 3 years	Diagnosis + 5 years	3 + 5 years	All time points
Siblings	0	0	1	1	1	6	5

2.2.3 Ethics

The study described in this thesis received ethics approval from the Great Ormond Street Hospital for Children NHS trust/Institute of Child Health Research Ethics Committee. To participate in the current study children were asked to attend two appointments at Great Ormond Street Hospital for Children NHS trust. At the appointments children completed an MRI scan and a detailed neuropsychological assessment. For all children the appointments occurred on the same day.

Parents and children received separate written information sheets about the study and discussed the study with the researchers before to taking part. Parents of all children, and young adults over the age of 16 years provided written consent prior to any testing or scanning. The Information Sheets and Consent forms used in the study can be seen in Appendix D.

³ In the normal population, siblings reared together have similar estimates of IQ (Kaufman 1994)

Ethical issues relating to consent

The current project was a new study of the UKALL XI cohort. Parents were informed that the current project should, therefore, be considered separately from the earlier UKALL XI Neuropsychology study. Children were not obliged to take part even though they had participated in the earlier study. Parents were also free to withdraw their child from the current project at any time.

Ethical issues relating to MRI scan

Prior to the scan, the radiographer discussed MRI safety issues with parents and anyone whose safety may have been compromised during the scan did not complete this part of the assessment. In the current study this included one sibling with a metal plate recently implanted in her body. The radiographer also discussed the potential of detecting brain abnormalities in healthy children. In the event that an abnormality was detected parents were told that they would be contacted within two weeks by the radiologist. If parents were not contacted then there were no abnormality detected in the MRI scan. In the event that such an abnormality was found in the scans the participant would have been invited back to the hospital and offered the appropriate medical help and counselling.

During the scan, participants listened to music to minimise the noise of the scanner environment. Children were also permitted to have a parent/researcher with them in the scanner room. The radiographer was also able to talk to the child periodically during the scan to check that the child was OK. Children held a buzzer throughout the scan. Children were instructed to press the buzzer if they became scared and wanted to come out of the scanner at any stage. In the present study, two children used the buzzer and were immediately removed from the scanning environment. Both children did not wish to re-enter the scanner at a later time and therefore scans were not acquired for these children.

Ethical issues relating to neuropsychological assessment

Following the assessment a short report summarising the performance for each child was produced. The report included performance on general, standardised measures of function (e.g. WISC, TeaCH). Performance on non-standardised experimental tasks was not included. A copy of the report was sent to each child's parents. All reports included information describing, in simple terms, how the results should be interpreted. All reports were co-signed by the supervising neuropsychologist on the project. Parents

were encouraged to contact the researchers if they had any queries about the content of their child's report.

Ethical issues relating to confidentiality

Data were stored in a lockable filing cabinet and coded to protect each child's identity. The master file revealing the code for each child was stored separately. Information that could identify an individual child was removed before data were presented for discussion or written up for publication.

2.3 Participant characteristics

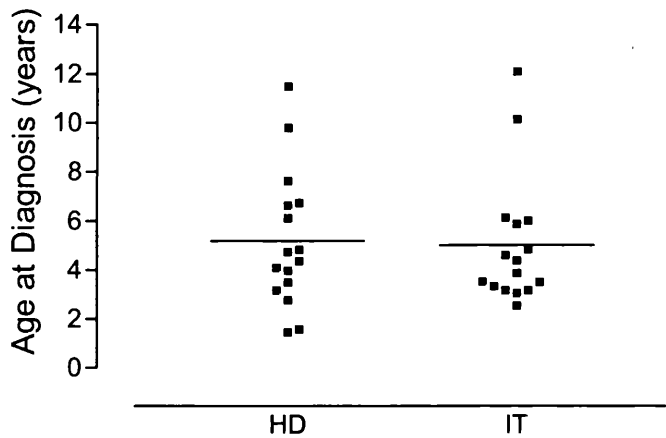
Within the literature the LT outcome of children treated for ALL has been associated with age-at-diagnosis, the length of follow-up, sex, and socioeconomic status (SES). For example, it has been reported that children treated with combined cranial irradiation and chemotherapy at a younger age show greater LT impairments than children treated at an older age (Meadows *et al.* 1981; Jannoun 1983 1136; Waber *et al.* 1990; Moore *et al.* 1991; Butler *et al.* 1994). Similarly, survivors show greater impairments the longer the time of assessment from diagnosis (Meadows *et al.* 1981; Cousens *et al.* 1988; Rubenstein *et al.* 1990). Furthermore, reports suggest that impairments are greater for girls than boys treated with combined irradiation and chemotherapy (Waber *et al.* 1990; Mulhern *et al.* 1991). Finally, lower parental education (Whitt *et al.* 1984) and SES (Butler *et al.* 1994) have been associated with poor performance across all domains of function in survivors of ALL. This relationship is particularly evident over the early years (1-3 years) following diagnosis (Copeland *et al.* 1996).

While the associations between these variables and outcome have not always been supported or evaluated (Rowland *et al.* 1984), particularly among LT survivors treated without cranial irradiation (Copeland *et al.* 1996), it was necessary to assess each factor as a potential confound of test performance in the following chapters. For this reason the next section describes each group participating in this research in terms of age-at-diagnosis, follow-up, age-at-test, sex, and SES.

2.3.1 Age-at-diagnosis

As expected, due to a higher rate of diagnosis in children aged less than 5 years than in older children, the distribution of age-at-diagnosis was positively skewed. However, the range in age-at-diagnosis was similar in the two treatment groups (HD group: 1.5 – 11.5 years; IT group: 2.6 –12.2 years) and there was no significant difference observed between the groups (Mann Whitney U (MWU): $z = -0.38$; $p = 0.71$)(Figure 2.1).

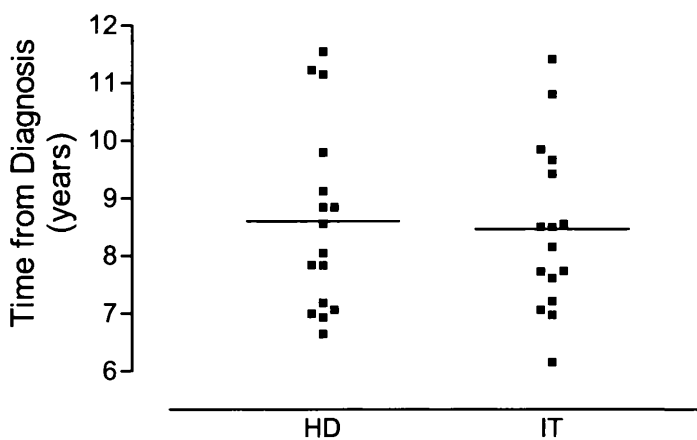
Figure 2-1 Age-at-diagnosis in each treatment group



2.3.2 Length of follow-up

There was no significant difference in the length of follow-up between the treatment groups (Independent samples t test (Indep.t test): $t = 0.27$; $p=0.80$; 95%CI (-0.96:1.25)(Figure 2.2).

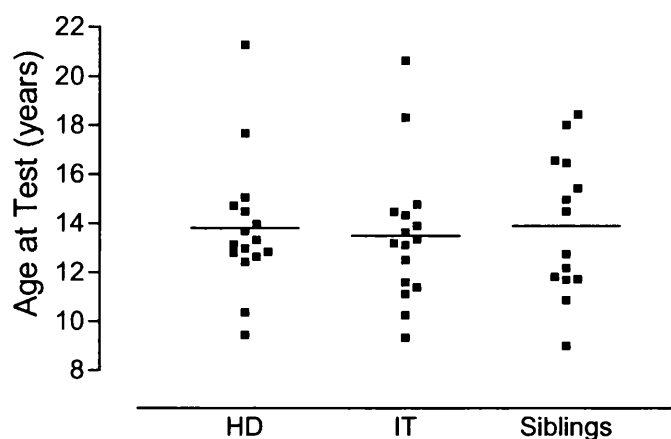
Figure 2-2 Length of follow-up in each treatment group



2.3.3 Age-at-test

The distribution of age-at-test was also positively skewed, presumably reflecting the higher rate of diagnosis in younger children and similar length of follow-up. Again, there was no significant difference observed between the groups (Kruskal Wallis (KW): $\text{Chi}^2 = 0.31$; $\text{df} = 2$; $p = 0.86$)(Figure 2.3).

Figure 2-3 Age-at-test



2.3.4 Sex

The number of boys and girls within and between treatment groups was similar. There were nearly twice as many girls as boys in the sibling group. However, this was not significant ($\text{Chi}^2 = 1.31$; $\text{df}=2$; $p=0.52$)(Table 2.5).

Table 2-5 Number of boys and girls

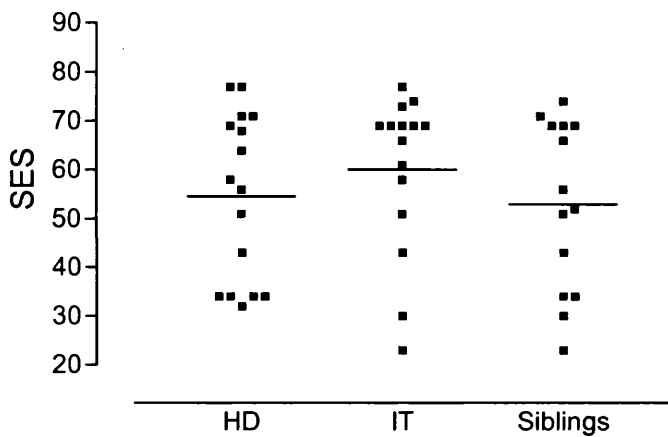
	Boys	Girls	Total
HD	9	7	16
IT	7	9	16
Siblings	5	9	14
Total	21	25	46

2.3.5 SES

SES was estimated using the International Socio-Economic Index of Occupational Prestige (ISEI). This scale attempts to measure the attributes of occupation that convert a person's education into income using the father's job title (Ganzeboom *et al.* 1996) 'Father' was defined as the major paternal caregiver for each child. A distinction was not made between biological and stepfathers.

Using the ISEI, job titles were scored between 16 and 90, where 16 represented the lowest SES score (e.g. farmhands, cleaners) and 90 the highest SES score (e.g. judge). There were no significant differences between groups on the estimate of SES (ANOVA: $F(2, 42) = 0.72, p = 0.49$)(Figure 2.4).

Figure 2-4 SES



2.3.6 Summary

To summarise, there were no significant differences between groups on the variables of age-at-diagnosis, follow-up, age-at-test, sex, or SES (Table 2.6).

Table 2-6 Summary characteristics of the participants

	N	Age-at-diagnosis (X ± SEM)	Follow-up (X ± SEM)	Age-at-test (X ± SEM)	Sex (M:F)	SES (X ± SEM)
HD	16	5.20 (0.7)	8.61 (0.4)	13.81 (0.7)	9:7	54.56 (4.3)
IT	16	5.04 (0.7)	8.47 (0.4)	13.51 (0.7)	7:9	60.07 (4.2)
Siblings	14	-	-	13.90 (0.8)	5:9	52.93 (4.7)

2.4 Statistical considerations

In an attempt to minimise the large number of analyses conducted as part of this thesis specific analyses were planned *a priori*. For normally distributed data⁴, statistical analyses (except where otherwise stated) were conducted using an analysis variance (ANOVA) model with planned comparisons. The contrasts used in the ANOVA models are shown in Table 2.7. These contrasts were based on the main hypotheses detailed in Chapter 1 (i.e. the Survivor group would be impaired relative to the Sibling group, and, the IT group would be impaired relative to the HD group). Results have been reported without the assumption of equal variance when the homogeneity of variance⁵ was violated. In addition, confounding factors (e.g. age-at-test) were included in a covariance model where appropriate.

Transformations were performed when data were not normally distributed and parametric analyses were conducted and reported on the transformed data. When the transformation failed to normalise the distribution of data, statistical analyses were conducted using non-parametric analyses (e.g. Kruskal Wallis test). The Chi square test was used when data were categorical.

Table 2-7 Planned contrasts used in analyses

Contrast	HD	IT	Siblings
Survivors vs.Siblings	-0.5	-0.5	1
IT vs. HD	1	-1	0

Power of the study

To calculate the power of the study it was assumed that the data were normally distributed, there was equal variance between groups, the hypotheses were unidirectional, and the confidence interval was 95% (i.e. $\alpha = 0.05$). Using these parameters, the power of the study to detect a reduction in the mean for the survivor group (Mean=85, SD=15, N=32) when compared to the sibling group (Mean=100, SD=15, N=14) of one standard deviation (Large effect size =1) was 0.92. The power of the study to detect a reduction in the mean for the survivor group when compared to the sibling group of half a standard deviation (Medium effect size = 0.5) was 0.46.

⁴ Normality was assessed using the Shapiro Wilks test.

⁵ The homogeneity of variance was assessed using the Levene's test.

The power of the study to detect a meaningful difference between treatment groups was lower. For example, the power of the study to detect a significant reduction in the mean for the IT group (Mean=85, SD=15, N=16) relative to the HD group (Mean 100, SD=15, N=16) of one standard deviation was 0.87. The power to detect a reduction of half a standard deviation in the IT group relative to the HD group was 0.40.

2.5 Retrospective neuropsychological analyses

2.5.1 Introduction

As all children participating in the current research were assessed previously, the next section describes some of the data collected for these children as part of the UKALL XI Neuropsychological study. On the basis of these data, deviations in performance at diagnosis and over the early years post-diagnosis can be identified. In addition, these data provide a LT assessment (i.e. at 5 years post-diagnosis) across many domains of function. Together, these data are important for the interpretation of task performance reported in subsequent chapters.

2.5.2 Methods

2.5.2.1 Developmental milestones

The age at which each child began sitting, walking, and talking was used as an index of early development. Parents provided this information and normal development was defined as sitting before 12 months, walking before 18 months, and talking before 24 months. Delayed development was defined as one or all of the following: first sitting after 12 months, first walking after 12 months, or first talking after 24 months.

2.5.2.2 Intelligence

Intelligence was assessed during the first five years post-diagnosis using the age-appropriate Wechsler scales (i.e. at diagnosis, at 3 years, and 5 years post-diagnosis). Children aged 2:0 – 5:11 years at the time of testing were assessed on the Wechsler Pre-school and Primary Scale of Intelligence – Revised UK Edition (WPPSI-R^{UK}) (Wechsler 1990). Children aged 6:0 – 16:11 years were assessed on the Wechsler Intelligence Scale for Children – 3rd UK Edition (WISC-III^{UK}) (Wechsler 1992) and participants 17:0 years or older were assessed on the Wechsler Adult Intelligence Scale – Revised UK Edition (WAIS-R^{UK}) (Wechsler 1986).

Each test consisted of a number of subtests designed to assess different aspects of function. All subtests were administered and scored in accordance with the appropriate manual. A standard score reflecting Verbal and Non-verbal IQ was obtained from each assessment. Briefly, the Verbal IQ (VIQ) score reflected performance on the Information, Similarities, Vocabulary, Arithmetic, Comprehension, and Digit span (included for the WAIS-R^{UK} only) subtests (Table 2.8). The Non-verbal IQ (PIQ) estimates reflect performance across the Picture completion, Block design, and Object assembly subtests combined with the Geometric design, and Mazes subtests on the WPPSI-R^{UK}, or the Picture arrangement, and Coding (Digit symbol) subtests on the WISC-III^{UK} and WAIS-R^{UK} (Table 2.9).

Supplementary indices of function were also calculated for children assessed on the WISC-III^{UK}. The Verbal comprehension index reflected performance on the Information, Similarities, Vocabulary, and Comprehension subtests. The Perceptual Organisation index was calculated from the Picture completion, Block design, Object assembly, and Picture arrangement subtests. The Freedom from distractibility and the Processing speed indices reflected performance on the Arithmetic and Digit span subtests, and the Coding and Symbol search subtests respectively.

Table 2-8 Subtests comprising the Verbal IQ estimate on each Wechsler scale

WPPSI-R ^{UK}	WISC-III ^{UK}	WAIS-R ^{UK}
Information	Information	Information
Similarities	Similarities	Similarities
Arithmetic	Arithmetic	Arithmetic
Vocabulary	Vocabulary	Vocabulary
Comprehension	Comprehension	Comprehension
		Digit span

Table 2-9 Subtests comprising the Non-Verbal IQ estimate on each Wechsler scale

WPPSI-R ^{UK}	WISC-III ^{UK}	WAIS-R ^{UK}
Picture completion	Picture completion	Picture completion
Block design	Block design	Block design
Object assembly	Object assembly	Object assembly
Geometric design	Coding	Digit symbol
Mazes	Picture arrangement	Picture arrangement

2.5.2.3 Receptive language

The British Picture Vocabulary Scale – Long form (BPVS)(Dunn 1982) was used to assess Receptive vocabulary at diagnosis, 3 years, and 5 years post-diagnosis in the LT survivors and their siblings. This test is a modified version of the Peabody Picture Vocabulary Test (PPVT)(Dunn & Dunn 1981) and has been adapted for the assessment of children living in Britain. All children aged 2:6 – 17:11 years at the time of assessment were assessed on this measure.

The BPVS was administered and scored in accordance with the manual. Briefly, children were asked to match a word spoken by the examiner to one of four images placed in front of them. Children complete items until a critical range of performance was established. The critical range for each subject spanned items between a basal level of performance (highest 8 consecutive responses) and a ceiling level of performance (lowest 8 consecutive responses containing 6 errors). The number of correct response obtained across this critical range was then converted to the standard score. The standard score was considered a measure of receptive vocabulary.

2.5.2.4 Fine motor function

The Annett peg-sorting task (Annett 1985) was used to assess fine motor function at diagnosis, 3 years, and 5 years post-diagnosis. In this task participants placed ten pegs in a ten holes on a board as quickly as possible. Average times were calculated for the dominant and non-dominant hands over five trials. Average times were then corrected for age using the norms in the manual. The age-corrected scores were considered measures of dominant and non-dominant fine motor skill.

2.5.2.5 Memory and learning

The Wechsler Memory Scale with age corrections for children (WMS)(Wechsler 1945)(Kimura & McGlone 1979) was used to assess memory and learning in the LT survivors and their siblings. As this measure was appropriate for children over the age of 6 years at the time of the assessment, memory and learning skills are reported for children at the 3 and 5 years post-diagnosis assessments only. Children at 3 years completed Form I and children at 5 years completed Form II.

Five measures of function provided indices of memory and learning at each assessment (i.e. Verbal immediate memory, Verbal delayed memory, Visual immediate memory, Visual delayed memory, and Paired associate learning)(Table 2.10). Verbal immediate memory reflected the average amount of information immediately recalled following the presentation of two short stories. Verbal delayed memory was the average amount of story information recalled after a filled 90-minute delay. Visual immediate memory reflected the child's ability to reproduce three geometric designs immediately following presentation. Visual delayed memory was the recall of these designs after a filled 40-minute delay. The index of Paired associate learning was a weighted score incorporating total number of correct paired associates learnt over three immediate trials and the number of correct paired associates recalled following a 90 minute delay.

For the verbal memory subtests children less than 12 years received the two stories developed by Kimura & McGlone (1979), while children older than 12 years received the Wechsler (logical memory) stories. For the visual memory subtests all children received the standard geometric designs. Similarly, all children received ten standard word-pairs for the paired associates subtest. This set consisted of six related items (e.g. up-down) and four unrelated pairs (e.g. cabbage-pen). Indices of memory and learning were converted to z scores using norms collected within the department.

Table 2-10 Indices of memory and learning taken from the WMS

Index of memory and learning	Measure
Verbal immediate memory	Immediate story recall
Verbal delayed memory	Delayed (+90 minutes) story recall
Visual immediate memory	Immediate design recall
Visual delayed memory	Delayed (+40 minutes) design recall
Paired associate learning	$\frac{1}{2} * (\text{Trial 1-3 PA learning}) + (\text{Delayed PA (+90 minutes)})$

PA = Paired associate

2.5.2.6 Visual perception

The Judgement of Line Orientation (Benton *et al.* 1983) was used to assess visual perception in the LT survivors and their siblings. This measure was administered to children over the age of 6 years. Visual perception has therefore been reported for children at the 3 and 5 years post-diagnosis assessments only. Children at 3 years completed Form V and children at 5 years completed Form H. Both versions presented the same test items but in a different order.

The Judgement of Line Orientation was administered in accordance with the manual beginning with 5 practice items and then the 30 test items. As norms were not available for all children aged > 6 – 16 years, raw scores reflecting the total number of trials correct are reported (maximum 30).

2.5.2.7 Academic attainments

The Wechsler Objective Reading Dimensions (WORD)(Wechsler 1993) and Wechsler Objective Numerical Dimensions (WOND)(Wechsler 1996) were administered to assess academic achievement in the LT survivors and their siblings. Each test was administered as part of the 5 years post-diagnosis assessment. All children completing these assessments were attending school.

Five indices of academic achievement were obtained; three from the WORD (i.e. Reading, Spelling, and Reading comprehension) and two from the WOND (i.e. Mathematical reasoning and Numerical operations). For each index, raw scores were converted to age corrected standard scores using the appropriate manual. The reading subtest involved reading single words of increasing difficulty, the spelling subtest involved writing single words from dictation, and the reading comprehension subtest required children to answer questions about short passages of text. The mathematical reasoning subtest involved problem solving and understanding of mathematical concepts (e.g. quantity, measurement, graphs, and geometry), while in the numerical operations subtest, children solved computational problems on paper (e.g. operations such as addition, subtraction, multiplication, and division). Each subtest began with easier items and progressed to include more difficult items. For example, the numerical operations subtest began with simple addition problems. Participants worked with decimals, fractions, and basic algebraic equations in later items.

2.5.3 Results

2.5.3.1 Developmental milestones

Generally, the developmental milestones of sitting, walking, and talking were achieved within the normal age range for all participants in each group (Table 2.11 (a), (b) and (c)). However, four children were considered to show signs of delay in language development. These children began talking at 27, 30, and 36 months (Table 2.12). The children were divided between the HD (N=1), IT (N=2), and sibling (N=1) groups.

With respect to the timing of diagnosis and early development, parents reported the majority of children were sitting, walking, and talking prior to diagnosis. This was true for all survivors except one child in the HD group. This child was diagnosed at 17 months of age and, although walking, had not begun to talk at that time. This child showed signs of developmental delay, not talking until 27 months.

Table 2-11 Developmental milestones for each group (in months).

(a) Sitting			(b) Walking		
Group	Median (range)	No delayed (>12 months)	Group	Median (range)	No delayed (>18 months)
HD	6 (4 –7)	0	HD	11.5 (9 – 16)	0
IT	6 (5 – 8)	0	IT	12 (10 –14)	0
Siblings	6 (5 – 9.5)	0	Siblings	12 (9-16)	0

(c) Talking		
Group	Median (range)	No delayed (>12 months)
HD	18 (12 – 27)	1
IT	18 (12 – 36)	2
Siblings	18 (6 – 30)	1

Table 2-12 Characteristics of the four children considered delayed (in months).

Group	Sitting	Walking	Talking	Age-at-diagnosis
HD	6	15	27	17
IT	5	12	27	42
IT	5	10	36	38
Siblings	9.5	11.5	30	-

2.5.3.2 Intelligence

The number of children with estimates of Verbal and Non-verbal IQ at each of the three assessments is shown in Table 2.13. As a limited number of children participated in all three assessments, individual ANOVA's were conducted at each time point instead of using a repeated measures design.

The number of children assessed on each of the Wechsler scales at diagnosis, 3, and 5 years post-diagnosis are shown in Table 2.14. At diagnosis, approximately 52% of all children assessed had been tested on the WPPSI-R^{UK} and 48% on the WISC-III^{UK}. In contrast, at 3 years (73%), and 5- years (93%) post-diagnosis the majority of children had been assessed using the WISC-III^{UK}.

Table 2-13 Number of participants with estimates of Verbal and Non-verbal IQ at diagnosis, 3 years and 5 years post-diagnosis.

	N	Assessed at diagnosis	Assessed at 3 years	Assessed at 5 years	Assessed at all points
HD	16	12	16	14	11
IT	16	12	16	14	10
Siblings	14	7	12	13	5
Total (missing)	46	31 (15)	44 (2)	41 (5)	26 (20)

Table 2-14 Number of participants assessed on each Wechsler scale at diagnosis, 3 years and 5 years post-diagnosis.

Group	N	Diagnosis			3 years			5 years		
		Wppsi	Wisc	Wais	Wppsi	Wisc	Wais	Wppsi	Wisc	Wais
HD	16	6	6	0	4	12	0	0	14	0
IT	16	7	5	0	4	12	0	0	13	1
Siblings	14	3	4	0	2	10	0	2	11	0

Wppsi = Wechsler Pre-school and Primary scale of Intelligence-R^{UK}

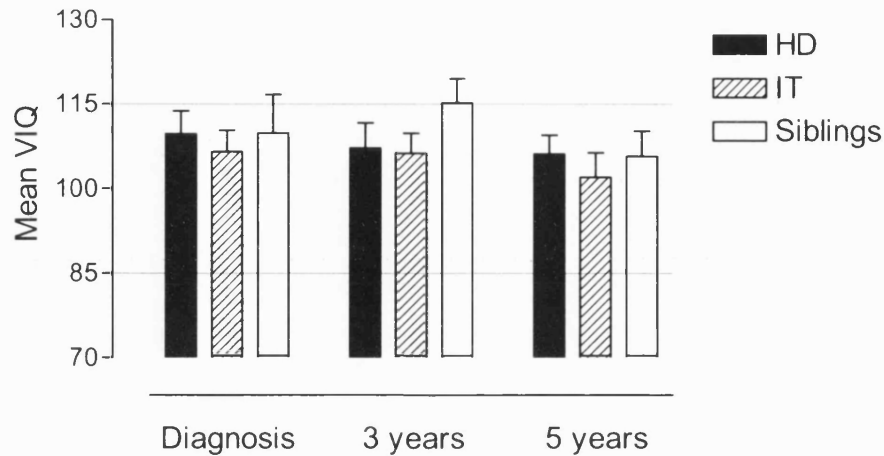
Wisc= Wechsler Intelligence Scale for Children-III^{UK}

Wais = Wechsler Adult Intelligence Scale-R^{UK}

Verbal IQ

There were no significant differences between groups on the estimate of Verbal IQ at diagnosis, 3 years, or 5 years post-diagnosis (Diagnosis ANOVA: $F(2, 28) = 0.18, p = 0.84$; 3 years ANOVA $F(2, 41) = 1.27, p = 0.29$; 5 years ANOVA $F(2, 38) = 0.32, p = 0.73$)(5 years Effect size =0.14)(Figure 2.5). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (5 years Matched sample t test: $t = 0.90, p = 0.34, \text{Effect size} = 0.32$).

Figure 2-5 Verbal IQ at diagnosis, 3 years, and 5 years post-diagnosis (Mean ± SEM)

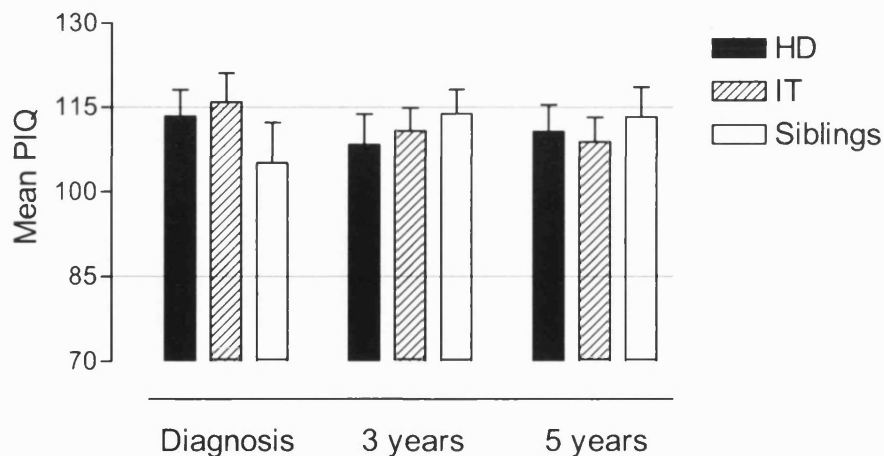


Lines on the graph represent the normal range (i.e. 85-115).

Non-verbal IQ

Similarly, there were no significant differences between groups on the estimate of Non-verbal IQ at diagnosis, 3 years, or 5 years post diagnosis (Diagnosis ANOVA: $F(2, 28) = 0.87, p = 0.43$; 3 years ANOVA: $F(2, 41) = 0.32, p = 0.73$; 5 years ANOVA: $F(2, 38) = 0.21, p = 0.81$)(5 years Effect size = 0.07)(Figure 2.6). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (5 years Matched sample t test: $t = 0.54, p = 0.59$, Effect size = 0.19).

Figure 2-6 Non-verbal IQ at diagnosis, 3 years, and 5 years post-diagnosis (Mean ± SEM)



Lines on the graph represent the normal range (i.e. 85-115).

IQ change over time

27 (HD=11; IT=10; and Sibling=6) participants completed IQ assessments at diagnosis and 5 years post-diagnosis. The children showing a decrease of 15 points or more are listed in Tables 2.15-2.16. A loss of 15 points was selected as this (1) reflected two to three times the amount of change expected when switching versions of the IQ test; (2) was at least one SD based on the test norms; (3) was three to four times the SE of measurement associated with each test; and (4) represented a clinically significant finding (Mulhern *et al.* 1991).

The five children showing a decline in VIQ were all girls. The majority (4/5) of children showing a decrease in PIQ had high PIQ estimates at diagnosis (i.e. > 118). There was no qualitative relationship evident between the children displaying the greatest decreases in IQ and group, SES, or age-at-diagnosis.

Table 2-15 Children showing a decrease of 15 or more VIQ points between diagnosis and 5 years

Group	Sex	SES	Age-at-diagnosis	VIQ diagnosis	VIQ 5 year	VIQ Change
HD	F	34	4.1	100	83	-17
HD	F	43	9.8	116	98	-18
IT	F	73	6.2	102	84	-18
IT	F	-	3.2	100	79	-21
IT	F	69	10.2	123	101	-22

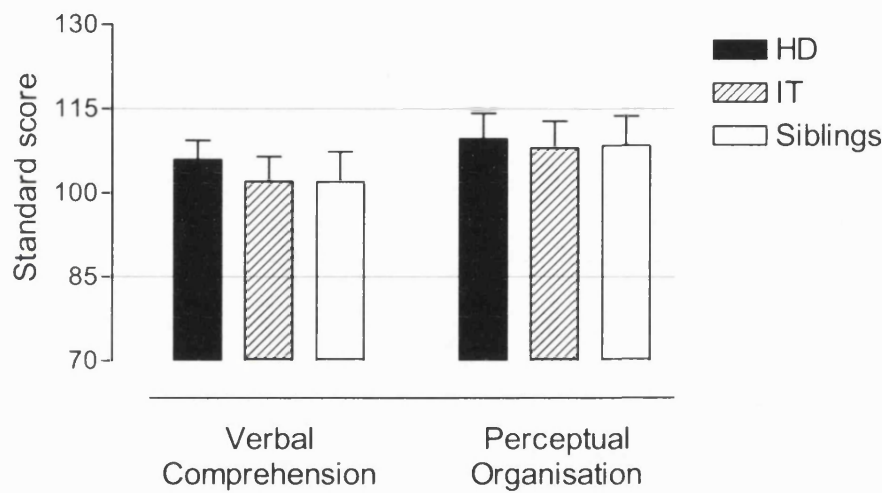
Table 2-16 Children showing a decrease of 15 or more PIQ points between diagnosis and 5 years

Group	Sex	SES	Age-at-diagnosis	PIQ diagnosis	PIQ 5 year	PIQ Change
HD	F	69	4.9	118	103	-15
IT	M	43	4.9	133	112	-21
HD	M	77	4.4	133	109	-24
HD	F	51	4.8	93	68	-25
HD	F	77	3.2	141	112	-29

Indices of function at 5 years

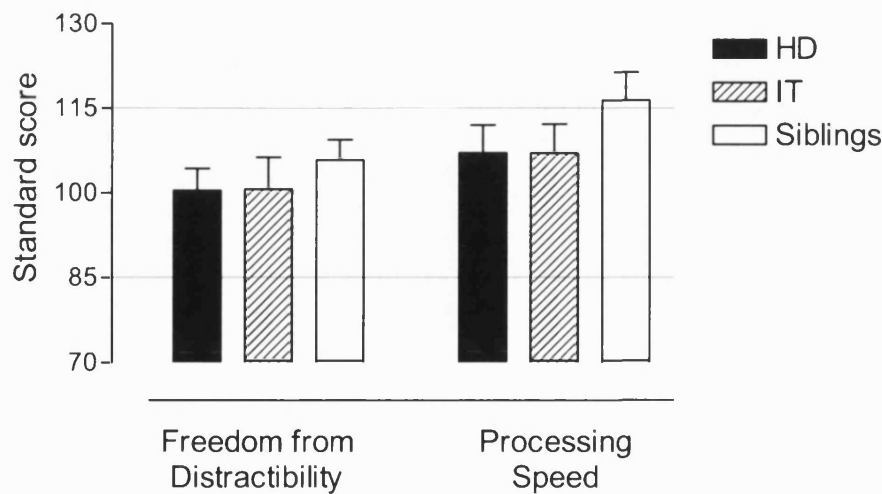
At 5 years post-diagnosis, there were no significant differences between groups on the indices of Verbal comprehension (ANOVA: $F(2, 35) = 0.28, p = 0.74$)(Figure 2.7); Perceptual organisation (ANOVA: $F(2, 35) = 0.03, p = 0.97$)(Figure 2.7); Freedom from distractibility (ANOVA: $F(2, 35) = 0.43, p = 0.67$)(Figure 2.8); or Processing speed (ANOVA: $F(2, 33) = 0.95, p = 0.40$)(Figure 2.8).

Figure 2-7 Verbal comprehension and Perceptual organisation at 5 years post-diagnosis (Mean \pm SEM)



Lines on the graph represent the normal range (i.e. 85-115).

Figure 2-8 Freedom from distractibility and Processing speed at 5 years post-diagnosis for each group (Mean \pm SEM)



Lines on the graph represent the normal range (i.e. 85-115).

2.5.3.3 Receptive language

The number of children completing the test of Receptive language at each of the three assessments is shown in Table 2.17. As a limited number of children participated in all three assessments individual ANOVA's were conducted at each time point instead of using a repeated measures design.

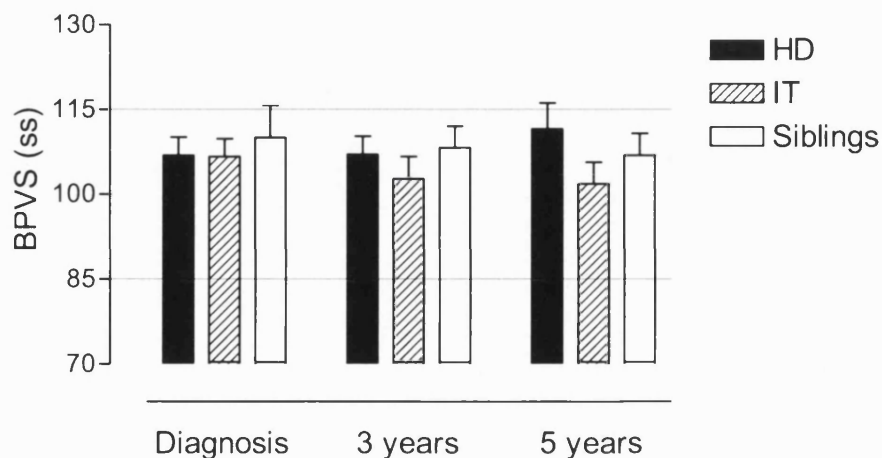
Table 2-17 Number of participants completing the test of receptive vocabulary at diagnosis, 3 years, and 5 years post-diagnosis.

	N	Assessed at diagnosis	Assessed at 3 years	Assessed at 5 years	Assessed at all points
HD	16	13	16	13	11
IT	16	12	16	14	10
Siblings	14	7	12	12	4
Total (missing)	46	32 (14)	44 (2)	39 (7)	25 (21)

Receptive language

There were no significant differences between groups on the test of receptive language at diagnosis, 3 years, or 5 years post-diagnosis (Diagnosis ANOVA: $F(2, 29) = 0.19, p = 0.83$; 3 years ANOVA: $F(2, 41) = 0.62, p = 0.55$; 5 years ANOVA: $F(2, 36) = 1.44, p = 0.25$)(5 years Effect size = 0.03)(Figure 2.9). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (5 years Matched sample t test: $t = 0.76, p = 0.47$, Effect size = 0.22).

Figure 2-9 Receptive vocabulary at diagnosis, 3 years, and 5 years post-diagnosis (Mean ± SEM)



Lines on the graph represent the normal range (i.e. 85-115).

2.5.3.4 Fine motor function

The numbers of children completing the test of fine motor function at diagnosis, 3 and 5 years post-diagnosis are presented in Table 2.18. As a limited number of children participated in all three assessments individual ANOVA's were conducted at each time point instead of using a repeated measures design.

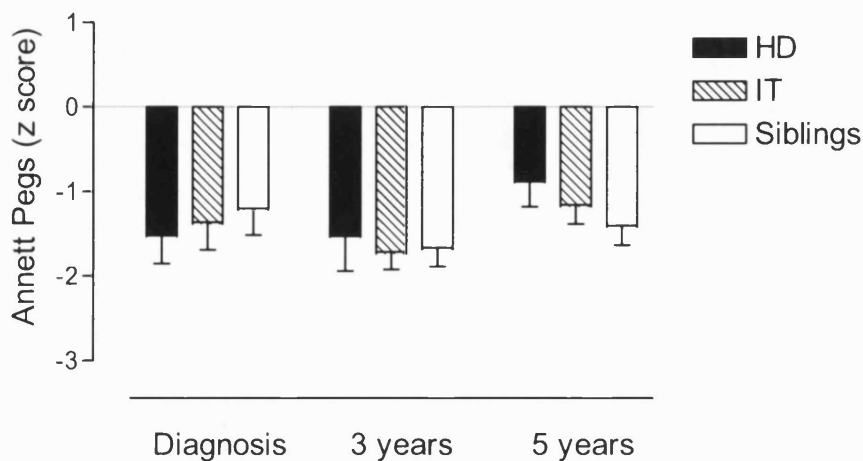
Table 2-18 Number of participants completing the test of fine motor function at diagnosis, 3 years, and 5 years post-diagnosis.

	N	Assessed at diagnosis	Assessed at 3 years	Assessed at 5 years	Assessed at all time points
HD	16	6	16	12	5
IT	16	8	15	14	6
Siblings	14	5	11	12	2
Total (missing)	46	21(25)	42 (4)	38 (8)	13(33)

Dominant hand

There were no significant differences between groups using the dominant hand at diagnosis, 3 years, or 5 years post-diagnosis (Diagnosis ANOVA: $F(2, 18) = 0.22, p = 0.81$; 3 years ANOVA: $F(2, 39) = 0.13, p = 0.88$; 5 years ANOVA: $F(2, 35) = 0.84, p = 0.31$)(5 years Effect size = 0.51)(Figure 2.10). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (5 years Matched sample t test: $t = 1.02, p = 0.34$, Effect size = 0.59).

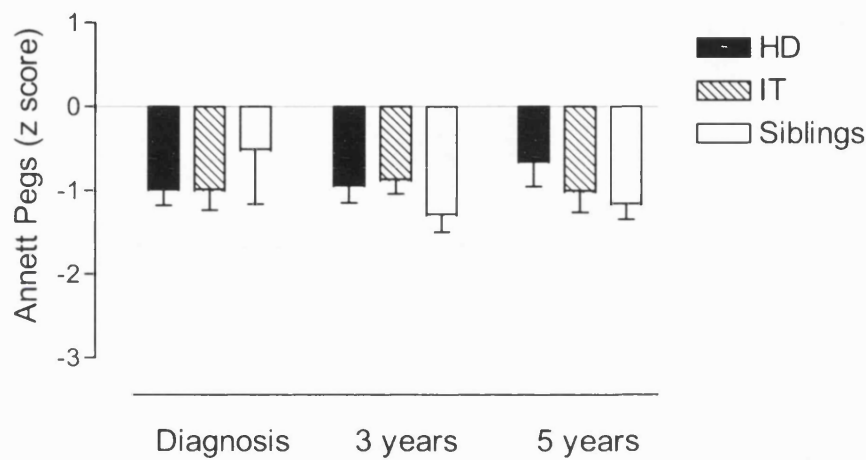
Figure 2-10 Fine motor function with dominant hand at diagnosis, 3 years, and 5 years post-diagnosis (Mean \pm SEM)



Non-dominant hand

Similarly, there were no significant differences between groups using the non-dominant hand at diagnosis, 3 years, or 5 years (Diagnosis ANOVA: $F(2, 18) = 0.56, p = 0.58$; 3 years ANOVA: $F(2, 39) = 1.32, p = 0.28$; 5 years ANOVA: $F(2, 35) = 7.12, p = 0.87$)(5 years Effect size = 0.54)(Figure 2.11). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (5 years Matched sample t test: $t = 0.66, p = 0.53$, Effect size = 0.30).

Figure 2-11 Fine motor function with non-dominant hand at diagnosis, 3 years, and 5 years post-diagnosis (Mean ± SEM)



2.5.3.5 Memory and learning

The number of children with memory and learning results at 3 and 5 years post-diagnosis are presented in Table 2.19.

Table 2-19 Number of participants completing the test of memory and learning at 3 years and 5 years post-diagnosis.

	N	Assessed at 3 years	Assessed at 5 years
HD	16	15	14
IT	16	15	14
Siblings	14	11	11
Total (missing)	46	41 (5)	39 (7)

Verbal immediate memory

There were no significant differences between groups on the estimates of Verbal immediate memory at 3 or 5 years post-diagnosis (3 years ANOVA: $F(2, 38) = 1.76, p = 0.19$; 5 years ANOVA: $F(2, 35) = 1.52, p = 0.23$)(Figure 2.12).

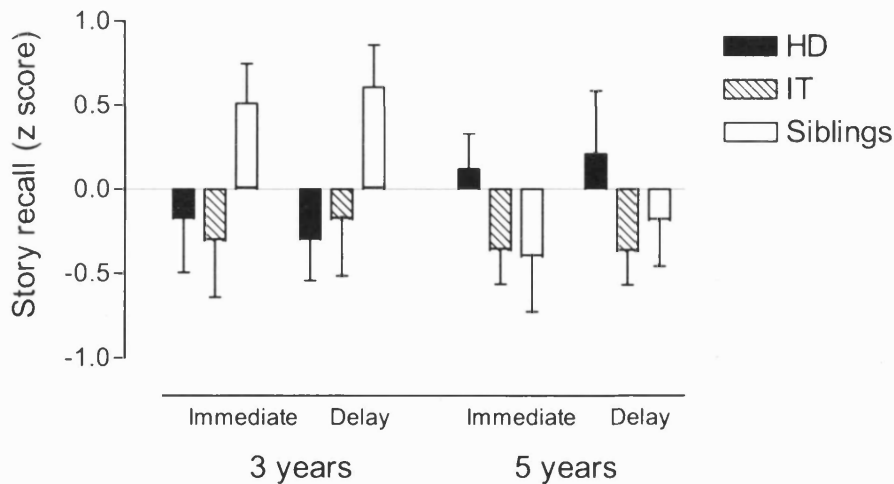
Verbal delayed memory

However, results from the Verbal delayed memory subtest indicated that there was a significant difference between the three groups, with the survivor groups performing significantly worse than the sibling group (ANOVA: $F(2, 37) = 2.62, p = 0.09$: Planned comparisons: Surv vs. Sibs $t = 2.28, df = 37, p = 0.03$; IT vs. HD $t = -0.30, df = 37, p = 0.76$, Effect size = 1.03). This difference was accounted for by Verbal IQ (ANCOVA: $F(2,36)=1.48, p=0.24$).

Despite this difference at 3 years, there were no significant differences between groups on Verbal delayed memory at 5 years post-diagnosis (ANOVA: $F(2, 35) = 1.19, p = 0.37$, Effect size = 0.12)(Figure 2.12).

The difference observed at 3 years post-diagnosis between survivors and siblings approached significance when the analysis was restricted to matched survivor-sibling pairs only (3 years Matched sample t test: $t = -1.78, p = 0.11$, Effect size = 0.96)(5 years Matched sample t test: $t = 0.69, p = 0.51$, Effect size = 0.25).

Figure 2-12 Verbal memory at 3 and 5 years post-diagnosis (Mean ± SEM)



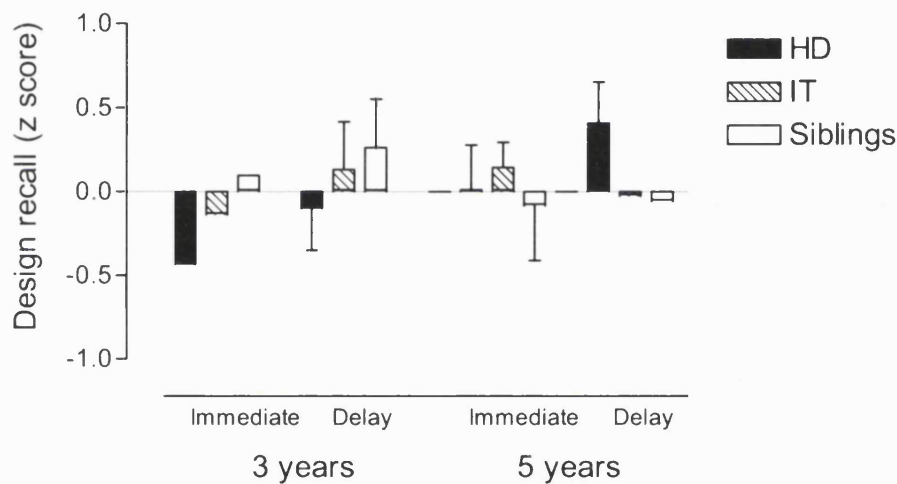
Visual immediate memory

There were no significant differences between groups on the estimates Visual immediate memory at 3 or 5 years post diagnosis (3 years ANOVA: $F(2, 38) = 0.69, p = 0.51$; 5 years ANOVA: $F(2, 36) = 0.21, p = 0.81$)(Figure 2.13).

Visual delayed memory

There were no significant differences between groups on the estimated of Visual delayed memory at 3 or 5 years post-diagnosis (3 years ANOVA: $F(2, 38) = 0.44, p = 0.65$; 5 years ANOVA: $F(2, 36) = 0.84, p = 0.45$)(Figure 2.13).

Figure 2-13 Visual memory at 3 and 5 years post-diagnosis (Mean \pm SEM)



Paired associate learning

Similarly, there were no significant differences between groups on Paired associate learning at 3 or 5 years post-diagnosis (3 years ANOVA: $F(2, 37) = 1.92, p = 0.16$ (Figure 2.14); 5 years ANOVA: $F(2, 36) = 0.66, p = 0.67$)(Figure 2.15).

Figure 2-14 Paired associate learning at 3 years post-diagnosis

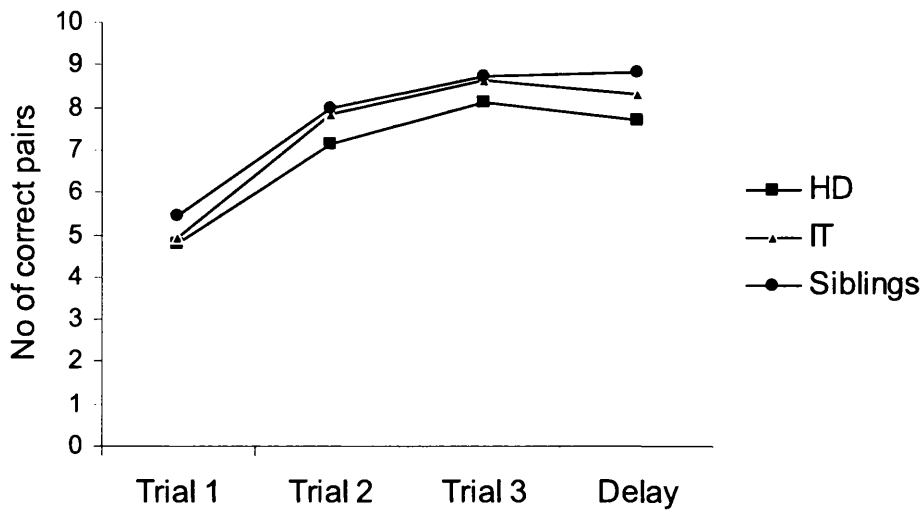
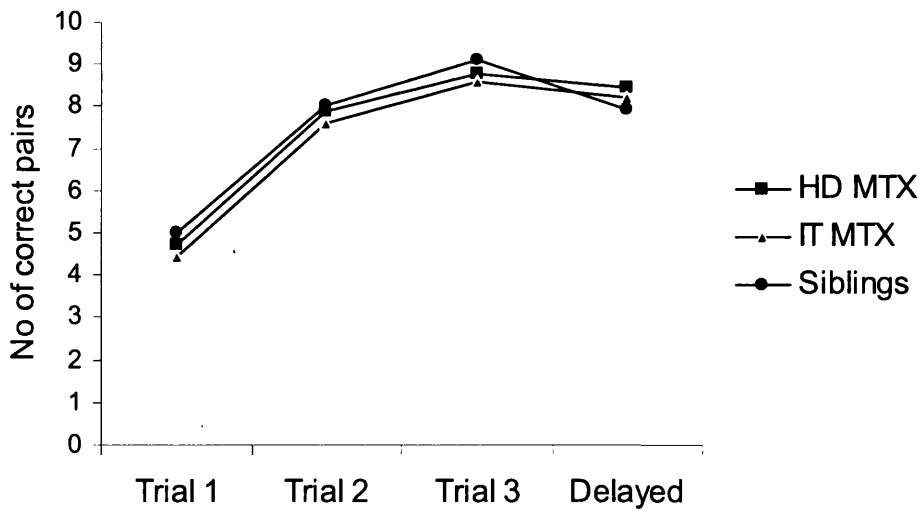


Figure 2-15 Paired associate learning at 5 years post-diagnosis



2.5.3.6 Visual perception

The numbers of children completing the test of visual perception at 3 and 5 years post-diagnosis are presented in Table 2.20.

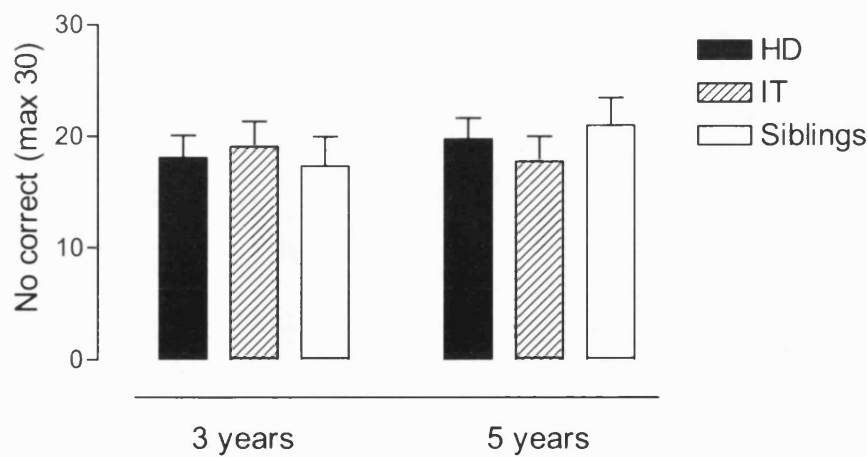
Table 2-20 Number of participants completing the test of visual perception at 3 years and 5 years post-diagnosis.

	N	Assessed at 3 years	Assessed at 5 years
HD	16	8	11
IT	16	8	9
Siblings	14	6	9
Total (missing)	46	22 (24)	29 (17)

Visual perception

There were no significant differences between groups on the test of visual perception at 3 or 5 years post-diagnosis (3 years: ANOVA: $F(2, 19) = 0.53, p = 0.60$; 5 years post-diagnosis ANOVA: $F(2, 26) = 7.12, p = 0.87$)(Figure 2.16).

Figure 2-16 Visual perception at 3 and 5 years post-diagnosis (Mean \pm SEM)



2.5.3.7 Academic attainments

The numbers of children completing the academic achievement tests at 5 years post-diagnosis are presented in Table 2.21.

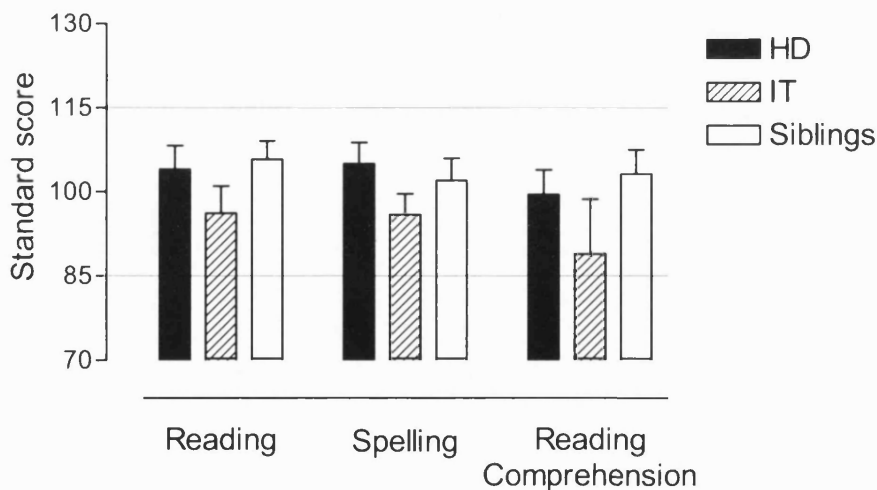
Table 2-21 Number of children completing the tests of academic attainment at 5 year assessments.

	HD (N=16)	IT (N=16)	Siblings (N=14)
Reading	11	11	10
Spelling	11	11	10
Reading comprehension	10	11	9
Mathematical reasoning	11	11	10
Numerical operations	13	12	11

There were no significant differences between groups on single word reading (ANOVA: $F(2, 29) = 1.47, p = 0.25$); spelling (ANOVA: $F(2, 29) = 1.52, p = 0.24$); or reading comprehension (ANOVA: $F(2, 27) = 1.12, p = 0.34$)(Figure 2.17).

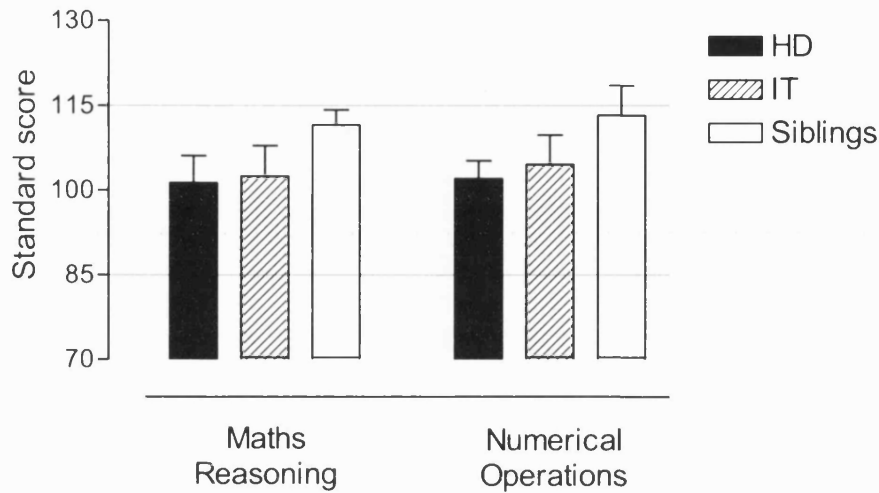
Similarly, there were no significant differences between groups on mathematical reasoning (ANOVA: $F(2, 29) = 1.44, p = 0.25$) or numerical operations (ANOVA: $F(2, 33) = 1.60, p = 0.22$)(Figure 2.18).

Figure 2-17 Reading, spelling, and reading comprehension at 5 years post-diagnosis (Mean ± SEM)



Lines on the graph represent the normal range (i.e. 85-115).

Figure 2-18 Mathematical reasoning and numerical skills at 5 years post-diagnosis (Mean \pm SEM)



Lines on the graph represent the normal range (i.e. 85-115).

Attainments and IQ

Table 2.22 shows the percentage of children from each group with reading, spelling, reading comprehension, mathematical reasoning, and numerical operation scores significantly ($p < 0.05$) below that predicted from their Full-Scale IQ score. These children are classified within the clinically significant range.

While reading and spelling scores were commensurate with IQ estimates for the majority of participants, 22-30% of children showed lower than predicted reading comprehension scores in each group. The majority of these children were boys (7/8).

Approximately one third (27-36%) of all survivors had mathematical reasoning scores within the clinically significant range. The majority of these children were girls (5/7). Similarly, 15-22% of all survivors had numerical operation scores falling within the clinically significant category. In contrast, poor maths skills were not seen in the sibling group. There was no qualitative relationship between children with poor academic achievement and age-at-diagnosis or SES (Table 2.23).

Table 2-22 Percentage of children with achievement scores falling within the clinically significant range

	HD (%)	IT (%)	Sibs (%)
Reading	9	0	0
Spelling	0	0	0
Reading comprehension	30	27	22
Maths reasoning	36	27	0
Numerical operations	15	17	9

Table 2-23 Details of children with academic achievement significantly below that predicted by IQ.

Group	Sex	SES	Age-at-diagnosis	Attainment(s)
HD	F	77	2.80	Mathematical reasoning
HD	F	32	6.76	Mathematical reasoning
HD	F	43	9.8	Mathematical reasoning
HD	M	71	3.19	Reading Reading comprehension Numerical operations
HD	M	77	4.39	Mathematical reasoning Numerical operation
HD	M	34	6.66	Reading comprehension
HD	M	71	11.50	Reading comprehension
IT	F	58	3.89	Numerical operations
IT	F	73	6.2	Reading comprehension Mathematical reasoning
IT	F	69	10.2	Mathematical reasoning Numerical operations
IT	M	51	3.19	Reading comprehension
IT	M	77	4.42	Mathematical reasoning
IT	M	69	5.90	Reading comprehension
Siblings	M	69		Reading comprehension Numerical operations
Siblings	M	69		Reading comprehension

2.5.4 Discussion

Generally, the results of the retrospective analyses accord well with other studies assessing outcome in the LT survivors of ALL. In particular, the results indicate that the mean level of function for all groups was within the normal range.

Developmental milestones

Based on parental reports the majority of children were developing normally prior to diagnosis. Within the literature, early development (including appropriate weight at birth and during the perinatal period) has been described in children who are diagnosed at a later stage with ALL (Appleton *et al.* 1990).

Intelligence

Results of the retrospective analyses failed to identify significant differences between groups on Verbal IQ and Non-verbal IQ at diagnosis, 3 years, and 5 years post-diagnosis. Similarly, analyses did not reveal significant differences between groups on the Verbal comprehension, Perceptual organisation, Freedom from distractibility, or Processing speed indices at 5 years post-diagnosis. These results are consistent with the literature where studies have reported no significant differences between survivors treated with chemotherapy-only and healthy controls (Smibert *et al.* 1996; Uberall *et al.* 1996; Anderson *et al.* 1997), or children treated for other cancers (Tamaroff *et al.* 1982). The results are, however, inconsistent with a report of significant decrease in VIQ and PIQ in survivors treated with TIT when compared to their siblings (Giralt *et al.* 1992). This discrepancy may result from the use of a short-form test in that study. For example, VIQ was estimated from performance on two subtests; Vocabulary and Similarities, and PIQ was estimated from performance on Block design and Coding.

On the basis of a limited number of children completing IQ assessments at diagnosis and 5 years post-diagnosis it was possible to assess changes in VIQ and PIQ with time. Results indicated that there was no significant group change in VIQ or PIQ over time. This is consistent with the longitudinal assessment of IQ in children treated with HD MTX over the first five years post-diagnosis (Mulhern *et al.* 1991; Ochs *et al.* 1991). However, while there were no significant changes with time observed on a group level, qualitative relationships between changes in LT VIQ and sex, and between initial PIQ and LT PIQ, were identified.

In particular, there was a clinically significant decrease in VIQ over the early years post-diagnosis for girls. A relationship between sex and changes in VIQ has not been described for girls and boys treated with chemotherapy-only over the early years post-diagnosis (e.g. first three years post diagnosis (Copeland *et al.* 1996). However, it has previously been reported that girls show a greater decrease in VIQ than boys when treated with combined chemotherapy and irradiation (Mulhern *et al.* 1991; Christie *et al.* 1995). In addition, these differences became more obvious at 5 years post-diagnosis. The current results indicate that girls may be particularly vulnerable to LT changes in Verbal IQ regardless of the type of PCNS therapy administered (i.e. with or without irradiation, and with or without HD MTX).

In addition, the current results indicated that the children with high Non-verbal IQ at diagnosis showed the greatest decrease in performance over time. A previous study has also reported a greater change in PIQ for children with high-average estimates of

PIQ at diagnosis. In particular, the decline in PIQ was greater when PIQ was higher to begin with and was apparent at three years post-diagnosis (Meadows *et al.* 1981). While this finding was reported in children treated with combined chemotherapy and irradiation, the current results indicate that this finding may be extended to included children treated with chemotherapy-only and assessed at 5 years post-diagnosis.

Language

The results of the current analyses indicate that performance on the test of receptive vocabulary did not differ between groups. This is consistent with the literature describing intact receptive vocabulary in the LT survivors of ALL treated with chemotherapy-only (Dowell *et al.* 1991; MacLean *et al.* 1995) or combined chemotherapy and irradiation (Copeland *et al.* 1996). Intact receptive vocabulary has also been reported in children receiving additional PCNS treatment for relapse (Longeway *et al.* 1990).

While it has been argued that the American equivalent of the BPVS (i.e. the PPVT) may not be sensitive enough to detect the subtle damage associated with the treatment of ALL (MacLean *et al.* 1995) detailed assessments of language functions (e.g. Clinical Evaluation of Language Fundamentals (CELF), Arizona Articulation Proficiency Scale, Token test, Boston naming test) in the LT survivors of ALL have also failed to reveal consistent language impairments within this population (Moehle *et al.* 1983; Williams *et al.* 1992; Butler *et al.* 1994; Iuvone *et al.* 2002).

Fine motor function

The results of the current analyses indicate that fine motor function was intact in the LT survivors of ALL treated with chemotherapy-only. Poor fine motor function has been reported for children treated with chemotherapy-only during treatment (Reinders-Messelink *et al.* 1999; Reinders-Messelink *et al.* 2001) and has been attributed to the use of vincristine (Copeland 1988). Beyond the treatment period, however, LT group impairments of fine motor function (Purdue pegboard and finger tapping) have not been identified in children treated with TIT (Kingma *et al.* 2002). Similarly, intact fine motor control (Grooved pegboard and finger tapping) has been reported in LT survivors treated with HD MTX + TIT for leukaemia and lymphoma (Rowland *et al.* 1984; Copeland *et al.* 1996), or other cancers (Moore *et al.* 1992 but see Dowell *et al.* 1991).

Memory and learning

Results of the current analyses indicated that there were no significant differences between groups with respect to immediate and delayed visual memory or learning through repetition with cued recall. This is consistent with the literature describing no LT memory impairment in survivors treated with chemotherapy-only when compared to their siblings (Giralt *et al.* 1992) or healthy controls (Kingma *et al.* 2002).

However, analyses indicated that survivors experienced transient verbal memory difficulties in the early years post-diagnosis. These difficulties were apparent on delayed story recall and were accounted for by differences in VIQ. The difficulties apparently resolved by the 5 years post-diagnosis follow-up assessment. Copeland (1988) has described a similar transient change in verbal memory in the survivors of ALL. In that study, children treated with chemotherapy-only showed a transient impairment in delayed verbal recall during treatment. In a further study, it has been reported that verbal recall of story information is significantly worse in children during treatment with triple intrathecal therapy (TIT) than in children four years post-diagnosis (1 year off therapy)(Brown *et al.* 1992). Together, these studies indicate that common treatment related factors, may result in transient verbal memory impairments. For example, the changes may be related to the administration of corticosteroids in the treatment for ALL. Consistent with this, acute memory impairments have been reported in children treated with corticosteroids (e.g. prednisone) for asthma (Bender *et al.* 1988)(Bender *et al.* 1991).

Visual perception

The results of the current analyses indicate that visual perceptual abilities were unaffected in the LT survivors of ALL. This is consistent with a similar study reporting no significant differences in performance (Motor Free Visual Perception Test) in a survivor treated with TIT and her twin (Williams *et al.* 1992). The results are also consistent with reports of intact visual perception (Judgement of Line orientation and Face Recognition Test) in survivors treated with IT MTX five years post-diagnosis when compared to children treated for other cancers (Butler *et al.* 1994).

Academic attainments

Current analyses indicated that there were no group differences in LT academic achievement. This is consistent with the literature for children treated with chemotherapy-only on similar tests (e.g. Wide range achievement test, Basic achievement skills test, Peabody individual achievement test) of reading, spelling and

arithmetic (Tamaroff *et al.* 1982; Brown *et al.* 1992; Anderson *et al.* 1994; Smibert *et al.* 1996; Anderson *et al.* 1997)(but see also Lansky *et al.* 1984; Brown *et al.* 1992). However, significant achievement deficits were identified in some children in each group (i.e. scores significantly below the level predicted on the basis of their IQ).

While reading and spelling were commensurate with IQ estimates for the majority of participants, 22-30% of children in each group (i.e. survivor and sibling groups) had lower than predicted reading comprehension scores. In addition, poor academic achievement in maths was also observed within the survivor group only. Poor academic achievement particularly in maths has been identified in children treated with chemotherapy (Ochs *et al.* 1991; Brown *et al.* 1992) or combined chemotherapy and irradiation (Lansky *et al.* 1984; Pfefferbaum-Levine *et al.* 1984; Peckham *et al.* 1988; Waber *et al.* 1990; Moore *et al.* 1991; Mulhern *et al.* 1991; Copeland *et al.* 1996; Anderson *et al.* 1997; Kingma *et al.* 2000). In addition, underachievement in maths was more common in girls than boys treated for ALL. This is consistent with reports in survivors treated with combined chemotherapy and irradiation (Waber *et al.* 1990; Waber *et al.* 1992)(but also see (Peckham *et al.* 1988)). The current results indicate that mathematical skills are vulnerable for children treated for ALL with chemotherapy-only treatment. In addition, maths skills are particularly vulnerable for girls.

Comparisons between the study participants and the wider UKALL XI cohort

Performance at 5 years post-diagnosis was also compared between the participants of the current study and the wider UKALL XI cohort treated with the same protocols (see Appendix B). The analyses indicated that the sample of children included in the current research was not entirely representative of the LT population of survivors participating in the UKALL XI Neuropsychological study. Specifically, the HD children involved in this research had a significantly higher general level of function than the wider UKALL XI LT cohort. In addition, there were greater proportions of HD and IT survivors showing maths underachievement in the current research than described in the UKALL XI cohort. Despite these differences, the HD and IT groups were representative of the larger UKALL XI population with respect to memory and learning, visual perception, fine motor function, single word reading, spelling and reading comprehension.

There were no significant differences between the siblings participating in this research project and the siblings participating in the wider UKALL XI study with respect to intelligence, language, memory and learning, visual perception, fine motor function, or academic achievement. This indicates that the siblings used in the current study were representative of the siblings participating in the wider UKALL XI study.

2.6 General summary

Three groups of children were studied in the current research: the HD group, the IT group, and the sibling group. The HD and IT survivors enrolled in the current study were selected to represent average, standard-risk children undergoing chemotherapy without irradiation for the treatment of ALL. In addition, all children were treated without complications and were randomised to one of two treatment protocols. All children were in first continuous remission and had been assessed over the first five years post-diagnosis as part of an earlier neuropsychological study. Siblings were selected to control for genetic and environmental factors associated with cognitive and brain development. All groups were well matched for age-at-diagnosis, length of follow-up, age-at-test, sex, and SES.

Retrospective analyses, of the test results collected over the early years post-diagnosis, indicated that survivors as a group showed a transient but significant difficulty with delayed recall of verbal material in the early years post-diagnosis. There was also weak evidence to indicate that verbal IQ may decline particularly in girls over these years, and, the greatest decrease in IQ may occur in children with highest estimates of IQ at diagnosis.

At five years post-diagnosis, approximately one quarter of all survivors and siblings showed poor reading comprehension. In addition, approximately one third of the survivors showed a lower than predicted performance on the LT tests of arithmetic. The majority of these children were girls. The LT difficulties at five years were not accompanied by reductions in the general level of performance (as indicated by intelligence) or deficits in language, fine motor function memory and learning, visual perception, reading, or spelling.

Part 2: Neuropsychological studies

3 Executive functions

As discussed in Chapter 1, the maturation of the frontal lobes may be vulnerable to the effects of PCNS therapy in children treated for ALL. Pathology of the frontal lobes can be associated with impairments in executive functions (Benton 1968; Luria 1973; Walsh 1978; Welsh & Pennington 1988). Few studies in the literature, however, have included measures of executive functions, and no studies have systematically reviewed executive functions within the LT survivors of children treated with chemotherapy-only. With this in mind, Chapter 3 describes a detailed investigation of executive functions in this population.

3.1 Introduction

'Executive function' (EF) is an umbrella term describing the mental operations necessary for purposeful, goal-directed behaviour (Lezac 1995). Executive 'dysfunction' or the dysexecutive syndrome can be characterised by reduced self-control, poor planning and organisation, difficulties initiating and using strategies for problem solving, and the inability to correct errors and use feedback (Benton 1968; Stuss & Benson 1986; Anderson *et al.* 2002).

For the purposes of the current study, EF can be conceptualised as high-level cognitive skills in three related areas⁶; (1) attentional control-processing speed; (2) cognitive flexibility-self monitoring, and (3) goal setting (Duncan 1986; Shallice 1988; Anderson *et al.* 2001).

Attentional control-processing speed

Attentional control refers to the ability to select relevant information from the environment while ignoring potential distractors and to sustain attention over time. In adults, frontal lobe lesions can be associated with poor sustained attention (Wilkins *et al.* 1987; Rueckert & Grafman 1996), an increase in distractibility (Woods & Knight 1986; Chao & Knight 1995), vulnerability to interference (Holzt & Vilkki 1988; Vendrell *et al.* 1995), and poor response inhibition (Leimkuhler & Mesulam 1985). Similarly, frontal lesions in children can be associated with reduced attention span (Anderson *et al.* 2002), impulsive responding (Levin *et al.* 1993; Eslinger & Biddle 2000), and vulnerability to interference (Mateer & Williams 1991).

⁶ This definition considers concept formation/reasoning and executive functions as separable (Sohlberg & Mateer 1989; Lezac 1995).

The role of the frontal lobes in the control of attention has also been highlighted by a number of functional imaging studies. For example, associations have been made between sustaining attention and the right frontal lobe including the dorsolateral prefrontal cortex (Pardo *et al.* 1991; Manly *et al.* 2003). Additional studies indicate that several areas of the frontal lobes are important in the suppression of interference and the inhibition of response. For example, the incongruent condition of the Stroop paradigm has been associated with increased activity of the lateral, medial, and anterior cingulate cortex in healthy subjects (Pardo *et al.* 1990; Brown *et al.* 1999; Stuss *et al.* 2001). Similarly, fMRI studies of healthy controls report involvement of the medial prefrontal cortex and inferior frontal sulcus in the inhibition of responses (no-go trials of a go/no-go paradigm)(Konishi *et al.* 1999).

Animal studies also support the role of the frontal cortex in the control of attention. For example, the lateral frontal cortex in primates is involved in the allocation of attention to novelty (Mesulam 1986). Single-cell recordings have also identified activity related to the detection of the target stimulus from distractors (Hasegawa *et al.* 2000)(Rainer *et al.* 1998a & b). Furthermore, lesions of the ventral prefrontal cortex in non-human primates have been associated with poor inhibition of no-go trials in a go/no-go learning task (McEnaney & Butter 1969; Iversen & Mishkin 1970).

Brain injury in children can also be associated with a slowing of processing speed (Ponsford & Kinsella 1992; Garth *et al.* 1997). Processing speed is the general rate an individual can execute cognitive operations (Fry & Hale 1996). Reports highlight a relationship between the speed of processing and tasks (e.g. working memory) dependent on the integrity of the frontal lobe in both adults (Salthouse 1992) and children (Fry & Hale 2000).

Cognitive flexibility-self monitoring

Cognitive flexibility refers to the ability to shift from one concept to another or change thought or actions according to the demands of the new situation (Walsh 1978; Grattan & Eslinger 1991). Adults sustaining frontal lobe lesions can show poor working memory (Freedman & Oscar-Berman 1986; Owen *et al.* 1990;Chao & Knight 1998; Chao & Knight 1995), verbal fluency (particularly when lesion is on the left)(Benton 1968; Perret 1974; Baldo & Shimamura 1998; Stuss *et al.* 1998), switching attention (Owen *et al.* 1993; Rogers *et al.* 1998; Stuss *et al.* 2001), and stimulus-reward learning (Rolls *et al.* 1994). Similarly, frontal lobe injury in children has been associated with poor performance on tests of fluency (verbal fluency)(Levin *et al.* 1993; Levin *et al.* 2001;

Anderson *et al.* 2002). In particular, children with focal frontal lesions generated fewer words on the test of verbal fluency (Anderson *et al.* 2002).

Functional imaging studies highlight the role of the frontal lobes in cognitive flexibility. For example, working memory (n-back paradigm) in healthy adults is associated with lateral prefrontal cortex activation (LaBar *et al.* 1999; Veltman *et al.* 2003). In addition, performance on a spatial working memory task is associated with an increase in lateral prefrontal cortex activity in adults (McCarthy *et al.* 1994; Owen *et al.* 1996; Courtney *et al.* 1998) and children (Nelson *et al.* 2000). Functional imaging studies also show that switching attention to a new task in comparison to continuing with the same task is associated with an increase in lateral prefrontal cortex activity (Omori *et al.* 1999; Dove *et al.* 2000; Sohn *et al.* 2000). Similarly, the lateral prefrontal cortex is involved in shifting attention between perceptual dimensions (Konishi *et al.* 1998; Nagahama *et al.* 1999; Nagahama *et al.* 2001; Pollmann 2001; Monchi *et al.* 2001).

Animal studies provided further support a role of the frontal lobes in cognitive flexibility. For example, single-unit recordings in primates show the lateral prefrontal cortex is involved in performing tasks with a delay (Rainer 1998a and b) and during spatial working memory tasks (Wilson *et al.* 1993). Similarly, ablation of the dorsolateral prefrontal cortex in primates is associated with impairments on the delayed response task (Goldman & Rosvold 1970) and spatial working memory task (Passingham 1985). Furthermore, ablation of the lateral aspects of the prefrontal cortex impairs attention switching in primates (Dias *et al.* 1997).

In addition, animal studies support the role of the ventral aspects of the frontal cortex in cognitive control. For example, ablation of the orbitofrontal cortices impairs stimulus-reward learning in primates (Butter 1969; Jones & Mishkin 1972; Meunier *et al.* 1997; Dias 1997). In particular, poor stimulus-reward learning is associated with continued selection of the previously rewarded stimulus (i.e. failure to break or adjust previously learned associations). Poor performance was also observed on the first occasion requiring a change in responding (i.e. first reversal), whereas the performance on subsequent reversals was not significantly affected (Dias *et al.* 1997). Furthermore, studies show the orbitofrontal neurons are sensitive to reward (Hikosaka & Watanabe 2000) and differentially respond to visual stimuli when reward contingencies are changed (Thorpe *et al.* 1983; Rolls *et al.* 1996). Similarly, the magnitude of reward and punishment associated with visual associations is represented in the orbitofrontal cortex (O'Doherty *et al.* 2001).

Goal setting

Goal setting includes the skills necessary to plan, initiate, and implement strategies to solve problems. Adults with frontal lesions show poor planning despite awareness of the task requirements (Owen *et al.* 1990; Bechara *et al.* 1994; Carlin *et al.* 2000), fail to generate and implement strategies to aid their performance (Shallice & Burgess 1991; Godefroy & Rousseaux 1997; Mangels 1997), and show poor performance on tests of problem solving (e.g. Wisconsin Card Sorting test (WCST)(Milner 1964; Eslinger & Damasio 1985; Shallice & Burgess 1991). Similarly, frontal lobe injury in children has been associated with poor problem solving (Tower of London)(Levin *et al.* 1994; Levin *et al.* 1997; Jacobs & Anderson 2002) where left frontal lesion volume was associated with planning time and percentage of items solved with one trial on the Tower of London task (Levin *et al.* 1997). In addition, children with focal frontal lesions have been shown to completed fewer categories on the WCST (Levin *et al.* 1997).

Functional imaging studies indicate that planning and problem solving are dependent upon areas of the frontal lobes. These areas include the anterior cingulate (Dagher *et al.* 1999) and dorsolateral prefrontal regions (Owen *et al.* 1996; Lazeron *et al.* 2000; van den Heuvel *et al.* 2003). In addition, functional imaging studies highlight the importance of the lateral prefrontal cortex for performance on the WCST (e.g. PET (Berman *et al.* 1995; Nagahama *et al.* 1996), SPECT (Rubin *et al.* 1991; Daniel *et al.* 1991; Marenco *et al.* 1993; Rezai *et al.* 1993) and fMRI (Konishi *et al.* 1998; Konishi *et al.* 1999; Monchi *et al.* 2001). The results of these studies indicate that performance of this task is associated with increased activation of the lateral prefrontal cortex.

The measurement of EF

Since EF involve self-direction and self-regulation of behaviour, the measurement of EF is compromised by the structured nature of the standard testing environment (Lezac 1995). In addition, Shallice (1988) and Walsh (1978) argue EF are particularly important in novel circumstances, where established routines for responding do not exist. This is supported by reports indicating that patients with lesions of the frontal lobes are disproportionately impaired in novel situations (Godefroy & Rousseaux 1997; Daffner *et al.* 2000).

For these reasons the investigation conducted in this chapter employed novel tests believed to tap everyday demands of the EF system (e.g. subtests from the Test of Everyday Attention for Adults (TEA)(Robertson *et al.* 1994) and Children (TEA-Ch)(Manly *et al.* 1999), and the Behavioural Assessment of the Dysexecutive Syndrome (BADS)(Wilson *et al.* 1996). In addition, traditional measures of EF (e.g. the

Tower of London (Shallice 1982), Controlled Oral Word Association Test (Anderson *et al.* 1996), the Stroop Colour and Word test (Golden 1978), and the Wisconsin Card Sorting test (Heaton 1981)) were administered as (1) these measures provide good diagnostic value, and (2) allow comparisons of the results of the current study with the wider literature. Furthermore, three experimental paradigms (e.g. spatial working memory, the IDED set shifting, and extinction learning) were administered. These paradigms have been shown to be sensitive to frontal lobe lesions in human and non-human primates.

Finally, the dysexecutive syndrome can be accompanied by emotional and behavioural problems. Symptoms include apathy, and a loss of initiative, creativity, and concentration, or in contrast, may include disinhibition, impulsivity, lack of insight, and judgement (Mesulam 2002). In addition, these problems may first appear in the longer-term following frontal lobe injury in childhood (Eslinger *et al.* 1992). To assess these aspects of behaviour, parents were asked to rate 'dysexecutive' type behaviours using the DEX questionnaire (Wilson *et al.* 1996). Parents also completed the Child Behaviour Checklist (CBCL)(Achenbach 1991) to serve as an index of general behaviour.

3.2 Methods

A number of tests were administered to assess EF in the LT survivors and their siblings. Tests were selected to reflect level of functioning across the three components of EF described above. A summary of the tests administered is provided in Table 3.1. This is followed by a brief description of each test.

Table 3-1 Summary of the tests administered to assess executive functions

Component of EF	Test administered (Index of function)
Attentional control-processing speed	
Attention span (auditory-verbal)	Digits forwards (number of items)
Attention span (visuo-spatial)	Corsi blocks (number of items)
Selective attention	Map mission/Map search (standard score)
Sustained attention	Score!/Elevator counting (percent correct)
Response inhibition	Walk don't walk (trials correct)
Resistance to interference	Stroop (interference t score)
Speeded response (verbal)	Stroop (word t score)
Speeded response (motor)	Map motor control (time per target)
Cognitive flexibility-self monitoring	
Working memory (auditory-verbal)	Digits backwards (number of items)
Working memory (visuo-spatial)	Spatial working memory (number of between-search errors)
Fluency	COWAT (z score)
Switching attention	Creatures/Visual elevator (standard score) IDED task (number of errors – ED shift)
Stimulus-reward learning (reversal)	IDED task (number of errors – first reversal)
Stimulus-reward learning (extinction)	Extinction (last error trial)
Goal setting	
Planning	Tower of London (raw score) Zoo map (BADS profile score)
Problem solving	WCST (number of categories completed) Action program (BADS profile score)
Strategic behaviour	Spatial working memory (strategy score) Key search (BADS profile score)
Organisation	6 elements (BADS profile score)
Other related measures	
General function	Vocabulary and Block design (IQ estimate)
Behaviour (general)	CBCL (total behaviour; internalising and externalising scores)
Behaviour (dysexecutive)	DEX questionnaire (total score)

3.2.1 Attentional control – processing speed

3.2.1.1 Attention span

Two tests were administered to assess auditory-verbal and visuo-spatial attention span in the LT survivors and their siblings. As norms were not available for all participants aged 9:0 – 21:4 the raw scores for auditory and visuo-spatial attention span have been reported.

Auditory-verbal

Auditory-verbal attention span was assessed using the Digits Forward part of the Digit Span subtest from the age-appropriate Wechsler Intelligence Scale (i.e. WISC-III or WAIS-III (Wechsler 1992; Wechsler 1999)). In this subtest children were asked to repeat strings of digits presented by the examiner. The level of difficulty increased in the task by increasing the number of digits in the string. Children had two attempts at each string length. The longest string of digits correctly recalled on at least one trial was considered to be the measure of auditory-verbal attention span.

Visuo-spatial

Visuo-spatial attention span was assessed using the Corsi blocks (Corsi 1972 cited in Isaacs & Vargha-Khadem 1989). In this test, children were asked to point to a series of wooden blocks as demonstrated by the examiner. The level of difficulty increased in the task by increasing the number of blocks included in the sequence. Children had two attempts at each sequence length. The longest sequence of blocks correctly recalled on at least one trial was considered to be the measure of visuo-spatial attention span.

3.2.1.2 Selective attention

Selective attention was assessed for children aged < 18 years using the Map Mission subtest from the TEA-Ch (N=36)(Manly *et al.* 1999), or for participants older than 18 years using the Map Search subtest from the TEA (N=5)(Robertson *et al.* 1994). Each test was administered and scored in accordance with the manual. As norms were not available on either version for children aged 16:0 - 17:11 standard scores for these children (N=3) were calculated in accordance with the published norms for children aged 15:11 as part of the TEA-Ch.

The subtests were similar between age-appropriate versions of the test and participants were asked to find as many target symbols as possible within one minute. Target symbols (maximum = 80) were scattered over a large map amongst equal numbers of other distractor symbols. The total number of targets identified within the time limit was then converted to an age-appropriate standard score (mean = 10; SD=3). The standard score was used as the index of selective attention.

3.2.1.3 Sustained attention

Sustained attention was assessed for children aged less than 18 years using the Score(!) subtest from the TEA-Ch (N=37)(Manly *et al.* 1999), or for participants older than 18 years using the Elevator Counting subtest from the TEA (N=5)(Robertson *et al.* 1994). Each subtest was administered in accordance with the manual. However, as age-appropriate standard scores were not available for participants completing the subtest from the TEA percentages of trials correct were analysed for all children.

The Score(!) and Elevator subtests involved counting a string of tones, presented at irregular intervals, over a number of trials. As part of the TEA-Ch children received 10 trials, whereas participants received 7 trials on the TEA. The percentage of trials correct was used as an index of sustained attention.

3.2.1.4 Response Inhibition

The Walk Don't Walk subtest from the TEA-Ch (Manly *et al.* 1999) was administered to all participants as a measure of response inhibition. As this subtest was part of the children's attention battery, norms were not available for all participants. Therefore, the total number of trials correct (raw score) has been reported.

In this subtest, participants were asked to take a step along a path with each tone heard on a tape. Unpredictably the tone would change to indicate that no further steps should be taken. The test required the ability to sustain attention to the task at hand and not to enter into an automatic style of responding. The total number of trials correct (maximum= 20) was considered an index of response inhibition.

3.2.1.5 Resistance to interference

The Stroop Colour and Word test (Golden 1978) was administered to assess resistance to interference in the LT survivors and their siblings. All parts of the test were administered and scored (using the age corrections) as described in the manual.

Briefly the test involved three trials where participants were asked to identify as many stimuli as possible within 45 seconds. In the first trial subjects were asked to read a series of words (either red, green, or blue) presented in black ink. The second trial involved naming the colour of 'XXXX' stimuli printed in red, blue, or green ink. In the final trial, children were asked to name the colour of ink for a series of words where the colours (either red, blue, or green) and words (either red, blue, or green) were incongruent. Using the number of items identified in each trial and the age corrections described in the manual, a measure of interference (mean = 0; SD=10) corrected for speed factors (word reading and colour naming) was then calculated. This score served as an index of resistance to interference.

3.2.1.6 Processing speed

Two tests of processing speed were administered to the LT survivors and their siblings. The tests were adapted from subtests included in the Stroop Colour and Word test (Golden 1978), and the TEA-Ch (Manly *et al.* 1999). Each test focussed on either verbal or motor speeded response. The speeded verbal response score was corrected for age using age correction in the manual. Norms unavailable for the speeded motor response therefore raw scores were reported.

Speeded verbal response

Speeded verbal response was assessed using the Word subtest from the Stroop Colour and Word test. In the Word subtest, subjects were asked to read a series of words (either red, green, or blue) presented in black ink. The number of words correctly identified within 45 seconds was then corrected for age using the procedure described in the manual. This produced a word reading 't' score. The 't' score was used as the index of speeded verbal response.

Speeded motor response

The test of speeded motor response was adapted from the Map Mission subtest in the TEA-Ch. In the speeded motor response test, participants were asked to circle 80 symbols (all targets and no distractors) scattered on an A3 page. The total time taken divided by the number of targets circled was considered to be the measure of speeded motor response. This score was not corrected for age.

3.2.2 Cognitive flexibility – self monitoring

3.2.2.1 Working memory

Two tests were administered to assess working memory. The test of auditory-verbal working memory was adapted from the Digit span subtest and the test of spatial working memory was based on an experimental paradigm. Norms were unavailable for both tests therefore raw scores have been reported.

Auditory-verbal

Auditory-verbal working memory was assessed using the Digits Backwards part of the Digit Span subtest from the age-appropriate Wechsler Intelligence Scale (i.e. WISC-III or WAIS-III)(Wechsler 1992; Wechsler 1999). In this subtest children were asked to recall a string of digits in the reverse order following presentation by the examiner. The level of difficulty increased in the task by increasing the number of digits in the string. Children had two attempts at each string length. The longest string of digits correctly recalled on at least one trial was considered to be the measure of auditory-verbal working memory. As norms were not available for all participants aged 9:0 – 21:4 the raw scores are reported.

Spatial working memory

The spatial memory task was based on the tasks described by Passingham (1985) and Owen *et al.* (1990). This computerised test was self-paced and increased in difficulty over five levels. On the first level (practice items), participants were presented with two squares displayed in random locations of the screen. Behind one of the squares was a token. Participants were asked to find the token by looking behind each square (in any order) using the computer mouse. Once the token was found another token was hidden for the child to find. Importantly, tokens were hidden behind each square only once through out a given trial.

To efficiently complete the task participants were required to remember the squares already selected and to avoid them of the subsequent searches. Returning to a square where a token had already been found constituted a between-search error. Following the practice items (N=4), participants completed four trials with three, four, six, and eight boxes (test items). The positions of the squares changed between each trial. The total number of between-search errors was calculated across the all test items was used an index of spatial working memory.

3.2.2.2 Fluency

The Controlled oral word association test (COWAT) was administered as a measure of fluency. Raw scores were corrected for age using the norms published by Spreen & Strauss (1998).

In this test participants were given three, one minute trials to generate as many words as possible beginning with a particular letter of the alphabet (i.e. F, A, and S respectively). Participants were also required to select words according to restricted conditions (e.g. no proper nouns, same-word variations, or repetitions). The total number of permissible words produced across the three trials was then converted to a z score for each participant. The z score served as an index of fluency.

3.2.2.3 Switching attention

Two tests of attention switching were administered to the LT survivors and their siblings. The switching attention task was selected from the age-appropriate attention test (i.e. TEA-Ch or TEA). A computerised task, based on the IDED set-shifting paradigm, was also administered.

Creature counting/Visual elevator

Attention switching was assessed for children aged < 18 years using the Creature counting subtest from the TEA-Ch (N=37)(Manly *et al.* 1999) or for participants older than 18 years using the Visual elevator subtest from the TEA (N=5)(Robertson *et al.* 1994). Each test was administered and scored in accordance with the manual. As norms were not available on either version for children aged 16:0 - 17:11 standard scores for these children (N=3) were calculated in accordance with the published norms for children aged 15:11 as part of the TEA-Ch.

The subtests were similar between age-appropriate versions and participants were asked to count the number of creatures (TEA-Ch) or floors (TEA) scattered along a path. Participants were also required to repeatedly switch between counting upwards and counting downwards on the basis of occasional arrows indicating a change in direction was to be made. The number of trials correct (maximum = 7 (TEA-Ch) or 10 (TEA)) was then converted to an age-appropriate standard score (mean = 10; SD=3). The standard scores were used as an index of shifting attention. The timing scores associated with these subtests were not analysed.

Extradimensional set-shifting

The intradimensional/extradimensional set-shifting task (IDED) was administered to assess extradimensional shifting and reversal learning. The paradigm was based on the task described by Luciana & Nelson (1998). As norms were unavailable for this task, raw scores have been reported.

The IDED task was a computerised task involving visual discrimination and reversal learning. The test was self-paced and increased in difficulty as participants completed a maximum of nine levels. At each level, participants aimed to achieve as many correct answers as possible and were encouraged to deduce the rule guiding the correct choices. The extradimensional set-shifting part of this task was Level 8.

Briefly, on the first level (Simple visual discrimination), participants selected one of two lined patterns (in two of four locations) displayed on the monitor. After selecting a pattern, the child was given immediate feedback on the computer to indicate if the choice was right or wrong. The patterns re-appeared (not necessarily in the same location) and the child made another choice. The child continued selecting patterns until criterion was reached (i.e. 6 consecutive correct response in a maximum of 50 trials).

Following successful completion of simple visual discrimination the stimulus-reward associations were then reversed (Level 2 – Simple Reversal). Now the first correct pattern was incorrect and the first incorrect pattern was correct. Again participants completed 6 consecutive correct trials to criterion.

Upon completion of simple reversal, a second dimension (shape) was introduced to accompany the lines. Participants were required to continue to respond to the first dimension (lines) while ignoring the new dimension (shape). Initially (Level 3 – Compound Discrimination) shapes were positioned next to lined patterns, while on Level 4 (Compound Discrimination – overlapping stimuli) patterns and shapes were overlapping. Following successful completion of the visual discrimination of compound stimuli there was a second reversal (Level 5 – Compound Reversal).

At Level 6 (Intradimensional Shift) new compound stimuli were introduced that had not previously been used. Participants were required to continue to discriminate the new stimuli according to the previously relevant dimension (i.e. to continue to discriminate stimuli on the basis of lines while ignoring shapes). Success at this stage required shifting attention within a dimension (i.e. to the new lines). Following success on this stage the stimulus-reward associations were reversed (Level 7 – Intradimensional Reversal).

At Level 8 (Extradimensional Shift) novel compound stimuli were again introduced. This time, however, participants were required to shift attention to the new dimension (i.e. shapes). Successful discrimination on this stage required participants to discriminate on the basis of shape while ignoring lines. The final stage (Level 9 – Extradimensional reversal) involved the reversal of the stimulus-reward associations.

The number of stages completed was taken as an index of overall performance on this task. In addition, the number of errors made on Level 8 (Extradimensional shift) was recorded as an index of Extradimensional set-shifting.

3.2.2.4 Stimulus-reward learning

Two experimental paradigms were used to assess stimulus-reward learning. The IDED set shifting and the extinction tasks were based on experimental paradigms. As norms were unavailable for both tasks, raw scores have been reported.

Reversal

As described above the IDED task required visual discrimination and reversal learning. An index of reversal learning was extracted from performance on the IDED task. This index of reversal learning was the number of errors made on the first reversal (i.e. IDED level 2 – Simple Reversal).

Extinction

The computerised test of extinction was based on the paradigm described by Rolls et al 1994. In this task, participants learned to discriminate two fractal images, A and B. For each trial, only one image (A or B) appeared and participants either touched or refrained from touching the image. Points were awarded based on the participants' response. Participants were encouraged to score as many points as possible. During the discrimination phase, one point was awarded for touching image A or not touching image B, and, one point was subtracted for not touching image A or touching image B.

Images disappeared immediately from the screen when selected or disappeared after seven seconds when not selected. After the image disappeared, feedback was given to indicate if the child's response was right or wrong and the child's score was shown on screen. This was followed by the next trial until the discrimination criterion was reached (i.e. 9 out of 10 consecutive trials correct, 30 maximum trials). After successfully completing the discrimination stage the stimulus-reward associations changed and participants were required to refrain from selecting both images (i.e. points were lost for touching image A or B). The number of the last error trial on this part of the test was used as an index of stimulus-reward extinction.

3.2.3 Goal setting

3.2.3.1 Planning

Two tests of planning were administered to the LT survivors and their siblings. The Tower of London (Shallice 1982; Anderson *et al.* 1996) and the Zoo map subtest from the BADS (Wilson *et al.* 1996). As norms were unavailable for all participants aged 9:0 – 21:4 years, raw scores have been reported.

Tower of London

The Tower of London involved rearranging three coloured balls on a series of pegs to match the configuration presented on a stimulus card. The child was required to complete the task in a set number of moves and adhere to several rules including moving one ball at a time and placing a maximum number of balls on each peg. Children were permitted as many attempts as required to complete the task within 60 seconds. Performance was assessed over 12 trials. The level of difficulty increased in the task by increasing the number of moves required to find the solution. An item score was calculated for each trial and reflected the time to complete the task, and the number of failed attempts (Anderson *et al.* 1996). The item scores across the 12 trials

were then summed to provide the overall raw score (maximum = 108). As norms were not available for all participants aged 9:0 – 21:5 raw scores served as an index of planning.

Zoo map

In the Zoo map subtest of the BADS participants were asked to show how they would visit a series of designated locations on a map of the zoo, while obeying a number of rules. In the first part of the test, participants were required to formulate a successful plan to visit the designated locations, while minimising the number of errors (i.e. rule breaks). In the second part of the test, the participants were required to follow a plan provided by the examiner again without breaking the rules. The subtest score was calculated in accordance with the manual using the number of errors and time taken over both components of the test. The maximum score possible was four. To facilitate analyses, performance scores on this subtest were categorised as either accurate and efficient (score = 4), or inaccurate and inefficient (score < 4).

3.2.3.2 Problem solving

Two tests of problem solving were administered to the LT survivors and their siblings. The Wisconsin Card Sorting task (WCST)(Heaton 1981) and the Action Program subtest from the BADS (Wilson *et al.* 1996). As norms were unavailable for all participants aged 9:0 – 21:4 years, raw scores have been reported.

WCST

The Wisconsin Card sorting test (WCST) was administered to assess problem solving. In this test, each participant was given a set of cards. Participants were then asked to match each card to one of four sample cards on the desk in front of them. After matching a card feedback was provided by the examiner to indicate if a choice was right or wrong, and the aim of the task was to obtain as many correct answers as possible. Initially participants received positive feedback while sorting to the category of colour. This continued until 10 consecutive correct responses were obtained (i.e. the 'colour' category was completed). At this point the sorting criteria changed without the participants' knowledge and participants attempted to complete the next category. Sorting categories were rewarded in the following order: colour, form, number, colour, form, number. The test continued until six categories were achieved or all cards were sorted (N=128). The overall number of categories completed was taken as an index of overall performance on this task.

Action program test

The Action program subtest from the BADS was administered to all participants as a measure of problem solving. In this subtest, participants were asked to solve a novel five-step problem using a variety of materials such as water, wire, and cork. The subtest score was calculated in accordance with the manual using the number of stages completed independently. The number of errors made to complete each stage and the time taken was not important for this test. The maximum score possible was four. To facilitate analyses, performance scores on this subtest was categorised as either accurate and efficient (score = 4), or inaccurate and inefficient (score < 4).

3.2.3.3 Strategic behaviour

Two measures of strategy were calculated for the LT survivors and their siblings. One measure was taken from the spatial working memory task, while the Key search subtest from the BADS (Wilson *et al.* 1996) provided a second strategy measure. As norms were unavailable for all participants aged 9:0 – 21:4 years, raw scores have been reported.

Spatial working memory

The measure of effective strategy was taken from performance on the spatial working memory task described above (Owen *et al.* 1990; Owen *et al.* 1996). The strategy score was calculated to reflect the use of a predetermined search sequence (i.e. to search the squares in a given order and to eliminate squares from the sequence as each token is found). This was estimated from the number of different locations the searches began. A high score indicated poor use of the systematic search, whereas a low score indicated use of a predetermined sequence. The strategy score was calculated from the four searches conducted at the hardest level (i.e. the trials with eight squares).

Key search

Strategic behaviour was assessed using the Key search task from the BADS. The test asked children to demonstrate how they would conduct a search of a field to find a lost item. The subtest score was calculated in accordance with the manual and points were awarded for strategy efficiency, covering all of the ground, and the likelihood of success. The maximum score possible was four. To facilitate analyses, performance scores on this subtest was categorised as either accurate and efficient (score = 4), or inaccurate and inefficient (score < 4).

3.2.3.4 Organisation

The Modified 6 elements test from the BADS (Wilson *et al.* 1996) was administered to assess organisation in the LT survivors and their siblings. As norms were unavailable for all participants aged 9:0 – 21:4 years, raw scores have been reported.

This test requires participants to attempt both parts (A and B) of three tasks (dictation, arithmetic, and picture naming) within a 10-minute period. Participants must also adhere to one rule: they are not allowed to attempt two parts of the same task consecutively. The subtest scores were calculated in accordance with the manual where points were awarded for the number of tasks attempted, the rules broken, and the maximum time spent on any part of task. The maximum score possible was four. To facilitate analyses, performance scores on this subtest was categorised as either accurate and efficient (score = 4), or inaccurate and inefficient (score < 4).

3.2.4 Overall indices of function

An index reflecting overall performance in each of the three functional domains was also calculated for the participants. To calculate the indices performance on each subtest was converted to a z score.

3.2.4.1 Attentional control-processing speed index

The Attentional control – processing speed index was the average performance across the following components of EF:

Attention span (Corsi blocks), Selective attention (Map mission/Map search), Sustained attention (Score!/Elevator counting), Response inhibition (Walk don't Walk), and Speeded response (Stroop word t score).

3.2.4.2 Cognitive flexibility-self monitoring index

The Cognitive flexibility-self monitoring index was the average performance across the following components of EF:

Working memory (Spatial working memory), Fluency (COWAT), Switching attention (Creatures/Visual elevator), and Stimulus-reward learning (IDED first reversal).

3.2.4.3 Goal setting index

The Goal setting index was the average performance across the following components of EF:

Planning (Tower of London), Problem solving (WCST), and Strategic behaviour (Spatial working memory).

3.2.5 Related measures of function

3.2.5.1 General function

A two subtest short-form IQ test was administered to provide an index of the general level of function for all participants. The subtests Vocabulary and Block design were selected from the age-appropriate Wechsler Intelligence scales. For children aged between 6:0 and 16:11 years (N=39) the subtests were selected from the WISC-III^{UK}(Wechsler 1992) and for subjects >17:0 years (N=6) the subtest were selected from the WAIS-III^{UK}(Wechsler 1999).

Both subtests were administered and scored in accordance with the manuals. In addition, the Vocabulary and Block design scaled scores were combined and converted to an overall IQ estimate using the data provided by Sattler (1992). The overall IQ estimate was considered the index of general function.

3.2.5.2 Behaviour

General behaviour

The Child Behaviour Checklist 4-18 (Achenbach 1991) was completed by parents and used to obtain standardised ratings of behavioural problems in the LT survivors and their siblings. This measure was scored in accordance with the manual to yield three composite scores and eight profile sub-scores. The three composite scores were Total Behavioural problems, Externalising Behavioural problems, and Internalising Behavioural problems. Composite scores less than 60 were considered within the normal range whereas composite scores greater than 63 (>90th percentile) were considered 'clinically significant'. Scores falling between 60 and 63 were considered to be within the borderline range.

The profile sub-scores from the Internalising scale were the withdrawn, somatic complaints, and anxious/depressed profiles. The sub-scores from the Externalising scale were the delinquent and aggressive behaviour profiles. Social, thought, and attention problems profiles were also generated. Profile scores less than 67 were considered within the normal range whereas composite scores greater than 70 (>98th percentile) were considered 'clinically significant'. Scores falling between 67 and 70 were considered to be within the borderline range.

Composite scores and profile sub-scores were used to identify behavioural problems in the participants. In addition, children receiving remedial services at school were identified from the answers given to Part 1 question 2 of the CBCL questionnaire (i.e. Does your child receive special remedial services or attend a special class or special school?).

Dysexecutive behaviour

Parents of the LT survivors and siblings completed the 20-item Dysexecutive Questionnaire associated with the BADS (Wilson *et al.* 1996). Questions sampled the range of problems associated with the Dysexecutive syndrome as identified in adults. The questions covered four main areas: emotional and personality problems, motivational problems, behavioural problems, and cognitive problems. Each item was rated on a five point Likert scale (ranging from Never to Very Often). The maximum score was 80. Based on the findings in adults with the dysexecutive syndrome, scores greater than 55 (90th percentile) were considered to be within the clinically significant range (Wilson *et al.* 1996).

3.3 Results

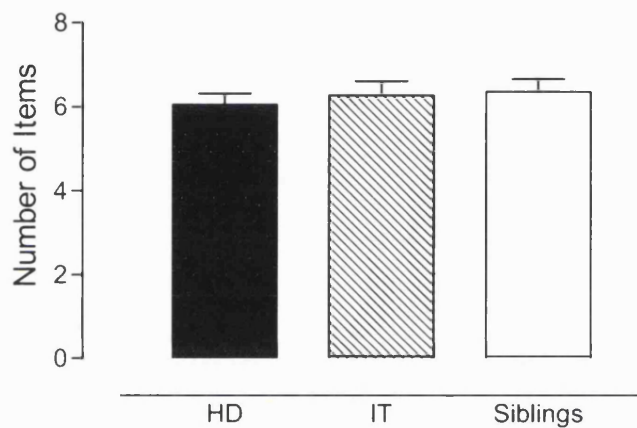
3.3.1 Attentional control – processing speed

3.3.1.1 Attention span

Auditory verbal (Digits forwards)

There were no significant differences between groups on the test of auditory verbal attention span (ANOVA: $F(2, 42) = 0.26, p = 0.78$)(Figure 3.1).

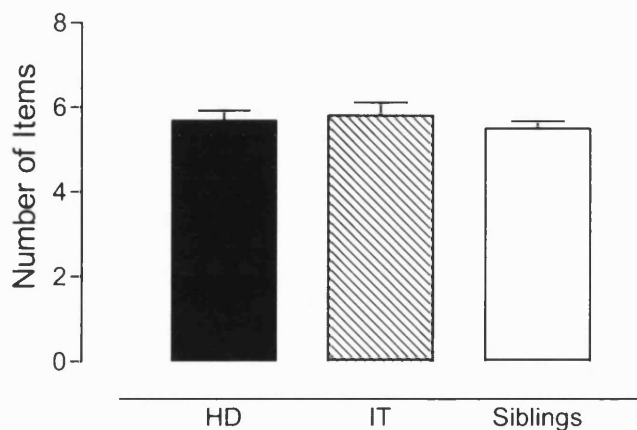
Figure 3-1 Auditory-verbal span in each group (Mean \pm SEM)



Visuo-spatial (Corsi blocks)

There were no significant differences between groups on the test of visuo-spatial attention span (ANOVA: $F(2, 42) = 0.22, p = 0.80$)(Figure 3.2).

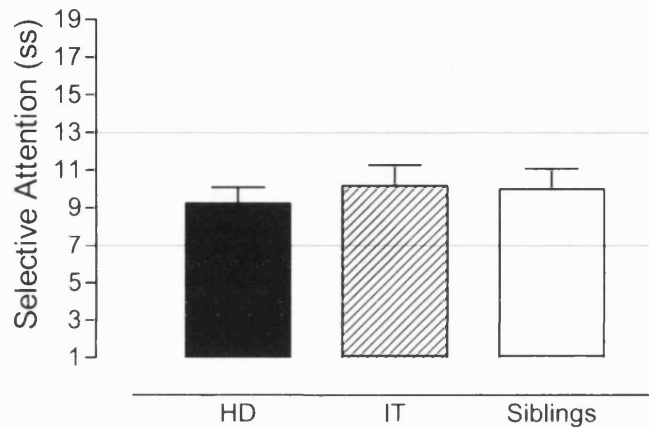
Figure 3-2 Visuo-spatial span in each group (Mean \pm SEM)



3.3.1.2 Selective attention (Map mission)

There were no significant differences between groups on the test of selective attention (ANOVA: $F(2, 41) = 0.24$, $p = 0.79$)(Figure 3.3).

Figure 3-3 Selective attention in each group (Mean \pm SEM)

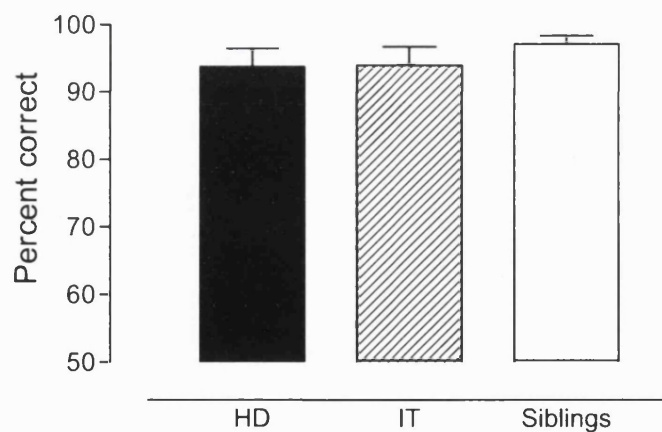


Lines on the graph represent the normal range (i.e. 7-13).

3.3.1.3 Sustained attention (Score!/Elevator counting)

The distribution of sustained attention scores was negatively skewed. Using non-parametric analyses there was no significant difference observed between groups (KW: $\chi^2 = 0.67$; $df = 2$; $p = 0.72$)(Figure 3.4).

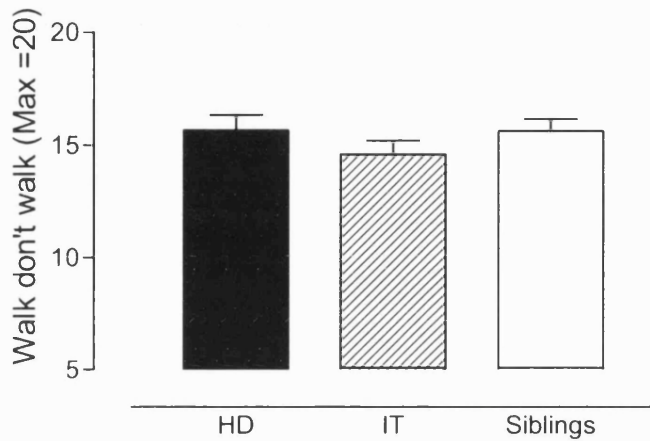
Figure 3-4 Sustained attention in each group (Mean \pm SEM)



3.3.1.4 Response inhibition (Walk Don't Walk)

The distribution of the Walk Don't Walk scores was negatively skewed. Using non-parametric analyses there was no significant difference observed between groups (KW: $\chi^2 = 2.66$; $df = 2$; $p = 0.26$)(Figure 3.5)

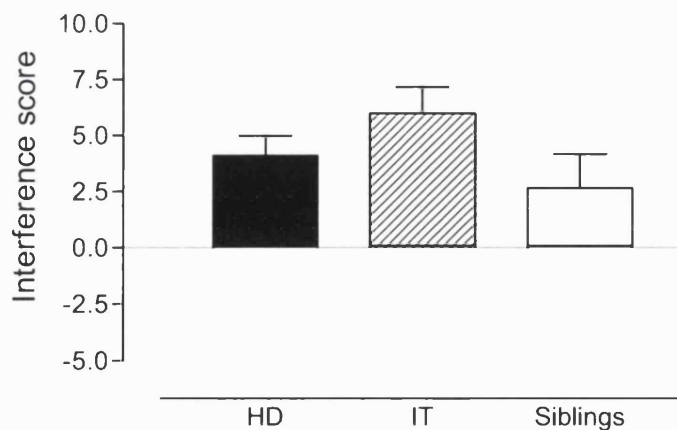
Figure 3-5 Response inhibition in each group (Mean \pm SEM)



3.3.1.5 Resistance to interference (Stroop interference)

There were no significant differences between groups on the test of interference (ANOVA: $F(2, 42) = 1.90$, $p = 0.16$)(Figure 3.6).

Figure 3-6 Resistance to interference in each group (Mean \pm SEM)

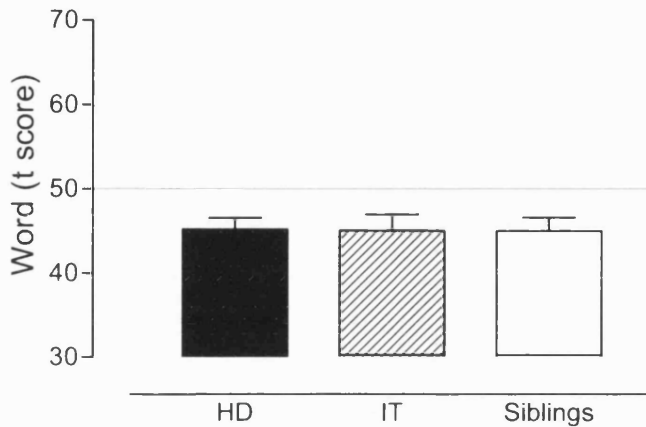


3.3.1.6 Processing speed

Speeded verbal response (Stroop word)

There were no significant differences between groups on the test of speeded verbal response (ANOVA: $F(2, 42) = 0.01, p = 0.99$)(Figure 3.7).

Figure 3-7 Speeded verbal response in each group (Mean \pm SEM)



Speeded motor response (Map motor control)

There was a significant difference between groups on the test of speeded motor response, where the HD survivors completed this task in a significantly slower time than the IT survivors (ANOVA: $F(2, 41) = 3.67, p = 0.03$: Planned comparisons: Surv vs. Sibs $t = -1.36, df = 41, p = 0.18$; IT VS. HD $t = 2.35, df = 41, p = 0.02$)(Figure 3.8). The difference between groups was not accounted for by age-at-test (ANCOVA: $F(2,40) = 8.54; p = 0.001$).

Within the HD, there was no significant difference between the times for girls and boys group (Indep. T test: $t = -0.04; p = 0.97$). While there was a significant correlation between time per target and age-at-diagnosis within the HD group (Corr: Pearson's $r = -0.72; p = 0.003$) there were insufficient data to describe this relationship independently from age-at-test (Figure 3.9).

Figure 3-8 Speeded motor response in each group (Mean \pm SEM)

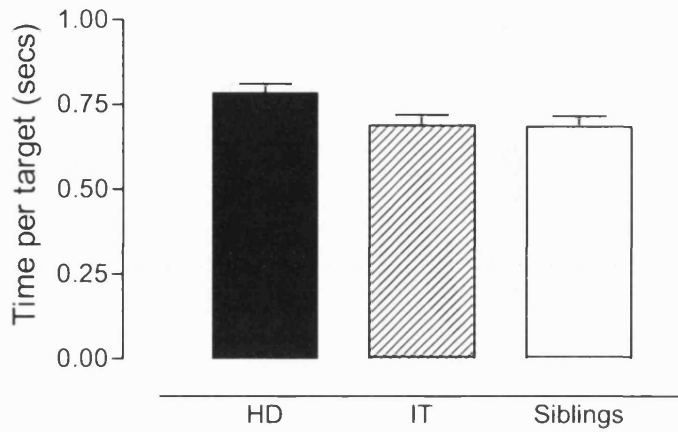
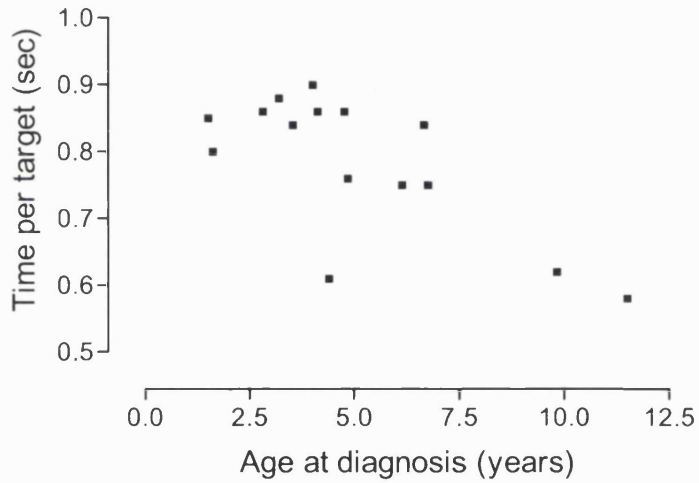


Figure 3-9 Relationship between speeded motor response and age-at-diagnosis in the HD group



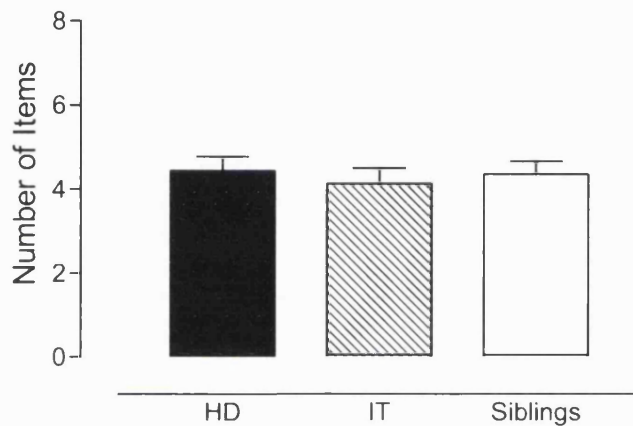
3.3.2 Cognitive flexibility – self monitoring

3.3.2.1 Working memory

Auditory-verbal (Digits backwards)

There were no significant differences between groups on the test of auditory-verbal working memory (ANOVA: $F(2, 42) = 0.35, p = 0.70$)(Figure 3.10).

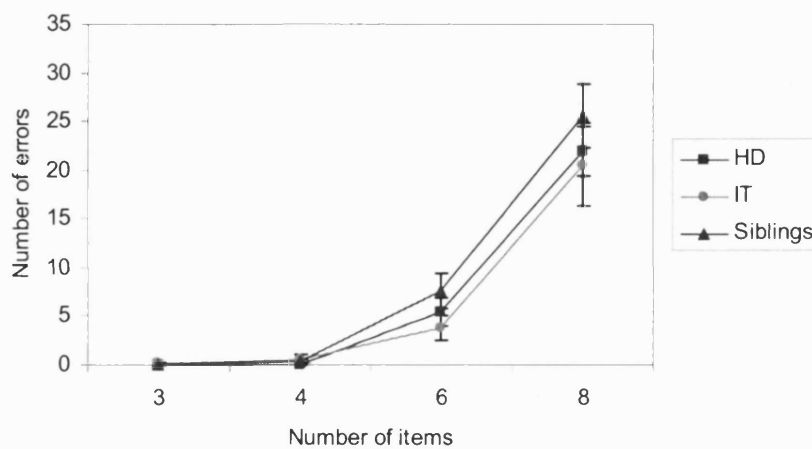
Figure 3-10 Auditory-verbal working memory in each group (Mean \pm SEM)



Spatial working memory

Parametric analyses of the square root transformed data revealed that the number of between-search errors increased significantly with set size (ANOVA: $F(1.35, 123) = 124.99; p < 0.001$ (with Greenhouse-Geisser correction))(Figure 3.11). However, there was no significant difference in the mean number of between-search errors made in each group (ANOVA: $F(2, 41) = 1.13, p = 0.33$).

Figure 3-11 The number of between-search errors by set size in each group (Mean \pm SEM)



3.3.2.2 Fluency (COWAT)

There was a significant difference between groups on the verbal fluency test with the HD survivors performing significantly worse than the IT survivors (ANOVA: $F(2, 42) = 4.22$, $p = 0.02$: Planned comparisons: Surv vs. Sibs $t = -1.27$, $df = 42$, $p = 0.21$; IT VS. HD $t = -2.59$, $df = 42$, $p = 0.01$)(Figure 3.12). The difference between groups was not accounted for by vocabulary (ANCOVA: $F(2,41) = 6.15$; $p = 0.005$).

The difference in the raw number of words produced by each group approached significance. This was accounted for a reduction in the number of words produced in the HD group when compared to the IT group (ANOVA: $F(2, 42) = 2.46$, $p = 0.10$: Planned comparisons: Surv vs. Sibs $t = 0.79$, $df = 42$, $p = 0.44$; IT VS. HD $t = -1.98$, $df = 42$, $p = 0.06$)(Figure 3.13). There was no significant difference between groups on the percentage of errors made (e.g. same word different endings or proper nouns)(Table 3.2)(MWU: $z = -1.34$; $p = 0.18$).

Within the HD group, there was no significant difference between the score for girls and boys (Indep. T test: $t = 0.28$; $p = 0.79$). Similarly, there was no significant correlation between verbal fluency scores and age-at-diagnosis within the HD group (Corr: Pearson's $r = 0.12$; $p = 0.65$).

Figure 3-12 Verbal fluency in each group (Mean \pm SEM)

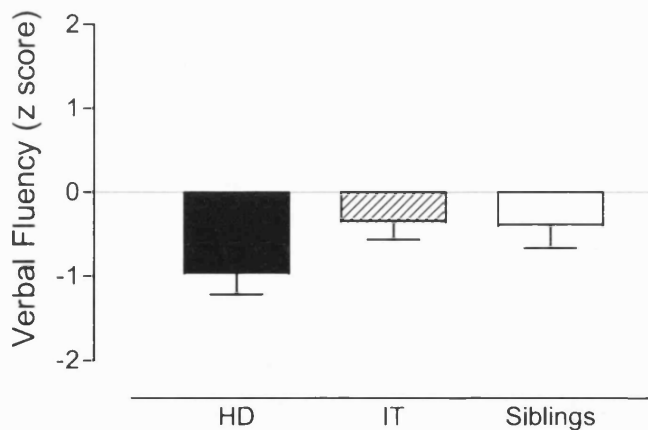


Figure 3-13 The number of words (including errors) made in each group (Mean \pm SEM)

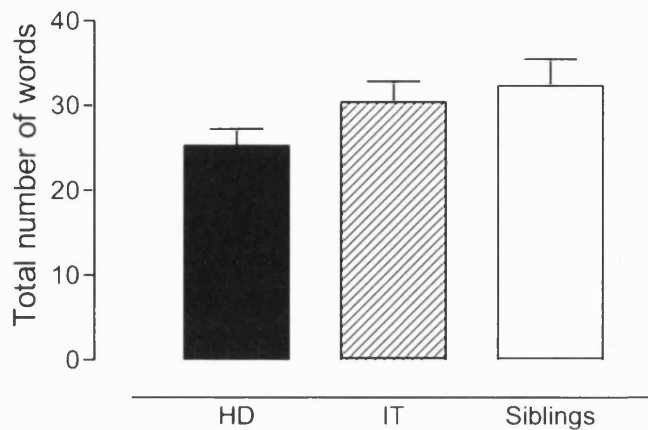


Table 3-2 Analysis of the errors made on the test of verbal fluency by the HD group

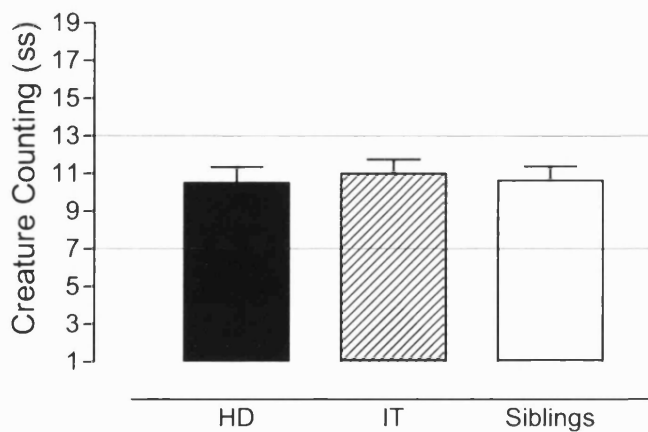
Type of errors	Percentage of total errors made
Repetitions (not perseveration)	4 %
Perseveration	0 %
Words beginning with other letters	15 %
Similar words with different endings	35 %
Proper nouns	42 %
Other (e.g. made up words)	4 %

3.3.2.3 Switching attention

Creatures counting/Visual elevator

There were no significant differences between groups on the test of switching attention (ANOVA: $F(2, 42) = 0.11, p = 0.90$)(Figure 3.14).

Figure 3-14 Performance on the Creature counting/Visual elevator task in each group (Mean \pm SEM)



Lines on the graph represent the normal range (i.e. 7-13).

ED shift (IDED task)**Stages completed**

The overall level of performance as indicated by the number of stages completed (max=9) achieved was similar between groups (Table 3.3). Two children failed to complete all nine stages. Both children were in the IT group and failed to achieve the extradimensional reversal stage.

Table 3-3 Percentage of children completing nine stages on the IDED task in each group

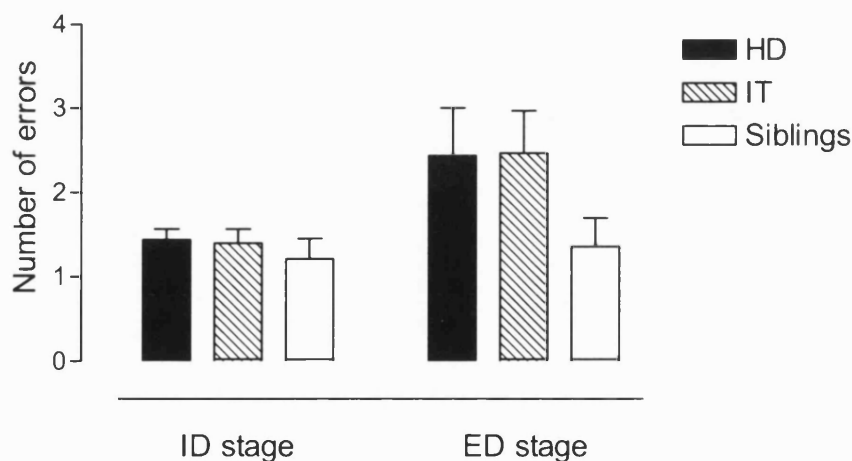
	Less than 9 stages	9 stages
HD	0%	100%
IT	13%	87%
Siblings	0%	100%

ED stage

Parametric analyses of the square root transformed data did not reveal significant difference between groups on the number of trials required to complete the ED shift (ANOVA: $F(2, 42) = 0.73, p = 0.49$)(Figure 3.16).

Repeated measures analyses indicated that the number of errors made at the ED stage was greater than the number of errors made at the ID stage⁷ ($F(1,42) = 18.03, p < 0.001$). However, again there was no significant difference between groups (ANOVA: $F(2,42) = 1.81, p = 0.18$)(Figure 3.16).

Figure 3-15 The number of errors made at the ID and ED stages in each group (Mean \pm SEM)



⁷ This is consistent with the literature indicating that the ED shift more difficult than the ID shift (Roberts *et al.* 1988).

3.3.2.4 Stimulus-reward learning

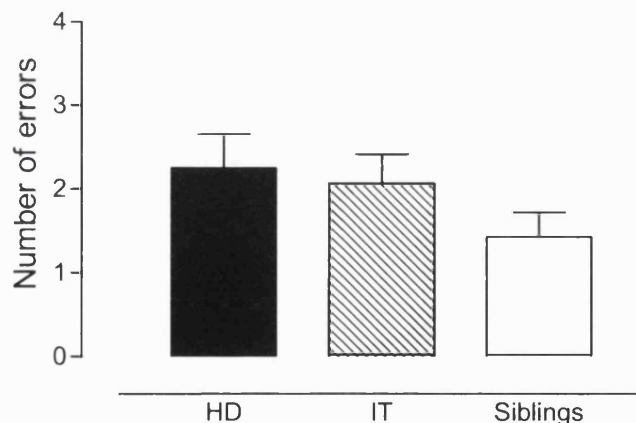
Visual discrimination (IDED task)

Parametric analyses of the square root transformed data revealed a significant difference between survivors and siblings on the number errors made on the simple visual discrimination (ANOVA: $F(2, 42) = 3.89$, $p = 0.03$; Planned comparisons: Surv vs. Sibs $t = -2.77$, $df = 42$, $p = 0.01$; IT VS. HD $t = 0.43$, $df = 42$, $p = 0.67$, Effect size = 0.85)(Figure 3.15).

Poor performance in the survivor group was not accounted for by age-at-test (ANCOVA: $F(2, 41) = 4.67$; $p = 0.02$). Poor performance in the survivor group was not related to sex (Indep. t test: $t = -0.40$, $p = 0.69$) or age-at-diagnosis (Pearson's: $r = 0.37$, $p = 0.12$).

The difference between survivors and siblings, however, failed to reach significance when the analysis was restricted to matched survivor-sibling pairs only (Matched sample t test: $t = 1.56$, $p = 0.14$, Effect size = 0.51).

Figure 3-16 The number of errors made at the visual discrimination stage in each group (Mean \pm SEM)



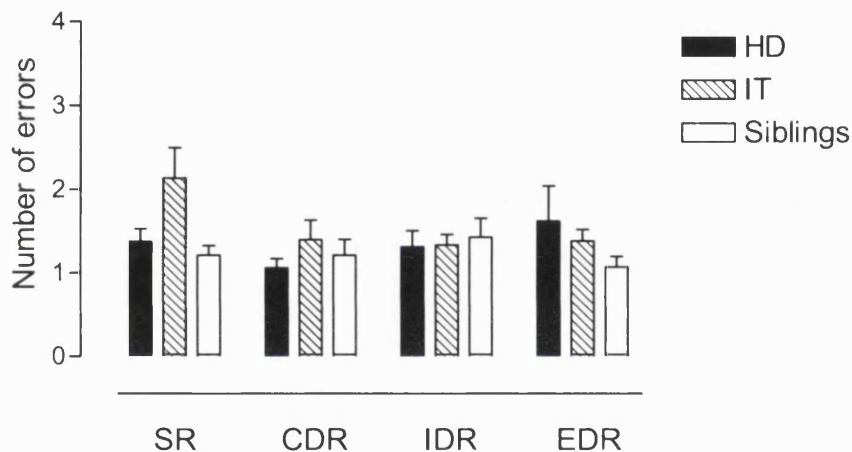
Reversal learning (IDED task)

Parametric analyses of the square root transformed data revealed that there was a significant difference between groups on the number of errors on the first reversal. This was a result of poor performance by the IT group in comparison to the HD group (ANOVA: $F(2, 42) = 2.70$, $p = 0.09$; Planned comparisons: Surv vs. Sibs $t = -0.93$, $df = 42$, $p = 0.36$; IT VS. HD $t = -2.10$, $df = 42$, $p = 0.05$)(Figure 3.17).

Poor performance in the IT group was not accounted for by age-at-test (ANCOVA: $F(2,41) = 5.09$, $p = 0.01$) or by the differences between groups observed on the simple visual discrimination stage (i.e. Level 1 of the IDED task)(ANCOVA: $F(2,41) = 2.59$, $df = 41$, $p = 0.09$). Poor performance in the IT group was also not related to sex (Indep. t test: $t = 0.06$, $p = 0.95$) or age-at-diagnosis (Pearson's: $r = -0.24$; $p = 0.51$).

There was no significant difference between groups on the number of errors made for subsequent reversals ((Compound reversal ANOVA: $F(2, 42) = 0.30$, $p = 0.74$)(Intradimensional reversal ANOVA: $F(2, 42) = 0.54$, $p = 0.59$)(Extradimensional reversal⁸ ANOVA: $F(2, 40) = 0.91$, $p = 0.41$)).

Figure 3-17 The number of errors at the reversal stages in each group (Mean \pm SEM)



SR = simple reversal; CDR = compound discrimination reversal; IDR = intradimensional reversal; EDR = extradimensional reversal.

Extinction learning

Two children in the HD group failed the discrimination stage of the extinction test. Therefore, the results from these children are not included in the analyses.

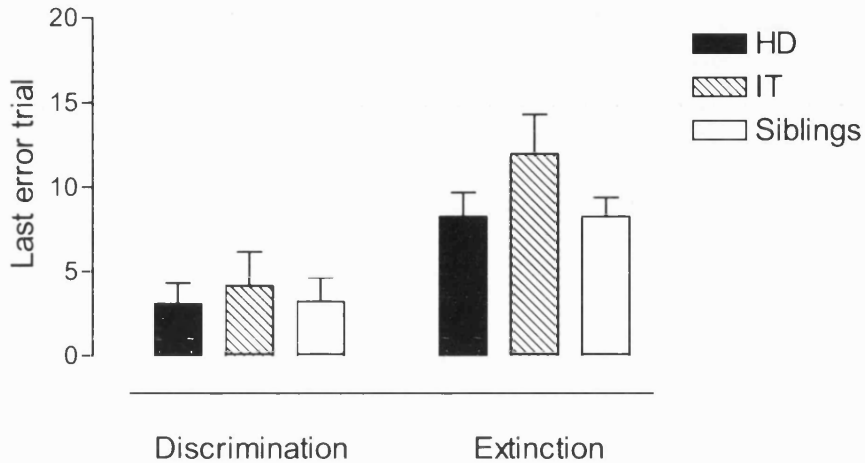
There was no significant difference between groups on discrimination learning (ANOVA: $F(2,40) = 0.13$, $p = 0.88$).

Parametric analyses of the square root transformed data revealed a difference between groups on the last error extinction trial that approached significance. This reflected poor performance of the IT group in comparison to the HD group (ANOVA: $F(2, 40) = 2.93$, $p = 0.08$: Planned comparisons: Surv vs. Sibs $t = -1.25$, $df = 34.7$, $p = 0.22$; IT VS. HD $t = -1.95$, $df = 23.5$, $p = 0.06$)(Figure 3.18).

⁸ Two children in the IT group failed this stage. Their data is not included in this analysis or at the EDR stage in Figure 3.17.

Poor performance in the IT group was not related to sex (Indep. t test: $t = 0.06$; $p = 0.95$) or age-at-diagnosis (Pearson's: $r = 0.08$; $p = 0.81$).

Figure 3-18 Last error trial for discrimination and extinction learning in each group (Mean \pm SEM)



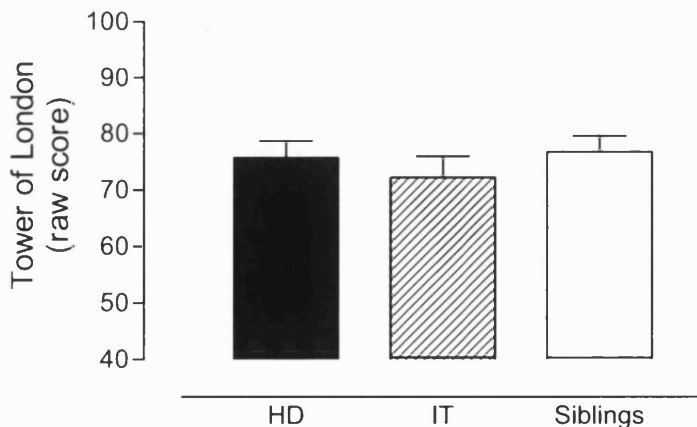
3.3.3 Goal setting

3.3.3.1 Planning

Tower of London

There were no significant differences between groups on the Tower of London (ANOVA: $F(2, 42) = 0.53$, $p = 0.60$)(Figure 3.19).

Figure 3-19 Performance on the Tower of London in each group (Mean \pm SEM)



BADS Zoo map

Performance on the BADS zoo map subtest was divided into scores less than, and scores equal to four (Table 3.4). There was no significant difference between groups on this task ($\text{Chi}^2 = 0.83$; $\text{df} = 2$; $p = 0.66$).

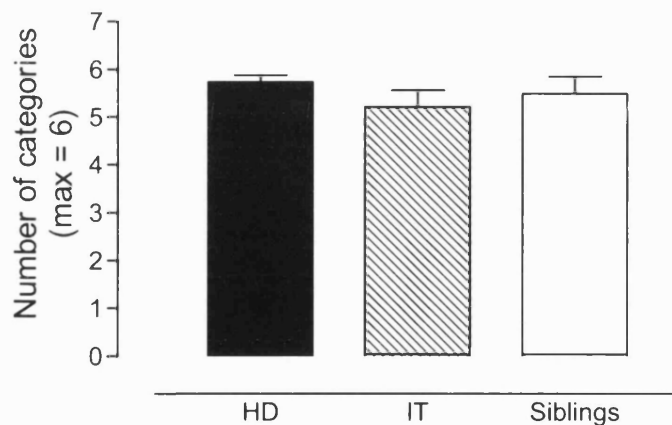
Table 3-4 Percentage of children with raw scores on the BADS zoo map subtest less than or equal to four

	Raw score < 4	Raw score = 4
HD	44 %	56 %
IT	60 %	40 %
Siblings	50 %	50 %

3.3.3.2 Problem solving**WCST**

There was no significant difference between groups on the number of categories completed on the WCST (ANOVA: $F(2,36) = 0.74$, $p = 0.48$)(Figure 3.20)

Figure 3-20 The number of categories completed on the WCST in each group (Mean \pm SEM)



BADS Action program

Performance on the BADS action program subtest was divided into scores less than, and scores equal to four (Table 3.5). There was no significant difference between groups on this task ($\text{Chi}^2 = 0.06$; $\text{df} = 2$; $p = 0.97$).

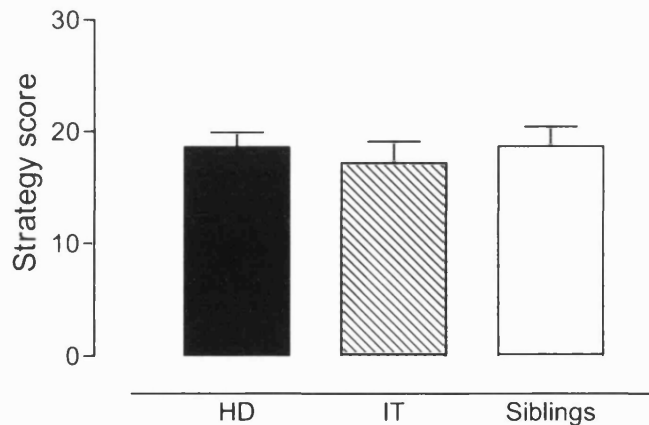
Table 3-5 Percentage of children with raw scores on the BADS action program subtest less than or equal to four

	Raw score < 4	Raw score = 4
HD	38 %	63 %
IT	40 %	60 %
Siblings	35 %	64 %

3.3.3.3 Strategic behaviour**Spatial working memory strategy score**

Using parametric analyses of the square root transformed data, there was no significant difference between groups on the strategy score (ANOVA: $F(2, 41) = 0.29$, $p = 0.75$)(Figure 3.21).

Figure 3-21 Strategy scores on the spatial working memory task in each group (Mean \pm SEM)

**BADS Key search**

Performance on the BADS key search subtest was divided into scores less than and scores equal to four (Table 3.6). There was no significant difference between groups on this task ($\text{Chi}^2 = 2.55$; $\text{df} = 2$; $p = 0.28$).

Table 3-6 Percentage of children with raw scores on the BADS key search subtest less than or equal to four

	Raw score < 4	Raw score = 4
HD	38 %	63 %
IT	60 %	40 %
Siblings	64 %	36 %

3.3.3.4 Organisation

BADS 6 elements

Performance on the BADS 6 elements subtest was divided into scores less than four and scores equal to four (Table 3.7). There was a significant difference between groups on this task with the sibling group more likely to achieve a score of four than the survivor group ($\text{Chi}^2 = 11.93$; $\text{df} = 2$; $p = 0.003$). There was no qualitative relationship between BADS 6 elements scores and sex (Table 3.8) or age-at-diagnosis (Table 3.9) within the survivor groups.

Table 3-7 Percentage of children with raw scores on the BADS 6 elements subtest less than or equal to four

	Raw score < 4	Raw score = 4
HD	63 %	38 %
IT	73 %	27 %
Siblings	14 %	86 %

Table 3-8 The number of boys and girls scoring less than or equal to four in the survivor groups

	Raw score < 4	Raw score = 4
HD Boys	6/16	3/16
HD Girls	4/16	3/16
IT Boys	4/15	3/15
IT Girls	7/15	1/15

Table 3-9 The mean age-at-diagnosis for children scoring less than or equal to four in the survivor groups

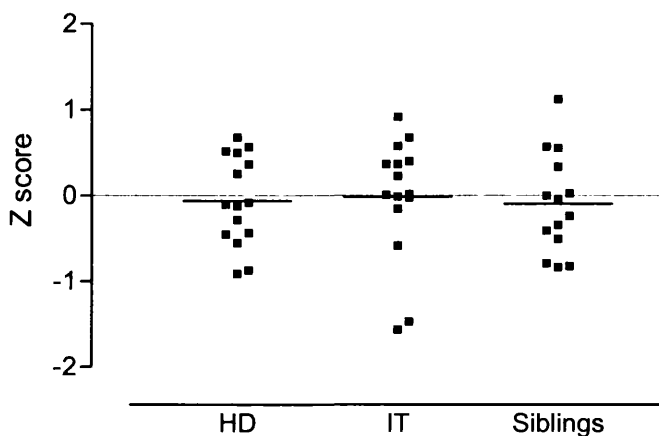
	Raw score < 4	Raw score = 4
HD Mean age-at-diagnosis (range)	4.90 (1.49 – 7.66)	5.71 (1.60 – 11.50)
IT Mean age-at-diagnosis (range)	4.90 (2.57 – 10.17)	5.53 (3.08 – 12.13)

3.3.4 Overall indices of function

3.3.4.1 Attentional control-processing speed index

There were no significant differences between groups on the Attentional control-processing speed index (ANOVA: $F(2, 41) = 0.17$, $p = 0.85$, Effect size = 0.17)(Figure 3.22). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (Matched sample t test: $t = -0.07$, $p = 0.95$, Effect size = 0.04).

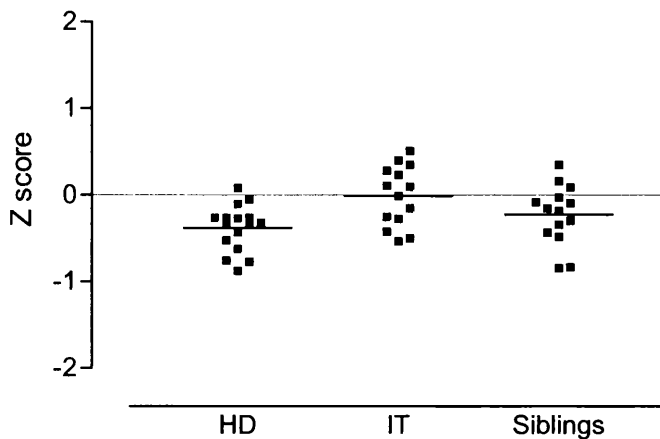
Figure 3-22 Attention control-processing speed index



Dashed line indicates z score = 0.

3.3.4.2 Cognitive flexibility-self monitoring index

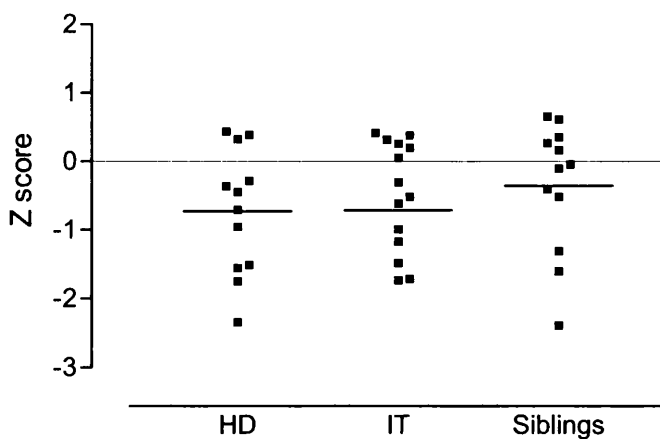
There were no significant differences between groups on the Cognitive flexibility-self monitoring index (ANOVA: $F(2, 41) = 2.06$, $p = 0.14$, Effect size = 0.01)(Figure 3.23). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (Matched sample t test: $t = 0.13$, $p = 0.90$, Effect size = 0.05).

Figure 3-23 Cognitive flexibility-self monitoring index

Dashed line indicates z score = 0.

3.3.4.3 Goal setting index

There were no significant differences between groups on the Goal setting index (ANOVA: $F(2, 36) = 2.43$, $p = 0.11$, Effect size = 0.35)(Figure 3.24). There was also no significant difference between survivors and siblings when the analysis was restricted to matched survivor-sibling pairs only (Matched sample t test: $t = -1.28$, $p = 0.22$, Effect size = 0.42).

Figure 3-24 Goal setting index

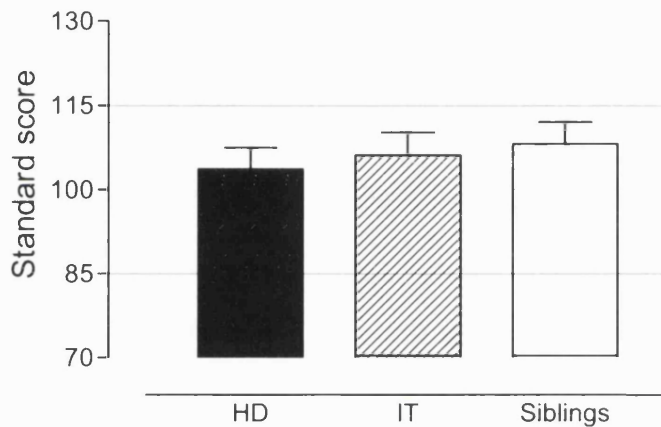
Dashed line indicates z score = 0.

3.3.5 Related measures of function

3.3.5.1 General function

Consistent with results of full IQ assessments conducted over the first five years post-diagnosis, there were no significant differences between groups on the estimate of IQ obtained in this assessment (ANOVA: $F(2, 42) = 0.33$, $p = 0.72$)(Figure 3.22).

Figure 3-25 IQ estimates in each group (Mean \pm SEM)



Lines on the graph represent the normal range (i.e. 85-115).

3.3.5.2 Behaviour

General behaviour

There was no significant difference between groups on the Total Behavioural Problems scale (ANOVA: $F(2,41) = 2.06$, $p = 0.14$)(Figure 3.23). In addition, there were no significant differences between groups on the Internalising Behavioural Problems scale (ANOVA: $F(2,41) = 1.23$, $p = 0.30$) or the Externalising Behavioural Problems scale (K-W: $\chi^2 = 1.14$, $df = 2$, $p = 0.57$).

Profile sub-scores

Profile sub-scores were within the normal range for all participants in the HD group. While the majority of profile scores were in the normal range for participants in the IT and sibling groups, five children in the IT group and three siblings had profile sub-scores within the borderline ($N=5$) or clinically significant range ($N=3$)(Table 3.10).

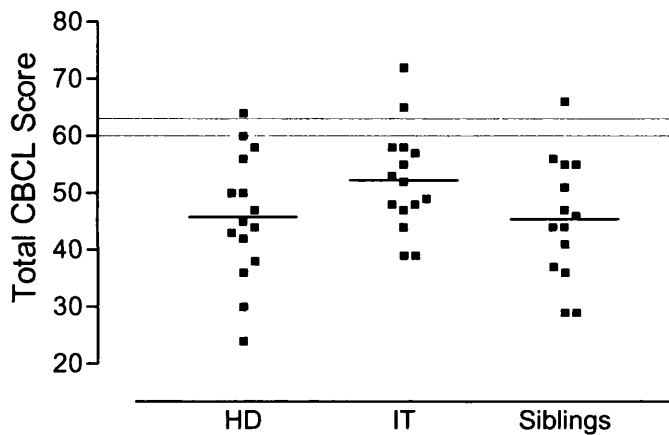
Borderline sub-scores were observed on the somatic complaints, anxious/depressed, thought problems, and attention problems profiles in the IT group. Within the sibling group borderline sub-scores were observed on the social problems profile.

Three children had clinically significant sub-scores (i.e score > 70). These were all on the internalising sub-score profiles. Two children were identified in the IT group with sub-scores exceeding 70 on the somatic complaints profile. A sibling was identified within the clinically significant range on the anxious/depressed profile.

Children receiving additional help at school

Parents indicated that six participants received additional help or attended special classes at school. These children were divided evenly across the groups (HD = 2; IT = 2; Siblings = 2). Children received additional help for reading (N=2), maths (N=1) or for several subjects (N=2). The remaining sibling attended a special class for advanced maths.

Figure 3-26 Total behavioural problem scores for the study participants



Solid line indicates mean for group; dashed line indicates the classification of score - normal range: score < 60; borderline range: 60 < score < 63; clinically significant: score > 63.

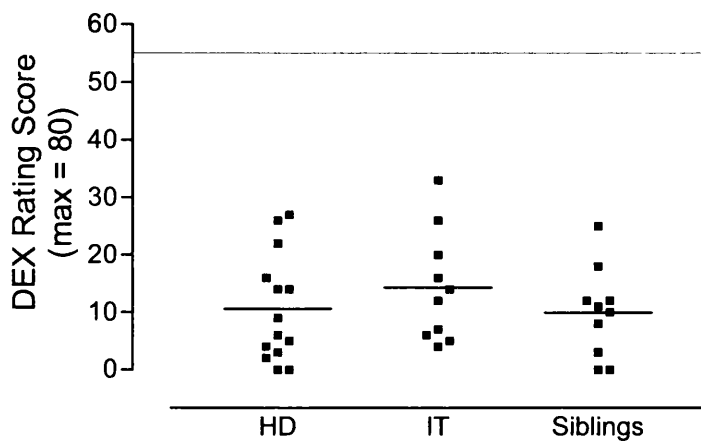
Table 3-10 Number of participants with profile sub-scores ≥ 67 (raw scores)

Profile	HD	IT	Siblings
Internalising			
Withdrawn	0	0	0
Somatic complaints	0	3 (67; 70; 90)	0
Anxious/depressed	0	1 (70)	1 (75)
Externalising			
Delinquent behaviour	0	0	0
Aggressive behaviour	0	0	0
Other			
Social problems	0	0	2 (68; 68)
Thought problems	0	2 (67; 68)	0
Attention problems	0	2 (67; 68)	0

Dysexecutive Behaviour

Scores for all participants on the dysexecutive questionnaire were within the normal range. Using non-parametric analyses there was no significant difference observed between the groups (KW: $\chi^2 = 1.53$; $df = 2$; $p = 0.47$)(Figure 3.24).

Figure 3-27 Dysexecutive behaviour scores for the study participants



Dashed line indicates the 90th percentile for adults with the dysexecutive syndrome (Wilson et al. 1996). Scores below this have been interpreted as falling within the normal range.

3.3.6 Summary of results

The results from the tests of executive functions are presented in Table 3.11.

Table 3-11 Summary of executive functions

Component of EF	Index	Significant ANOVA	Surv vs. Sibs	IT vs. HD
Attentional control-processing speed				
Attention span (auditory verbal)	Digits forwards	N		
Attention span (visuo-spatial)	Corsi blocks	N		
Selective attention	Map mission	N		
Sustained attention	Score!/Elevator counting	N		
Response inhibition	Walk Don't Walk	N		
Resistance to interference	Stroop interference score	N		
Speeded verbal response	Stroop word t score	N		
Speeded motor response	Map motor control	Y (p=0.03)	N	Y (HD<IT) ^{a,b} (p=0.02)
Cognitive flexibility-self monitoring				
Working memory (auditory-verbal)	Backwards digits	N		
Working memory (spatial)	Between-search errors (SWM)	N		
Fluency	FAS	Y (p=0.02)	N	Y (HD<IT) ^{a,c} (p=0.01)
Switching attention	Creature/Elevator counting	N		
Switching attention	ED shift (IDED)	N		
S-R learning (reversal)	First reversal (IDED)	Y (p=0.09)	N	Y (IT<HD) ^{a,c} (p=0.05)
S-R learning (extinction)	Last error trial	Y (p=0.08)	N	Y (IT<HD) ^{a,c} (p=0.06)

Executive Functions

Component of EF	Index	Significant ANOVA	Surv vs. Sibs	IT vs. HD
Goal setting				
Planning	Tower of London	N		
Planning	Zoo map (BADs)	N		
Problem solving	WCST	N		
Problem solving	Action program test (BADs)	N		
Strategic behaviour	SWM strategy score	N		
Strategic behaviour	Key search (BADs)	N		
Organisation	6 elements	Y ($p < 0.01$)	Y (Surv < Sibs) ^{a,c}	N
Related measures				
General function	IQ estimate (vocabulary + block design)	N		
General behaviour	Total problems CBCL	N		
Executive behaviour	DEX questionnaire	N		

^(a) finding not related to sex

^(b) finding related to age-at-diagnosis but there was insufficient data to describe this relationship independently from age-at-test

^(c) finding not related to age-at-diagnosis

3.4 Discussion

The results of the current study indicated that there were no significant differences between groups on the overall indices of EF. These included measures of Attentional control-processing speed, Cognitive flexibility-self monitoring, and Goal setting. Subtle differences in test scores associated with each of the domains of EF are discussed in the following sections.

Attentional control

The results of the current study failed to identify significant differences between groups on the measures of attention span, selective attention, sustained attention, response inhibition, or resistance to interference.

In the HD group, this finding is consistent with reports of intact selective attention and vigilance (D2 concentration test or the Dot cancellation test) in LT survivors treated with similar HD MTX protocols and compared to age-matched controls (Uberall *et al.* 1996; Kingma *et al.* 2002). The results in the IT group are also consistent with performance of children treated with more aggressive intrathecal therapy (i.e. TIT) when compared to their siblings, on other tests of attention and vigilance (e.g. the Double mark test of attention or the Continuous performance test) (Giralt *et al.* 1992; Williams *et al.* 1992). In addition, no significant differences in attention were observed in children six years from diagnosis treated with IT MTX (Tamaroff *et al.* 1982).

Furthermore, age-appropriate resistance to interference scores on the Stroop Colour and Word test have also been reported in children treated with IT MTX (Butler *et al.* 1994). Similarly, no significant differences have been reported on measures of impulsivity (e.g. Matching familiar figures test) in the LT survivors treated with either HD MTX + FAR (Mulhern *et al.* 1988) or children treated with TIT (Brown *et al.* 1992), and test norms.

The literature reports both intact span (Schatz *et al.* 2000; Kingma *et al.* 2002) and reduced span (Harila-Saari *et al.* 1998) within the LT survivors treated with chemotherapy-only. For example, intact verbal and spatial span were observed in survivors treated with heterogeneous chemotherapy-only protocols (30 months post diagnosis) when compared to age-, sex- matched controls (Schatz *et al.* 2000). In contrast, impaired digit span has been reported in a LT survivor treated with chemotherapy-only with evidence of white matter abnormalities on MRI (Case 1, Harila-Saari *et al.* 1998). This child has also been born prematurely and suffered from

epilepsy. The inconsistency may therefore be related to the additional problems experienced by this child.

Processing speed

Results of the current study also failed to identify significant differences in processing speed when a verbal response was required. In contrast, results highlighted poor performance of the HD group when compared to the IT group on a processing task requiring a speeded motor response. This was not accounted for by age-at-test. Poor performance on this test, requiring rapid circling of target symbols (without distractors), may occur as a consequence of reduced visuo-motor integration.

The literature supports reduced visuo-motor integration in the LT survivors of ALL. Furthermore, the results of the current study are consistent with the literature describing poor visuo-motor integration in children treated with HD MTX but not IT MTX-only. For example, poor visuo-motor integration has been reported in LT survivors treated with HD MTX when compared to siblings on the Bender-Gestalt test (Moss *et al.* 1981). In particular, errors were commonly distortions or copying inaccuracies. Similarly, poor visuo-motor integration (Beery's test of visuo-motor integration or Wechsler Coding subtest) has been described in survivors treated with more aggressive therapy (i.e. TIT), three (Giralt *et al.* 1992), or four (Brown *et al.* 1992) years post-diagnosis (but see also Williams *et al.* 1992). In addition, non-significant declines in performance have been reported in survivors treated with HD MTX + TIT compared to age matched controls (Kingma *et al.* 2002). In contrast, no significant differences in visuo-motor integration were reported in survivors treated for various cancers with IT MTX (Butler *et al.* 1994).

Poor visuo-motor intergration has been associated with calcifications and the presence of white matter changes on MRI in the LT survivors of ALL treated with combined irradiation and chemotherapy (Iuvone *et al.* 2002). In particular, calcification of the subcortical arcuate fibres (cortico-medullary junctions), or white matter pathology of the posterior portion centrum semiovale and/or deep parietal white matter (notable sparing the subcortical U fibres), was associated with low scores on the test of visuo-motor integration. These studies highlight the importance of white matter in visuo-motor integration.

The results of the current study also indicated that the performance within the HD group was not related to sex. This is consistent with reports in the literature showing neither sex is more vulnerable to LT impairments in visuo-motor intergration (Brown *et al.* 1992; Copeland *et al.* 1996; Kingma *et al.* 2002). However, it was unclear if poor

performance by the HD group on the speeded motor task was related to age-at-diagnosis. Within the literature poor visuo-motor integration has inconsistently been related to age-at-diagnosis in survivors treated with HD MTX. For example, poor performance was observed in survivors treated at a young age for leukaemia or lymphoma at five years post-diagnosis (Copeland *et al.* 1996). In contrast non-significant declines in visuo-motor integration were not related to age-at-diagnosis in survivors treated with TIT (Kingma *et al.* 2002).

While the relationship with age-at-diagnosis is unclear, visuo-motor integration is likely to be an important factor limiting the performance of the HD group on this task given the literature. A specific investigation of visuo-motor integration in the survivors (especially the HD group) using standardised measures would help to evaluate this relationship (e.g. the Beery's test of Visuo-motor integration).

Cognitive flexibility – self monitoring

The results of the current study failed to reveal significant group differences on the tests of working memory or switching attention.

This is consistent with the literature describing intact working memory (Digits reversed) between survivors (>5 years post-diagnosis) treated with HD MTX and test norms (Mulhern *et al.* 1988). In addition, no significant differences in working memory (accuracy of responses on an auditory verbal Sternberg working memory paradigm) were reported for survivors treated with HD MTX (4/11 had received additional CI) when compared to healthy controls or survivors of solid tumours (Lahteenmaki *et al.* 1999). Furthermore, intact verbal and spatial working memory has been reported in survivors treated with heterogeneous chemotherapy-only protocols and age-, sex-matched controls (Schatz *et al.* 2000).

In contrast, poor attention switching (Trail making test part B) has been reported in LT survivors treated with HD MTX + TIT or aggressive intrathecal treatment (e.g. TIT) when compared to age-matched controls (Lesnik *et al.* 1998; Kingma *et al.* 2002). Similarly, poor attentional switching has been described in survivors treated with HD MTX when compared to test norms (Moore *et al.* 1992). The inconsistency in these findings may be related to the demands of the task involved. For example, in the current study the switching task involved verbal responses to visual stimuli, whereas the Trail Making B task requires a visuo-motor output. As described above survivors treated with HD MTX have difficulty completing tasks requiring complex visuo-motor integration. It is possible therefore that the changes observed on the Trail making task are related to an underlying visuo-motor integration difficulties.

Verbal fluency

The results of the current study indicated that the HD group was significantly impaired on the test of verbal fluency. This is consistent with the literature describing poor performance on this test in survivors treated with aggressive intrathecal therapy (TIT) when compared to their siblings (uncorrected for multiple comparisons)(Giralt *et al.* 1992). In the current study, poor performance was associated with a reduction in productivity (i.e. poor word generation over time). However, there was also a qualitative increase in the number of errors produced (i.e. word extensions and proper nouns) in the HD group. The significant group difference in verbal fluency was not accounted for by a decrease in vocabulary.

LT pathology following treatment with combined chemotherapy and radiation for ALL has been associated with poor performance on the test of verbal fluency. For example, three children developing calcifications of frontal lobes and basal ganglia (Cases 4 and 12), or calcification of frontal lobes and dilation of the lateral ventricles and subarachnoid spaces (Case 6) as revealed by CT showed poor word fluency when compared to test norms (Appleton *et al.* 1990). In addition, poor verbal fluency has been associated with calcification of the basal ganglia and/or atrophy as revealed by CT when compared to survivors with normal scans (Brouwers *et al.* 1985). In particular, the poor performance in that study was associated with a decrease in the number of words produced.

Frontal lobe lesions in adults are associated with a reduction in word production on the verbal fluency task (Baldo & Shimamura 1998). Similarly, the performance of the verbal fluency task in healthy adults is associated with an increase in activity of the anterior cingulate and inferior frontal gyrus (Phelps *et al.* 1997). Furthermore, word fluency (Mendez *et al.* 1989) and expressive language deficits (Wallesch *et al.* 1983) have been associated with basal ganglia pathology. Together these studies highlight the importance of the subcortical structures and frontal lobes in performing this task.

Visual discrimination

The results of the current study indicated that the survivor groups showed poor visual discrimination learning when compared to the sibling group. In particular, the survivor groups made more errors than the sibling group on level 1 of the IDED task. This finding may be related to (1) visual-perceptual problems, (2) poor visuospatial memory, or a (3) learning deficit.

Visual-perceptual and visuospatial memory problems have not been described in LT survivors treated with chemotherapy-only when compared to their siblings (Giralt *et al.* 1992; Williams *et al.* 1992), healthy controls (Kingma *et al.* 2002), or children treated for other cancers (Butler *et al.* 1994). Similarly, intact visual perceptual and visual memory skills were identified in the current study participants at 5 years post-diagnosis (see Chapter 2).

Intact learning was also described for the study participants at 5 years post-diagnosis. However, the test used at that assessment was a verbal based test (i.e. verbal paired associates). Learning with non-verbal material was not assessed in that assessment. The results of the current study, suggest that non-verbal learning (e.g. visual discrimination learning) is reduced in the LT survivors treated with chemotherapy-only. Consistent with this, poor non-verbal learning (Porthus labyrinths test) has been described in LT survivors of ALL treated with TIT when compared to their siblings (Giralt *et al.* 1992).

Non-verbal learning problems may be a direct result of the administration of MTX. For example, experimental studies highlight the vulnerability of learning processes following treatment with MTX. Rats (Lewis-inbred) receiving a single dose of MTX at 17 days were impaired in learning the association between a conditioned stimulus and an aversive unconditioned stimulus using an aversive Pavlovian fear-conditioning paradigm (Yanovski *et al.* 1989) (but see also Stock *et al.* 1995 for a similar paradigm with Sprague-Dawley rats). In particular, the treated rats took longer to acquire the conditioned response than a deprived control group or normal control group. This study suggests that MTX can affect the acquisition of classical conditioning paradigms in rats (Yanovski *et al.* 1989).

The role of folate in learning has been described in other experimental studies. For example, rats born to folate-deficient mothers show poor learning capacity (Whitley *et al.* 1951). In addition, learning deficiency in rats can be reversed by the administration of folate (Bachevalier *et al.* 1981). Furthermore, large doses of folate impair learning in animals (Prakash & Petrie 1982).

Experimental studies also indicate that visual (pattern) discrimination learning is dependent upon the caudate nucleus (specifically the tail) and the inferior temporal cortices (Teng *et al.* 2000). For example, combined lesions of the tail or the caudate nucleus and hippocampus (but not the hippocampus-only) impaired pattern discrimination learning in monkeys. In addition, damage to tail of caudate nucleus in monkeys results in impaired pattern discrimination learning (Divac *et al.* 1967). Lesions

of area TE (which projects to the tail of the caudate nucleus) also impair performance in pattern discrimination (Dean 1976; Phillips *et al.* 1988; Buffalo *et al.* 1998; Buffalo 1999). Similarly, object-reward association is dependent upon the inferior temporal cortex (Parker & Gaffan 1998). Together these studies highlight the role of non-frontal (specifically inferior temporal cortex and the caudate nucleus) in visual discrimination learning. It would be advantageous to explore the function of the cortico-striatal learning system in future studies in this population.

Reversal and extinction

While significant differences in visual discrimination learning were observed between the survivor and sibling groups, there was also a significant difference observed between survivor groups on the reversal learning part of the IDED task (i.e. IT group < HD group). This was apparently not accounted for by poor visual discrimination learning. In addition, weak evidence indicated that extinction learning was also affected in the IT group when compared to the HD group.

Poor reversal and extinction learning is associated with lesions of the orbitofrontal cortex (Butter 1969; Jones & Mishkin 1972; Rolls *et al.* 1994; Meunier *et al.* 1997). For example, patients with orbitofrontal lesions produce more errors during reversal learning than patients with damage in other frontal or extra-frontal areas (Rolls *et al.* 1994). However, it has been shown that lesions of the orbitofrontal cortex do not impair the acquisition of simple visual discriminations. In addition, the reversal impairment following orbitofrontal lesions is restricted to the first reversal only (Iversen & Mishkin 1970; Dias *et al.* 1997).

The results of the current study are inconsistent with this literature describing the functional impairments associated with orbitofrontal pathology. For example, the IT group also showed impaired visual discrimination learning. In addition, the IT group showed a non-significant decrease in performance on the second reversal in the IDED task (i.e. compound discrimination reversal). Furthermore, two children in the IT group failed the reversal following the extradimensional shift. This was the fourth reversal of the task.

On the basis of the current findings it is unlikely that exclusive orbitofrontal pathology is responsible for the poor performance observed in the IT group. The results suggest that other brain areas are involved. In particular, the caudate nucleus and thalamus are important for stimulus-reward learning. For example, in the monkey lesions of the ventral caudate have been shown to impair visual discrimination reversal (Divac *et al.* 1967). In addition, neurons of the ventral caudate and ventral striatum mirror

orbitofrontal responses (Rolls *et al.* 1983; Williams *et al.* 1993). Furthermore, functional imaging studies have recorded an increase in activation of the caudate during the reversal part of the IDED task. This was unaccompanied by changes in the orbitofrontal cortex (Rogers *et al.* 2000).

The thalamus has also been implicated in stimulus-reward (reversal) learning. For example, excitotoxic lesions of the rat dorsomedial thalamus (but not the anterior thalamic nuclei or prelimbic cortex) have been associated with an increase in the number of errors made during reversal learning (Chudasama *et al.* 2001). In addition, lesions of the dorsomedial nuclei in the monkey are associated with impaired stimulus-reward reversal (Gaffan & Murray 1990).

On the basis of this literature therefore it is likely that the pathology responsible for the stimulus-rewards learning deficit seen in the IT group includes subcortical (e.g. caudate nucleus and/or thalamus) and/or cortical sites (e.g. orbitofrontal cortex).

Goal setting

The results of the current study did not reveal significant group differences on the tests of planning, problem solving and strategic behaviour.

These results are consistent with a report of WCST performance in LT survivors treated for ALL with chemotherapy-only (Butler *et al.* 1994). In that study, there was no significant difference in performance on the WCST (number of categories achieved) between survivors treated with IT MTX and children treated without PCNS therapy for other cancers (e.g. osteogenic sarcoma).

In contrast, the results of the current study revealed a significant difference between survivor and sibling groups on the 6 elements test of organisation. Non-significant declines in constructional organisation (Organisational score on the Rey-Osterrieth Complex Figure) have been identified in survivors treated with HD MTX when compared to test norms (Waber *et al.* 1995). Furthermore, the changes observed in that study were of a similar magnitude to the changes seen in survivors treated with combined chemotherapy and irradiation. While further investigation is required these results indicate that poor organisation may be a common LT consequence of PCNS therapy.

Importantly, impaired performance of the survivors on this task may reflect poor sustained attention. The six elements task required participants to sustain attention for a period of 10 minutes. Therefore, attention may be one factor responsible for reduced performance on the six elements task. While groups did not differ on sustained

attention (see above), the task administered in the current study involved relatively short trials of attention (i.e. < 2 minutes). It has been suggested that sustained attention is moderated by different neural mechanisms depending on the duration of the task. For example, sustained attention over relatively short periods of time (e.g. seconds) may be related to the posterior attention systems, whereas the anterior attention system may be involved in sustaining attention over a longer period of time (eg from 10 to 20 minutes) (Posner & Petersen 1990). It would therefore be advantageous to measure attention in the survivors over a much longer time period. This would help to assess the role of sustained attention in performance on the six elements task.

A change in sustained attention (i.e. fatigue effect) has been reported in survivors treated with combined radiation and chemotherapy. In particular, survivors with abnormalities identified on CT (atrophy or calcification), showed greater intra-individual variation in performance over time (e.g. reaction time task over 30 minutes), than survivors without abnormalities on CT. Furthermore, performance was poorest in children with basal ganglia or parietal area calcifications (Brouwers *et al.* 1984). Significant differences in sustained attention (d2 concentration test) have also been observed in LT survivors treated with chemotherapy-only or combined therapy and white matter changes on MRI (Hertzberg *et al.* 1997).

Overall performance

Despite poor performance on the speeded motor response, verbal fluency, stimulus-reward leaning, and the six elements tasks, there was no significant difference between groups on IQ estimate. This is consistent with reports of no correlation between IQ and tests of EF in children (Dennis *et al.* 1991; Welsh *et al.* 1991) and adults (Warrington *et al.* 1986). Similarly, early brain damage can result in impairments of EF but relatively intact intellectual functions (Asarnow *et al.* 1991; Garth *et al.* 1997; Levin *et al.* 1997; Pentland *et al.* 1998). This suggests that the impairments observed in the LT survivors were specific and not detected at general level of function. In addition, the results highlight the importance of using specific tests rather than reliance on IQ as only estimate of outcome in this population.

Behaviour

While an increase in behavioural problems, particularly on the internalising scale of the CBCL, have been reported for children with cancer (56% ALL) at diagnosis (Sawyer *et al.* 2000)(but see also Michalowski *et al.* 2001), these problems resolve with time and are reported with a similar frequency to that documented within the community in the longer term (Sawyer *et al.* 2000). Similarly, few behavioural problems have been

reported for children treated with chemotherapy-only for ALL at four (Noll *et al.* 1997) or five (Meadows & Evans 1976; Mulhern *et al.* 1988) years post-diagnosis. The results of the current study are consistent with this literature indicating that the LT psychosocial impact of ALL and its treatment during childhood is minimal. In addition, parents reported few dysexecutive type behaviours on the DEX questionnaire.

Importantly, anxiety may contribute to poor performance on tests of executive function (Tamaroff *et al.* 1982; Williams *et al.* 1992). Group increases in anxiety were not observed in the current study. This suggests that anxiety, as measured by the CBCL, is unlikely to explain the group differences observed on the measures of EF in this study (i.e. speeded motor response, fluency, stimulus-reward learning, and organisation).

Cautions to interpretation

While the results of the current study indicate that subtle difficulties in the survivor groups when compared to the sibling group, there are a number of factors worthy of consideration.

Firstly, inter-individual differences in performance may have masked group differences. For example, on the extradimensional shifting level of the IDED task there was large inter-individual variation in all groups. The level of inter-individual variation at this stage of the task is not surprising since performance at the ED stage of the task shows the greatest improvement with development over late childhood and adolescence (Luciana & Nelson 1998). However, inter-individual variation may have masked any group differences in performance on this task and suggests that this task may not have been sensitive to subtle group differences in attention shifting.

In addition, the tasks administered may not be as sensitive to frontal dysfunction as described in the literature for adults. For example, the spatial working memory task was selected as an index of frontal function on the basis of performance of this task in adult populations. The use of this task has assumed that the same cognitive processes and functional networks are used in children and adults to perform the task. While a functional imaging study has supported the recruitment of similar networks in adults and children on a task of spatial memory (Nelson *et al.* 2000), it has also been suggested that underlying cognitive processes may differ between children and adults. For example, the spatial working memory task may place a greater demand on non-executive memory in children because of the child's reduced spatial span when compared to adults (Luciana & Nelson 1998). Consistent with this, improvements in spatial span have been observed across the age range 7 – 15 years (Isaacs & Vargha-

Khadem 1989). Thus, the test may be less sensitive to frontal dysfunction in children than it is known to be in adults (Williams *et al.* 2000)

3.5 Summary

A systematic assessment of EF across three aspects of function was conducted within the LT survivors and their siblings. No significant differences were observed between groups on the indices of Attentional control-processing speed, Cognitive flexibility-self monitoring, and Goal setting. The results suggested that EF were intact within sample. Furthermore, parents reported minimal behavioural concerns for the study participants. The results of current investigation provided limited support for specific underlying pathology of the frontal lobes in the survivors of ALL.

4 Bimanual coordination

As discussed in Chapter 1, the maturation of the corpus callosum may be vulnerable to the effects of PCNS therapy in children treated for ALL. Pathology of the corpus callosum can be associated with impairments in bimanual coordination. Chapter 4 investigates bimanual coordination in the LT survivors and their siblings.

4.1 Introduction

The corpus callosum

The corpus callosum is the largest fibre tract linking the two hemispheres of the brain. In primates and humans, the fibres of the corpus callosum show topographical organisation where neocortical areas of the frontal lobes are reciprocally connected through the anterior two thirds of the corpus callosum, while the posterior section of the corpus callosum connects the parietal, temporal, and occipital cortices (Pandya *et al.* 1971; de Lacoste *et al.* 1985; Pandya & Seltzer 1986).

Bimanual coordination

The importance of the corpus callosum has been highlighted in studies of bimanual coordination. In particular, the coordination of the hand and finger movements is dependent upon communication through the corpus callosum, since hand and finger movements are controlled to a large degree by the contralateral hemisphere. This contrasts with the control of proximal limb movements (e.g. arms), which receive significant control from the ipsilateral hemisphere (Gazzaniga *et al.* 1967; Brinkman & Kuypers 1973; Goldman-Rakic & Schwartz 1982; Eliassen *et al.* 2000).

Morphological abnormalities of the corpus callosum can result in poor bimanual coordination. For example, poor coordination is reported in adults with complete callosotomies (Zaidel & Sperry 1977). Similarly, adults with partial callosotomies (including the anterior section of the corpus callosum) performed significantly worse than controls on a bimanual coordination task requiring subjects to draw diagonal lines of varying degrees using an X-Y plotter. In this task, each hand controlled either the X- or Y- axis and to perform the task subjects needed to make coordinated left and right hand movements (Preilowski 1972; Preilowski 1975). Poor performance was associated with slow execution and a greater number of errors on the task. Moreover, performance was especially poor when patients were asked to complete angles requiring greater left than right hand speed. Agenesis of the corpus callosum in adults

and children has also been associated with slow and inaccurate performance on this task despite extensive practice (Jeeves 1986; Jeeves *et al.* 1988).

The role of the corpus callosum in bimanual movements is also suggested by research correlating activity between neuronal populations in the two hemispheres. For example, transient interhemispheric correlations have been observed between neuronal groups in the primary motor cortex, where the strength of the correlation was related to the type of bimanual task. Importantly, the correlations between activity and behaviour were only observed in interhemispheric interactions and not in intra-hemispheric interactions suggesting that the task required interhemispheric cortico-cortical connections (Donchin *et al.* 2001). The authors propose that this connection is mediated by the corpus callosum.

Development of bimanual coordination in children

The integrity of the corpus callosum is also important during the development of bimanual coordination in children. In particular, children show a significant increase in the ability to make parallel (i.e. non-mirror) movements with advancing age (Fagard *et al.* 1985; Fagard *et al.* 2001). The increase in performance has been observed in children when compared to young adults on the Preilowski task (Jeeves *et al.* 1988). Similarly, improvements in performance (speed to complete unimanual and bimanual angles) were noted on the Etch-a-sketch adaptation of the Preilowski task in children aged 6-11 years (Steese-Seda *et al.* 1995) and 6-14 years (Marion *et al.* 2003). In particular, age related changes were observed in bimanual tasks requiring differential contributions of the left and right hands (Marion *et al.* 2003).

The improvements in bimanual coordination during childhood may be related to an increase in interhemispheric efficiency allowing the inhibition of ipsilateral motor cortex (Dennis 1976; Chiarello 1980; Geffen *et al.* 1994; Reddy *et al.* 2000). This inhibition presumably acts to suppress mirror activity, thereby facilitating bimanual motor performance. Alternatively, the improvements may reflect the increasing bilateral integration and use of visual and tactile feedback to moderate performance (Steese-Seda *et al.* 1995).

Additional studies suggest that the development of bimanual skills may be particularly sensitive to aberrations of development (MacNeilage *et al.* 1984). For example, poor bimanual tapping performance (alternating tapping task) has been observed in children with learning disabilities (Badian & Wolff 1977; Njiokiktjien & Ramaekers 1991) and dyslexia (Wolff *et al.* 1984; Rousselle & Wolff 1991). Poor performance (increase in errors) on the bimanual coordination task has also been observed in children and

adults with dyslexia (Moore *et al.* 1991; Moore *et al.* 1995), particularly, when the left hand contribution is greater than right, or when visual feedback is withheld (Gladstone *et al.* 1989). Similarly, tests of coordination and interhemispheric integration have been used to identify children with delayed development or cognitive impairments (Steese-Seda *et al.* 1995).

The experiments described in this chapter were carried out with a view to assess the functional integrity of the corpus callosum in the LT survivors of ALL and their siblings. Two tests of bimanual coordination were administered that required participants to make parallel (i.e. non mirror) movements. These tests were the bimanual tapping task and the bimanual coordination test.

4.2 Methods

4.2.1 Experiment 1 – bimanual tapping task

Subjects

All participants completed the bimanual tapping task. The number of left- and right-handed children is shown in Table 4.1. Handedness was defined as the hand used for writing.

Table 4-1 Handedness in each group

	Left-handed	Right-handed	Total
HD	3	13	16
IT	1	14	15
Siblings	3	11	14

Bimanual tapping task

The bimanual tapping task was based on the paradigm described by (Leonard *et al.* 1988) using the Thurstone tapping apparatus. Briefly, this apparatus consisted of two circular plates mounted on a board. Each plate was divided into four segments and each segment was numbered 1, 2, 3, or 4. Numbers were out of phase between the two plates (Figure 4.1). Participants used a stylus to complete three tapping tasks: unimanual sequencing, bimanual sequencing, and unimanual tapping. A counter recorded the number of contacts made between the stylus and the plates.

Unimanual sequencing involved tapping the segments in order (i.e. 1, 2, 3, 4) as many times as possible in 30 seconds. Participants completed two trials with each hand. Errors made while completing the sequences were noted by the examiner and categorised as either perseverative (e.g. 1, 1, 2, 3, 4), sequential (e.g. 1, 3, 2, 4), or misses (i.e. when stylus did not make contact with the plate). The number of taps minus the number of perseverative and sequential errors was averaged across two trials for each hand and was taken as an index of unimanual sequencing⁹.

Bimanual sequencing involved tapping the segments in order simultaneously with both hands. Participants completed one trial of 30 seconds and errors were recorded as above. The number of simultaneous taps minus the number of perseverative and sequential errors was the index of bimanual sequencing.

To explore the relationship between unimanual and bimanual sequence tapping a ratio score was also calculated. This ratio was:

$$\text{Bimanual tapping} / (\text{Dominant tapping (Trial 1)} + \text{Non-dominant tapping (Trial 1)})$$

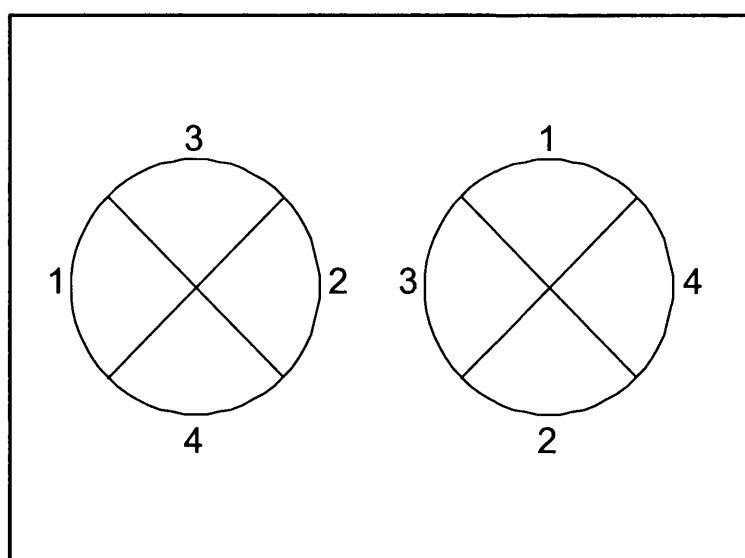
According to this ratio a score of 0.5 indicated that the participant performed equally well with both hands as with each hand separately. A score less than 0.5 indicated that performance was compromised when using both hands compared with using each hand independently.

Unimanual tapping involved tapping in one segment with the stylus as fast as possible for 15 seconds. The number of taps made with each hand was used as the index of unimanual tapping.

The three tapping tasks were completed in the following order: Unimanual sequencing (dominant hand)(Trial 1); Unimanual sequencing (non-dominant hand)(Trial 1); Bimanual sequencing; Unimanual sequencing (non-dominant hand)(Trial 2); Unimanual sequencing (dominant hand)(Trial 2); Unimanual tapping (dominant hand), and Unimanual tapping (non-dominant hand).

⁹ It was necessary to subtract the perseverative and sequential errors as these errors still made contact with the plates and therefore contributed to the overall tapping score as recorded by the counter.

Figure 4-1 Segment numbering for the bimanual tapping task



4.2.2 Experiment 2 – bimanual coordination test

Subjects

15 right-handed participants completed the bimanual coordination test. The groups were matched on age at diagnosis (MWU: $z = -1.36$; $p = 0.18$), years since diagnosis (MWU: $z = -0.52$; $p = 0.60$), age at test (KW: $\text{Chi}^2 = 4.46$; $df = 2$; $p = 0.11$), sex ($\text{Chi}^2 = 1.67$; $df = 2$; $p = 0.42$), and SES (KW: $\text{Chi}^2 = 1.75$; $df = 2$; $p = 0.42$) (Table 4.2).

Table 4-2 Characteristics of the children completing the bimanual coordination task

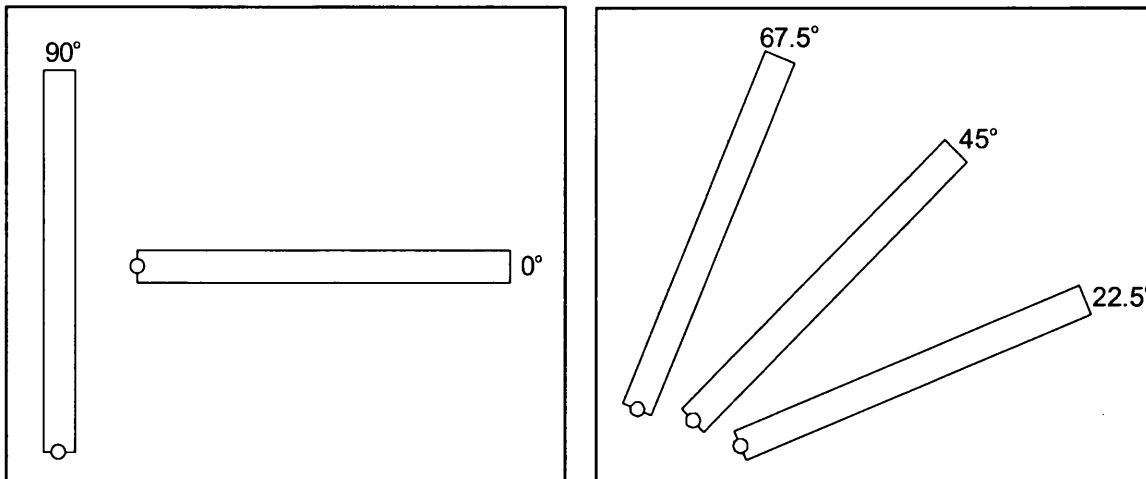
	N	Age at diagnosis ($X \pm \text{SEM}$)	Years from diagnosis ($X \pm \text{SEM}$)	Age at test ($X \pm \text{SEM}$)	Sex (M:F)	SES ($X \pm \text{SEM}$)
HD	5	3.81 (0.4)	8.62 (0.9)	12.44 (1.1)	2:3	65.20 (4.7)
IT	5	4.70 (0.6)	7.84 (0.6)	12.54 (0.8)	3:2	58.60 (5.1)
Siblings	5			15.58 (1.1)	4:1	55.40 (8.0)

Bimanual coordination test

The bimanual coordination test (BCT) was based on the paradigm described by (Steese-Seda *et al.* 1995).

Briefly, the task involved two Etch-a-Sketch (Ohio Art) boards each with a different transparency taped to each screen. The transparencies displayed sets of parallel pathways to be traced as part of the task. The first transparency contained two pathways; a vertical (90°) and a horizontal path (0°). The second transparency displayed three pathways; at angles of 22.5° , 45° and 67.5° (Figure 4.2). Each path was 120 mm long and 10 mm wide.

Figure 4-2 Pathways to be completed in the bimanual coordination test



To complete the task participants were instructed to move the cursor from the start position of each pathway to the end point as quickly as possible while keeping within the guidelines. The left hand knob controlled the vertical displacement of the cursor, while the right hand knob controlled the horizontal movement. One complete rotation of either knob moved the cursor 35 mm.

The 90° and the 0° degree paths required clockwise movement of the right and left hands separately. In contrast, the angled pathways required synchronised movement of both hands. The 45° path required participants to turn both knobs clockwise (i.e. parallel movements) at similar speed. The 22.5° and the 67.5° paths required faster turning of either the left hand or right hand respectively. To ensure the task was complete using finger and hand movements, participants were instructed to keep their forearms and wrists on the table. In addition, participants were instructed to use both hands together to create a continuous line on the angled pathways rather than a stair-like pattern.

Initially, participants were given the Etch-a-sketch toy for one minute and shown how to control the cursor using the left and right hand knobs. Participants then completed two trials of each path on the first transparency (i.e. right unimanual (90°) and then left unimanual (0°)) followed by two trials of the angled pathways. For the angled pathways subjects completed two trials of the 45° path and then the 22.5 or 67.5 pathways in counterbalanced order. For each trial the examiner moved the cursor into the starting circle at one end of the pathway. The participant then guided the cursor to the end of the pathway.

The average time taken to complete each path and the number of errors made (i.e. moving the cursor outside the guidelines) on the angled paths were recorded and used as indices of performance. In addition, two interhemispheric coordination scores were calculated for the angles requiring differential contributions from the left and right hands (i.e. the 22.5° and 67.5° paths). This reflected the additional interhemispheric requirements of performance for these pathways above the ability to turn each knob at the same time simultaneously. These scores were calculated as follows -

L faster than right = time to complete 22.5° / time to complete 45°, and

R faster than left = time to complete 67.5° / time to complete 45°.

General motor coordination

Parents of the children completing the bimanual coordination task were asked to complete the Movement Assessment Battery for Children Checklist (Henderson & Sugden 1992). The questionnaire assessed motor skills in four conditions: the child stationary and environment stable (e.g. tie shoelaces); the child moving and the environment stable (e.g. jumping, skipping or hopping); the child stationary and environment changing (e.g. catching or kicking a ball); and the child moving and the environment changing (e.g. riding a bike). Parents rated each item on a scale ranging from 0-3 (0 = child performs task very well and 3 = child is not close to successfully performing the task). The maximum score possible was 48.

4.3 Results

4.3.1.1 Bimanual tapping task

Unimanual tapping

A two way ANOVA revealed that the number of taps was greater with the dominant hand than the non-dominant hand ($F(1, 42) = 51.62$; $p < 0.01$). However, there was no significant difference between groups ($F(1, 42) = 0.04$; $p = 0.96$)(Figure 4.3).

Unimanual sequences

A two way ANOVA revealed that the number of sequential taps was also greater with the dominant hand than the non-dominant hand ($F(1, 42) = 16.51$; $p < 0.01$). However, there was no significant difference between groups ($F(1, 42) = 0.14$; $p = 0.87$)(Figure 4.4).

Bimanual sequences

There was no significant difference between groups on the number of bimanual taps (ANOVA: $F(2, 42) = 0.65$, $p = 0.53$)(Figure 4.5).

Thurstone ratio-score

There was no significant difference between groups on the Thurstone ratio score (ANOVA: $F(2, 42) = 0.89$, $p = 0.42$)(Figure 4.6).

Figure 4-3 Number of unimanual taps made in each group (Mean \pm SEM)

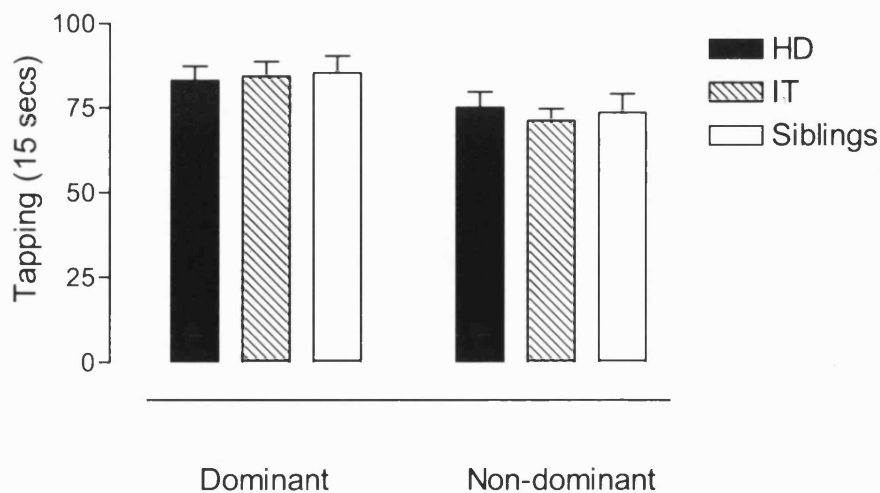


Figure 4-4 Number of unimanual sequences completed in each group (Mean \pm SEM)

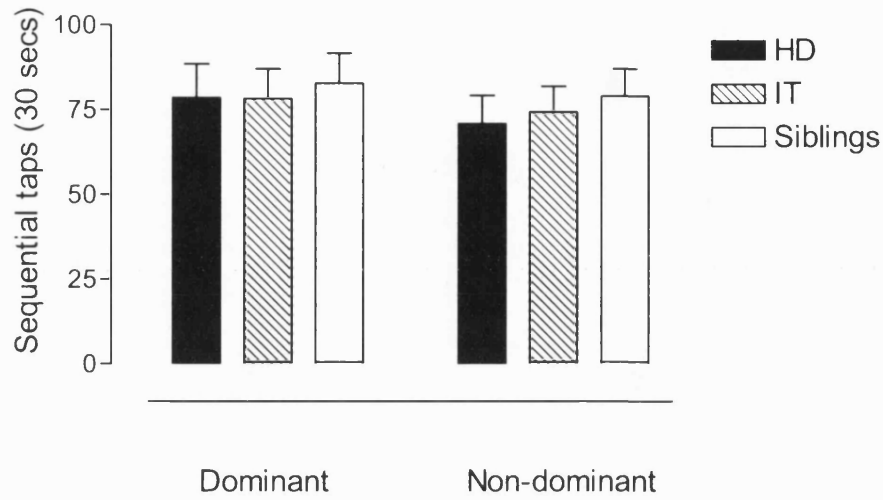


Figure 4-5 Number of bimanual sequence completed in each group (Mean \pm SEM)

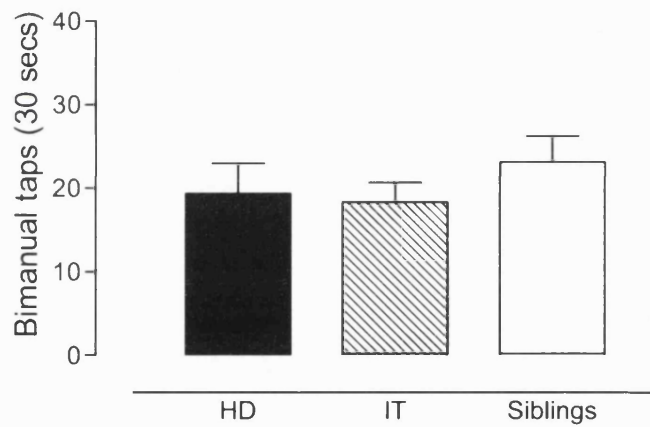
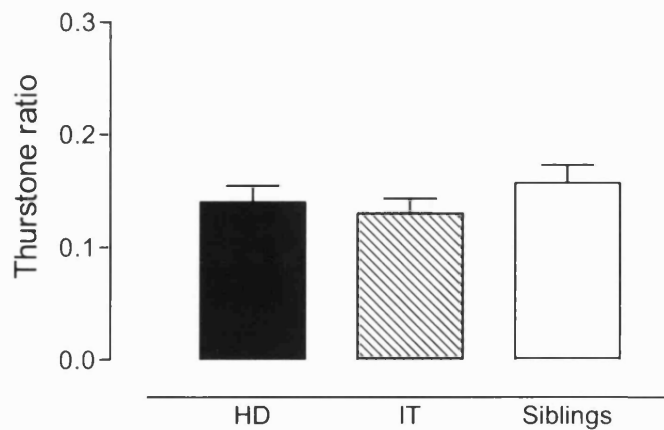


Figure 4-6 Thurstone ratio score for each group (Mean \pm SEM)



Errors

Few errors were made on the unimanual sequences. These were generally sequential (sequential errors 90%, perseverative errors 8% and misses 2%). There were no significant differences between groups on the number of errors made while completing sequences with either hand (Dominant ANOVA: $F(2, 42) = 0.94$, $p = 0.40$)(Non-dominant ANOVA: $F(2, 42) = 0.90$, $p = 0.41$).

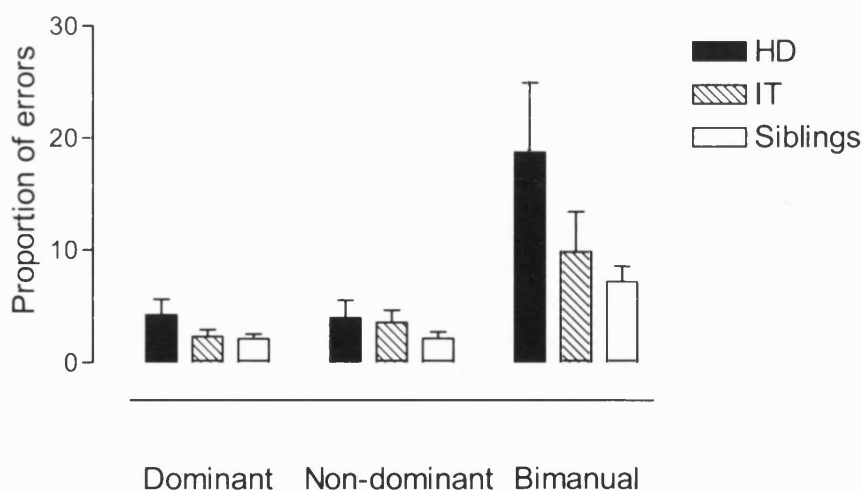
Similarly, there were few errors made on the bimanual sequences. Again these were generally sequential (sequential errors 96%, perseverative errors 2% and misses 2%). There were no significant differences between groups on the number of errors made on the bimanual sequences (ANOVA: $F(2, 42) = 1.48$, $p = 0.24$).

Proportion of errors made on the sequencing tasks

There was no significant difference in the number of errors made per correct response between groups on the unimanual sequencing tasks (Dominant: ANOVA: $F(2, 42) = 1.56$, $p = 0.22$)(Non-dominant: ANOVA: $F(2, 42) = 0.67$, $p = 0.52$)(Figure 4.7).

Despite a qualitative increase in the proportion of errors made by the HD group there was no significant difference observed between groups on the percentage of errors on the bimanual task (ANOVA: $F(2, 42) = 1.96$, $p = 0.15$)(Figure 4.7).

Figure 4-7 Proportion of errors made in each group (Mean \pm SEM)



4.3.1.2 Bimanual coordination task

Angles requiring unimanual response (0° and 90°)

The average time to complete the task was greater with the left hand than the right (Friedman: $\text{Chi}^2 = 15.00$; $p < 0.01$). However, there was no significant difference between groups (Left KW: $\text{Chi}^2 = 0.03$; $df = 2$; $p = 0.98$)(Right KW: $\text{Chi}^2 = 0.07$; $df = 2$; $p = 0.96$)(Figure 4.8).

Angles requiring bimanual response (22.5°, 45° and 67.5°)

The time to complete the bimanual tasks was longer than the time to complete the unimanual tasks (Friedman: $\text{Chi}^2 = 15.00$; $p < 0.01$). However, there was no significant difference between groups on the average time to complete any of the angles requiring bimanual responses (L faster R KW: $\text{Chi}^2 = 2.42$; $df = 2$; $p = 0.30$)(L=R KW: $\text{Chi}^2 = 1.44$; $df = 2$; $p = 0.49$)(R faster L KW: $\text{Chi}^2 = 3.90$; $df = 2$; $p = 0.14$)(Figure 4.9).

Interhemispheric coordination

There was no significant difference between groups on the interhemispheric scores (L faster R KW: $\text{Chi}^2 = 0.69$; $df = 2$; $p = 0.71$)(R faster L KW: $\text{Chi}^2 = 0.98$; $df = 2$; $p = 0.60$)(Figure 4.10).

Figure 4-8 Average time taken to complete unimanual tasks in each group (Mean \pm SEM)

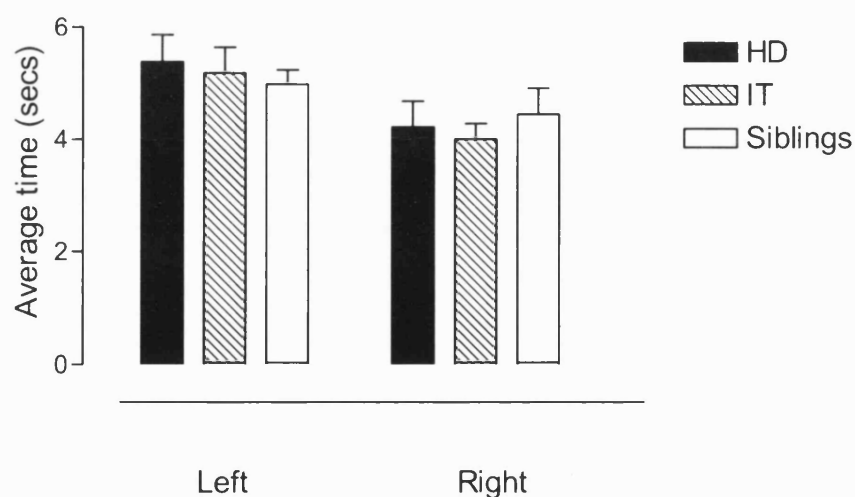


Figure 4-9 Average time taken to complete bimanual tasks in each group (Mean \pm SEM)

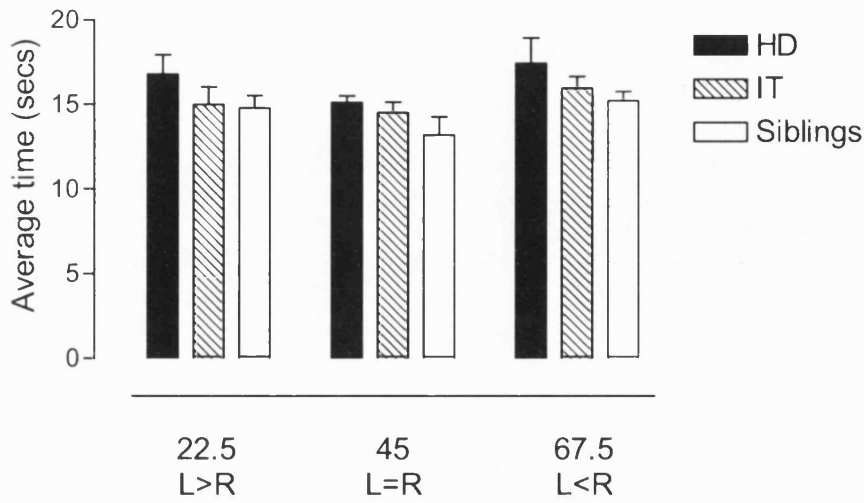
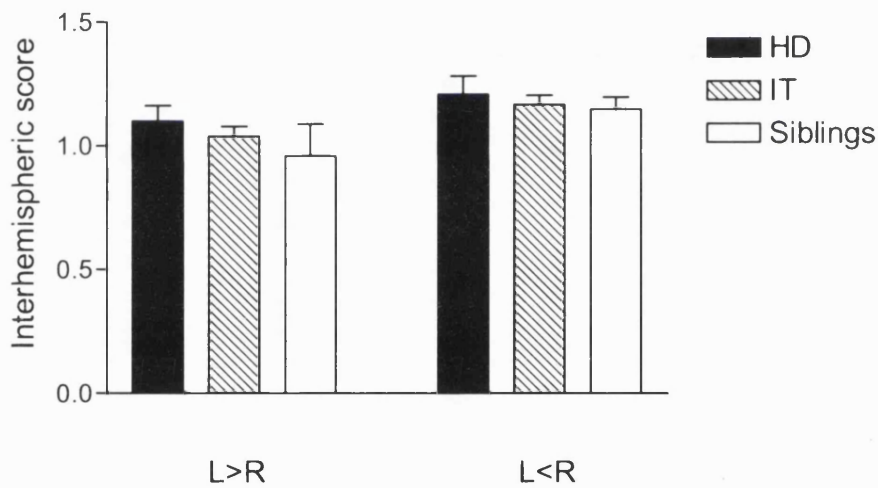


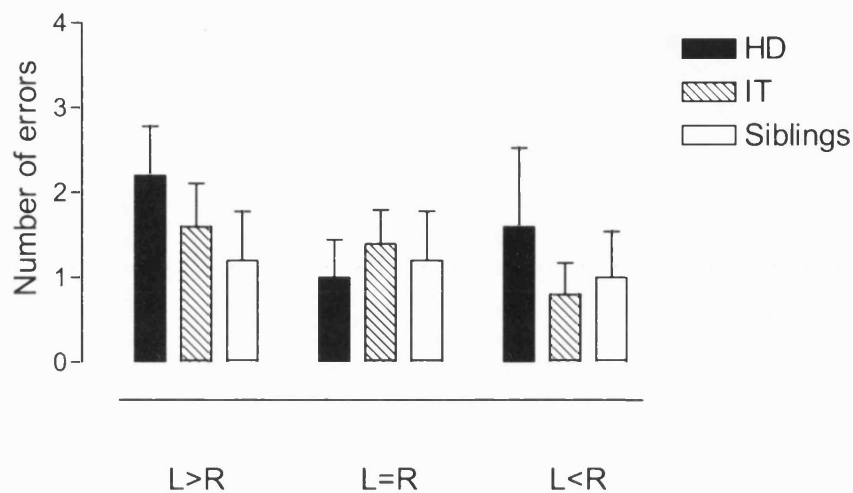
Figure 4-10 Interhemispheric scores in each group (Mean \pm SEM)



Errors

There was no significant difference between groups on the number of errors made on the angles requiring bimanual coordination (L faster R KW: $\text{Chi}^2 = 0.52$; $\text{df} = 2$; $p = 0.77$)(L=R KW: $\text{Chi}^2 = 0.07$; $\text{df} = 2$; $p = 0.96$)(R faster L KW: $\text{Chi}^2 = 1.34$; $\text{df} = 2$; $p = 0.44$)(Figure 4.11).

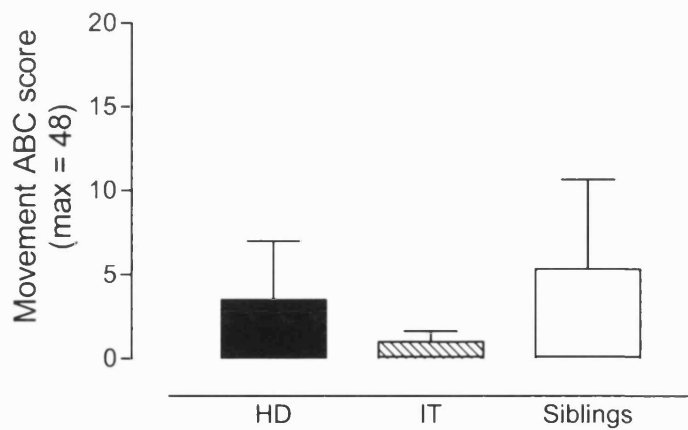
Figure 4-11 Number of errors on the bimanual tasks in each group (Mean \pm SEM)



4.3.1.3 General motor coordination

There was no significant difference between groups on the Movement ABC score (KW: $\chi^2 = 0.28$; $df = 2$; $p = 0.87$)(Figure 4.12).

Figure 4-12 Movement ABC scores in each group (Mean \pm SEM)



4.4 Discussion

The current investigations of bimanual coordination failed to reveal any significant differences between groups. Similarly, significant differences were not identified between groups on the Movement ABC questionnaire. However, general differences were noted across all groups in performance on several aspects of the bimanual tasks (e.g. between dominant and non-dominant hands, and different levels of task complexity).

Bimanual tapping test

The results of the bimanual tapping task indicated that unimanual tapping was faster with the dominant than non-dominant hand. Consistent with this, the right hand has been shown to tap faster than the left hand on a similar tapping task in right-handed adults (Wyke 1967; Corkin 1968) and children (Denckla 1973). In addition, participants were slower to complete bimanual when compared to unimanual sequences. This is consistent with reports using this task (Leonard *et al.* 1988) and other bimanual motor tasks (Wyke 1969; Wyke 1971).

Furthermore, there was no significant difference between groups on the number of errors made on the unimanual and bimanual sequence tasks. However, the majority of errors for all participants on both tasks were sequential. Sequential errors are believed to reflect the control of sequencing and timing of movements from direct visual input. It is expected that over time the errors would decrease as control would become less dependent on constant visual feedback and move towards an internal form of control (Leonard *et al.* 1988). The rate of errors over time as an index of this learning system may be of interest in further studies.

Significant differences were not observed between groups on the bimanual tapping task. However, this result may be due to a number of factors. For example, there was large inter-individual variation in performance on the bimanual aspect of this task. In particular, the proportion of errors per correct response ranged from 0-85% amongst members of the HD group (0-50% in the IT group and 0-15% in the sibling group). This suggests that additional factors were important for performance on this task. The task required attending to visual and kinaesthetic cues, and switching attention between to the eight different spatial locations. Auditory feedback of the stylus hitting the board might also contribute to the control of movement sequences (Leonard *et al.* 1988). Variable performance in the HD group may therefore reflect different attentional and motivational factors between subjects. However, there was no significant correlation

between the proportion of errors and the switching or sustained attention scores obtained in Chapter 3 within the HD group.

In addition, subjects were not assessed on the degree of handedness. Intermanual coordination (alternating tapping) is reported to be significantly greater in left than right handers. This is especially the case when accompanied by a low score of motor lateralisation (i.e. no consistent hand preference on items of the Edinburgh Handedness Questionnaire)(Oldfield 1971; Gorynia & Egenter 2000). Similarly, smaller variation is observed between unimanual performance of the left and right hands in left handed healthy adults (Peters 1987). This suggests that the degree of handedness is an important factor in bimanual coordination. It would be advantageous to control for the degree of handedness in future studies. Handedness could be assessed using the Laterality portion of Physical and Neurological Examination for Soft signs (PANESS)(Denckla 1985), or the Handedness questionnaire adapted from (Crovitz & Zener 1962) and described by Isaacs *et al.* (1996).

Bimanual coordination test

There were no significant differences observed between groups on the test of bimanual coordination. However, all groups completed the unimanual angles requiring a right handed (0°) response faster than they completed the angles requiring a left handed response (90°). Similarly, the time to complete the unimanual angles was faster than the time to complete bimanual angles. These results are consistent with developmental studies employing this task (Steese-Seda *et al.* 1995).

No significant differences were observed between groups on the tasks requiring bimanual responses. In addition, performance was not significantly different across the bimanual angles. In particular, there was no significant difference between the time and accuracy to complete the angles requiring equal (L=R) or differential (L>R or R>L) input from each hand in any group. This is in contrast to reports indicating that angles requiring differential contributions from the left and right hands are more difficult than angles requiring left and right hands turning at similar speeds (Marion *et al.* 2003).

A reason for this inconsistency may be related to differences in the administration and scoring of the bimanual coordination task between the two studies. For example, the bimanual coordination task used in the (Marion *et al.* 2003) study implemented a computerised version of the task. As noted by the authors this allows greater precision in the measurement of both time and accuracy to complete the task than the Etch-a-sketch version.

Future studies may therefore benefit from the more sensitive computerised version of the task. In addition, the computerised version facilitates the measurement of performance in a greater range of conditions (e.g. angles requiring mirror movements) and trials without visual feedback. Performance trials withholding visual feedback have been shown to be particularly sensitive to anterior corpus callosum lesions (Preilowski 1972; Preilowski 1975; Eliassen *et al.* 2000).

Within the literature, reports indicate that fine motor function is not impaired in the LT survivors treated with TIT (Kingma *et al.* 2002) or HD + TIT (Rowland *et al.* 1984; Moore *et al.* 1992; Copeland *et al.* 1996). Despite a number of caveats listed above, the current findings indicate that these results can be extended to include intact bimanual coordination. This is in contrast to children treated with combined CRT + IT MTX where motor immaturity, coordination and right/left discrimination problems have been reported (Eiser & Lansdown 1977; Appleton *et al.* 1990; Christie *et al.* 1994).

4.5 Summary

Participants completed two tasks of bimanual coordination sensitive to lesions of the corpus callosum. Significant differences in performance were not observed between groups on either task. The current findings suggest that the functional integrity of the corpus callosum was preserved within the LT survivors treated with chemotherapy-only.

Part 3: Neuroimaging studies

5 Conventional MRI

Chapters 3-4 describe changes in cognitive and behavioural function experienced by the LT survivors of leukaemia. In the following chapters, an attempt is made to identify the underlying brain pathology responsible for these impairments using MRI. Chapter 5 describes gross changes in brain structure as revealed by conventional MR techniques. In Chapter 6, an objective method, voxel based morphometry, was used to quantify more subtle brain abnormalities.

5.1 Introduction

The imaging abnormalities identified in children treated for leukaemia include; white matter changes, calcifications, and cerebral atrophy. The incidence of each type of abnormality is associated with the type and combination of therapies received (e.g. methotrexate (MTX), triple intrathecal therapy (TIT), or cranial irradiation (CI)) and the time elapsing between treatment and follow-up. MR imaging has proven to be the tool of choice for identifying treatment related changes in ALL. However, calcifications are more readily identified using CT (Asato *et al.* 1992; Mulhern *et al.* 1992; Hertzberg *et al.* 1997; Iuvone *et al.* 2002).

White matter changes have been documented during the treatment phase in children treated with MTX-only (Ochs *et al.* 1983; Asato *et al.* 1992; Paakko *et al.* 1996). White matter changes are, however, more common in children treated with triple intrathecal therapy (TIT) than MTX-only (Wilson *et al.* 1991; Asato *et al.* 1992; Mahoney *et al.* 1998). Generally, white matter changes improve by the end or within the months following treatment (Ochs *et al.* 1983; Wilson *et al.* 1991; Asato *et al.* 1992; Paakko *et al.* 2000). However, not all changes resolve with time (Harila-Saari *et al.* 1998) and white matter changes can still be detected in the longer term (Hertzberg *et al.* 1997; Kingma *et al.* 2001). In addition, white matter changes may first appear in the long-term in children treated with MTX-only (Patient 4 (Harila-Saari *et al.* 1998))(Ochs *et al.* 1983). White matter changes commonly involve periventricular regions and the centrum semiovale (Asato *et al.* 1992; Kingma *et al.* 1993). White matter changes during treatment and in the months following treatment with HD MTX have been related to age-at-diagnosis (Wilson *et al.* 1991; Paakko *et al.* 2000)(but see also Price & Jamieson 1975). In the long-term, however, white matter changes have not been associated with age-at-diagnosis (Harila-Saari *et al.* 1998).

Calcifications have been observed in the LT survivors of leukaemia using CT (Brouwers *et al.* 1985; Brouwers & Poplack 1990; Chessells *et al.* 1990; Iuvone *et al.*

2002) and MRI (Kingma *et al.* 1993; Hertzberg *et al.* 1997; Harila-Saari *et al.* 1998; Cetingul *et al.* 1999). Calcifications are most often identified in the basal ganglia but may also affect the deep layers of the cerebral cortex or dentate nuclei of the cerebellum (Flament-Durand *et al.* 1975; Price & Birdwell 1978; Brouwers *et al.* 1985; Brouwers & Poplack 1990; Chessells *et al.* 1990). The incidence of calcifications is greatest in children treated with combined MTX + CI (Price & Jamieson 1975; Price & Birdwell 1978; Ochs *et al.* 1991; Hertzberg *et al.* 1997; Iuvone *et al.* 2002). However, calcifications have also been observed following HD MTX + TIT (Lovblad *et al.* 1998). In children treated with combined MTX + CI the changes first appear in the long-term (e.g. five (Harila-Saari *et al.* 1998) or seven (Riccardi *et al.* 1985) years after treatment). Calcifications are more common in LT survivors treated at a younger age (<10 years)(Price & Birdwell 1978).

Cortical atrophy has been reported in children during treatment with HD MTX (Paakko *et al.* 1996) or TIT (Wilson *et al.* 1991). It has also been demonstrated in long-term (>5years post diagnosis) survivors treated with MTX-only (Harila-Saari *et al.* 1998; Kingma *et al.* 2001). During treatment it has been found that the degree of atrophy is related to the use of IT MTX (Crosley *et al.* 1978). Atrophy identified during treatment may resolve with time (Wilson *et al.* 1991) or persist in the longer term (Harila-Saari *et al.* 1998). Atrophy may also first appear in the long-term (Brecher *et al.* 1985). The incidence of atrophy in LT survivors (>6 years post diagnosis) is not significantly different between children receiving MTX and children receiving combined MTX + CI (Hertzberg *et al.* 1997).

In this study, conventional MRI techniques were used to reveal gross structural abnormalities in LT survivors and their siblings. In addition, an attempt was made to relate the abnormalities identified to indices of cognitive and behavioural function.

5.2 Methods

5.2.1 Data acquisition

All subjects were scanned unsedated using a 1.5T Siemens Vision scanner. Four imaging sequences were acquired for each subject (Table 5.1). The neuroradiological assessment was based on the coronal and axial T2 weighted images. Analyses of the 3D structural (3D FLASH) images and of the diffusion tensor imaging (DTI) data are described in Chapter 6.

Table 5-1 Imaging protocol

Sequence	TR (ms)	TE (ms)	Other
T2 Coronal	3458 ms	96 ms	19 slices, 5 mm thick
T2 Axial	3458 ms	96 ms	19 slices, 5 mm thick
3D FLASH	16.80 ms	5.70 ms	Voxel size = 0.8 X 0.8 x 1mm
DTI		110 ms	Voxel size = 1.5 x 1.5 x 3 mm

5.2.2 Data analyses

T2 weighted images were reviewed by a paediatric neuroradiologist. In this assessment particular attention was given to changes occurring within the white matter.

5.3 Results

Abnormalities identified as part of the clinical assessment are shown in Table 5.2.

Table 5-2 Gross structural abnormalities observed in the LT survivors and their siblings

Abnormality	HD (N=14)	IT (N=14)	Siblings (N=12)
Bilateral loss of white matter bulk			
Frontal lobes	0	0	0
Temporal lobes	0	0	0
Occipital lobes	0	3	0
Parietal lobes	0	3	0
Cerebellum	0	1	0
Pons	0	1	0
Ill defined bilateral white matter change			
Corona radiata	0	1	0
Internal capsule	0	1	0
Gliotic scar			
Centrum semiovale	2	0	0
Thalamus	1	0	0
Signal change basal ganglia			
Atrophy	0	0	0
Ventricular asymmetry	1	1	0
Other abnormality	2	1	3
Total number with abnormal scan	6	6	3

As shown in Table 5.2, abnormalities were observed in all groups. Abnormalities were seen more frequently in survivor group (43%) than sibling group (25%), however, this failed to reach significance ($\text{Chi}^2=1.14$ $\text{df}=1$ $p=0.29$). The number of children with abnormal scans was similar for the two treatment groups (HD = 6; IT = 6).

In the HD group, the survivors with abnormal scans had only one visible change. These abnormalities included gliotic scars in the centrum semiovale (left hemisphere=1; right hemisphere=1), gliotic scar of the thalamus (N=1), ventricular asymmetry (N=1); developmental arachnoid cyst in the posterior fossa (N=1) or developmental arachnoid cyst in the right frontal lobe (N=1).

Similarly, of the children with abnormal scans in the IT group, 5/6 displayed one abnormality. The remaining child in the IT group had two abnormalities visible on MRI (i.e. white matter change and ventricular asymmetry). Within the IT group, the most common abnormalities were bilateral white matter changes. White matter changes always involved two anatomical regions, co-occurring in the parietal + occipital lobes (N=3); the cerebellum + pons (N=1), or the internal capsule + corona radiata (N=1). One child had a congenital cyst located in the cerebellar-pontine angle.

Three children in the sibling group had abnormalities visible on MRI. These included a left hemisphere cerebellar venous anomaly (N=1); enlarged pituitary (N=1), or enlarged perivascular spaces in the midbrain (N=1). Atrophy or signal changes in the basal ganglia and/or cerebral cortex suggestive of calcification were not observed in any of the participants.

The majority (66%) of abnormalities in survivor groups were white matter changes (e.g. gliotic scars, bilateral white matter volume loss, or ill defined white matter signal change). White matter changes were not reported in the sibling group. Among the survivors the incidence of white matter abnormalities was not related to age-at-diagnosis ($t=-1.57$; $p=0.13$), age-at-test ($t=-1.39$; $p=0.18$) or length of follow-up ($t=0.36$; $p=0.72$)(Table 5.3). However, the type of white matter abnormality observed was related to the PCNS treatment received. For example, white matter abnormalities in the HD group were gliotic scars, whereas, the white matter changes observed among the IT group were qualitatively different (e.g. ill defined signal change and bilateral volume loss).

Table 5-3 Details of survivors with WM abnormalities visible on T2 weighted images.

Group	White matter (WM) abnormality	Sex	Age Diagn (Years)	Age Test (Years)	Follow-up (Years)	IQ est.	Select attn (SS)	Switch attn (SS)	Sustain attn (%)	Motor (time/target)	B/w digits (raw)	Stroop (t)	Verbal fluency (z)	Tower London (raw)	BADS (SS)
HD	Gliotic scar Left Thalamus	M	1.60	12.82	11.23	115	16	10	90	0.80	7	5.1	-0.23	73	100
IT	Bilateral WM change Cerebellum + Pons	M	3.08	12.50	9.43	100	15	5	90	0.61	3	6.7	-1.31	78	85
IT	Bilateral WM change Occipital + Parietal lobes	M	3.19	9.33	6.16	94	2	3	100	0.83	2	-1.1	-1.01	49	80
HD	Gliotic scar Left Centrum semiovale	M	3.19	10.38	7.19	94	8	9	90	0.88	5	10.7	-1.74	51	90
IT	Bilateral WM change Internal capsule + Corona radiata	M	3.53	14.42	10.81	91	13	8	60	0.66	4	3.3	-2.01	76	104
IT	Bilateral WM change Occipital + Parietal lobe + Ventricular asymmetry	M	4.85	13.33	8.51	112	5	8	90	0.79	5	9.5	-0.75	59	100
HD	Gliotic scar Right Centrum semiovale	F	4.85	13.98	9.13	109	6	5	100	0.76	6	0.8	-1.23	81	104
IT	Bilateral WM change Occipital + Parietal lobes	F	6.03	13.67	7.62	117	13	16	100	0.61	4	5.2	+0.32	80	109
	Survivors with WM abnormalities	X (sd)	3.79 (1.4)	12.55 (1.8)	8.76 (1.8)	104 (10.4)	9.75 (5.2)	8.00 (4.0)	90.00 (13.1)	0.74 (0.1)	4.25 (1.7)	5.01 (4.0)	-1.00 (0.8)	68.38 (13.3)	96.50 (10.3)
	Survivors without WM abnormalities	X (sd)	5.41 (2.8)	13.94 (2.6)	8.52 (1.5)	106.4 (15.0)	10.00 (3.1)	8.16 (2.67)	95.48 (8.5)	0.73 (0.1)	4.29 (1.2)	4.13 (5.2)	-0.73 (0.9)	76.71 (11.43)	99.00 (14.6)
	Comparisons between survivors with and without WM abnormalities	t (p)	-1.57 (0.13)	-1.39 (0.18)	0.36 (0.72)	-0.43 (0.67)	-0.16 (0.88)	-0.12 (0.91)	-1.49 (0.16)	0.25 (0.81)	-0.80 (0.94)	0.44 (0.66)	-0.75 (0.46)	-1.78 (0.14)	-0.44 (0.67)

There was no significant relationship between white matter changes observed and indices of function. For example, mean cognitive performance for the eight survivors with visible white matter changes on MRI was not significantly different from that of the survivors with normal MRI findings on estimates of IQ, selective attention, sustained attention, and switching attention. Similarly, there were no significant differences on measures of motor response, short-term memory (backwards digits), and executive functions (Interference, Verbal fluency, Tower of London and the BADS)(Table 5.3). Furthermore, poor performance on tests of attention and executive functions was noted in children with and without white matter abnormalities on MRI (Table 5.4).

Table 5-4 Percentage of children with test scores less than 1.64 SD below group mean (lowest 5% under the standard normal curve)

	Survivors with WM abnormality (%)	Survivors without WM abnormality (%)	Siblings (%)
IQ estimate	0	12.5	11.1
Selective attention	12.5	0	0
Switching attention	0	6.3	11.1
Sustained attention	12.5	6.3	0
Motor (time per target)	0	0	0
Backwards digit span	0	0	0
Stroop	0	6.3	11.1
Verbal fluency	0	12.5	0
Tower of London	25.0	0	0
BADS	0	6.3	0

5.4 Discussion

In the present study, 43% of the survivors had abnormal MRI scans. The incidence of abnormality was similar in each survivor group (i.e. HD = 6/14; IT = 6/14). The incidence of abnormality in the survivor groups was higher than the incidence observed in the sibling group (i.e. 3/12). However, this failed to reach significance.

Incidence of abnormalities in the HD group

The frequency of long-term changes in the HD group was similar to the incidence reported in the literature. For example, six of a group of 16 children (38%) treated with similar HD MTX protocols had abnormal MRI scans more than six years after diagnosis (Kingma *et al.* 2001). This is similar to a study of children treated with slightly different HD MTX protocols where 15/39 (38%) had abnormal scans four years after treatment (Hertzberg *et al.* 1997). In another study, two of seven children (29%) had visible MRI changes five years after therapy (Harila-Saari *et al.* 1998).

Incidence of abnormalities in the IT group

The incidence of abnormalities identified in the IT group was also similar to results in the literature. For example, 9/29 (31%) children treated with IT MTX had abnormal CT scans six years after PCNS treatment (Brecher *et al.* 1985). Similarly, 40% of children treated with TIT had persistent changes from baseline MRI three years post-treatment (Wilson *et al.* 1991).

The incidence of abnormalities observed in survivors enrolled in the current study was similar to that seen in children treated with combined CI + MTX for ALL. For example, using MRI, abnormalities have been identified with a similar frequency in LT survivors treated with combined therapy four years (51%)(Kingma *et al.* 1993), more than five years (66%)(Cetingul *et al.* 1999), and more than seven years post-diagnosis (43%)(Laitt *et al.* 1995). The incidence of abnormalities in the present study was also similar to the LT survivors treated with combined CI + chemotherapy for other cancers (Paakko *et al.* 1994).

Number of abnormalities observed

All children with abnormal scans in the HD group had only one type of visible abnormality (e.g. white matter change). Similarly, 5/6 in the IT group had only one type of change, although white matter changes always occurred in two anatomical regions. One child had two abnormalities (i.e. white matter change and ventricular asymmetry). This is again similar to previous MRI reports of children treated with MTX. For example, both children with abnormal MRI scans reported by Harila-Saari *et al.* (1998) had only one abnormality. In addition, the majority of survivors with abnormal scans had only one visible change in two other studies (5/6 (Kingma *et al.* (2001), and 11/15 survivors reported by Hertzberg *et al.* (1997)). In addition, all children with abnormal scans and treated with MTX had one abnormality in a long-term CT study (Brecher *et al.* 1985).

White matter abnormalities and atrophy

In the current study, the majority of changes in survivors were observed in white matter and atrophy was not seen in any participant. White matter changes were the most common finding reported by Kingma *et al.* (2001) with 5/16 children having changes to white matter, while only 2/16 had signs of atrophy. Both survivors with abnormal scans reported by Harila-Saari *et al.* (1998) had white matter changes without atrophy. In contrast, atrophy was the most common abnormality in the study by Hertzberg *et al.* (1997) study, with widening of sulci or ventricles evident in 14/39 survivors and white matter changes in only 2/39. In a similar CT study, atrophy was the only finding (Brecher *et al.* 1985). However, the detection of white matter changes with CT is limited.

Calcifications

In the present study signal changes indicative of calcifications were not seen in the basal ganglia. This is consistent with the literature as calcification is rare in children treated without combined MTX + CI therapy. Indeed calcifications have not been observed in LT survivors treated with MTX without additional CI (Mulhern *et al.* 1992; Hertzberg *et al.* 1997). Similarly, basal ganglia signal change on MRI (Harila-Saari *et al.* 1998; Kingma *et al.* 2001) or calcifications at autopsy (Price & Jamieson 1975) have not been reported in children receiving MTX-only. Calcification of the basal ganglia and subcortical U-fibers has been reported following HD MTX + TIT (Lovblad *et al.* 1998). Calcifications are most likely related to the combined neurotoxicity of MTX and CI and/or cytarabine as part of TIT.

Other abnormalities

In the present study additional abnormalities were observed in the survivor groups including ventricular asymmetry (N=2), arachnoid cysts (N=2), and a congenital cyst (N=1)). Ventricular asymmetry has been reported in one child during treatment with HD MTX (although it is not clear if this child also received combined MTX + CI therapy) (Paakko *et al.* 1996). Cysts have also been reported in LT survivors treated with combined CI + MTX five years (Ciesielski *et al.* 1994; Paakko *et al.* 1994), and eight years post-diagnosis (Laitt *et al.* 1995). In addition, an arachnoid cyst was evident before treatment in one child with ALL (Chessells *et al.* 1990).

MRI studies of healthy children (N=225) participating in a range of research studies (age birth – 18 years, majority > 7 years) have identified ventricular asymmetry in 1/225 and arachnoid cysts in 2/225 (Kim *et al.* 2002). In a similar study of healthy controls from a larger age range (3-83 years), arachnoid cysts were detected in 0.3% of the subjects using MRI (Katzman *et al.* 1999). Together, the studies indicate ventricular asymmetry and arachnoid cysts are relatively rare within the normal population. Ventricular asymmetry and/or cysts may therefore be related to the treatment and/or disease process in this population.

Incidence of abnormality in the sibling group

In the current study, the incidence of abnormality within the sibling group was 25%. The incidence of 'silent' abnormalities in the normal population has been reported as 21% in children (birth – 18 years)(Kim *et al.* 2002) and 18% in a greater age range (3-83 years)(Katzman *et al.* 1999). These reports indicate that some changes identified in our survivors would be expected to occur within the normal population and are therefore unlikely to be related to treatment. Despite this, there was a trend for more survivors to have abnormal scans than siblings indicating that the disease process and/or treatment may have LT effects visible on MRI.

Furthermore, white matter changes were not observed in the sibling group whereas white matter change was the most common abnormality in the survivor groups. In healthy children, white matter abnormalities on MRI are rare incidental findings occurring with a frequency of approximately 2.2% (Kim *et al.* 2002). In another study including a larger age range, white matter changes suggestive of demyelination (e.g. non-specific focal hypointensities on T2- weighted images) were observed in only 0.3% of subjects (Katzman *et al.* 1999). As the majority of changes observed within the survivors were changes to white matter and this is a rare finding in healthy controls,

this suggests that the white matter is particularly vulnerable to the LT effects of treatment and/or disease in this population.

Relationship between white matter abnormalities and type of treatment

Findings of the present study also suggested that the type of white matter change observed was related to the type of PCNS therapy administered. For example, within the IT group, changes were generally bilateral and resulted in a diffuse loss of white matter. In contrast, changes in the HD group were typically old white matter injuries (gliotic scars).

For the survivors in the current study, treatment was equivalent in all phases except during PCNS therapy. At this time the HD group received IT MTX (3 x 7.5-12.5 mg/m²) + HD MTX (3 x 6-8 mg/m²) + FAR. In contrast, the children in the IT group received IT MTX-only (3 x 7.5-12.5 mg/m²). This suggests that the additional HD MTX, FAR or the combination with IT MTX may be responsible for the differences in the type of white matter changes seen in the present study.

MTX and/or FAR have been related to the presence of white matter changes. For example, white matter changes appear on MRI after at least one trial of IT MTX (8-12 mg/m²) + HD MTX (1-5 g/m²) + FAR in ALL. In addition, the size of the white matter lesion (but not number) increases with continuing MTX treatment. Alternatively interrupting further administration of IT MTX improves the abnormalities observed (Asato *et al.* 1992).

However, the effect of total MTX dose and its relationship with FAR is unclear. For example, white matter changes were detected on MRI in 3/7 children diagnosed with standard-risk ALL and treated with HD MTX (8 x 1-5 g/m²) + IT MTX (13 x 12 g/m²) + FAR. In contrast, white matter changes were not observed in children (N=7) diagnosed with intermediate-risk ALL and treated with higher dose HD MTX (9 x 5 g/m²) + IT MTX (13 x 12 g/m²) + FAR (Paakko *et al.* 2000).

In addition, the administration of leucovorin (FAR) can modulate the MTX toxicity seen in children treated for ALL (Winick *et al.* 1991). In the short-term, patients receiving more FAR relative to MTX dose had significantly fewer acute neurotoxic events and fewer white matter changes on CT/MRI (Mahoney *et al.* 1998). Furthermore, the administration of FAR has been shown to improve white matter changes observed on MRI in children with inborn errors of folate metabolism (Surtees *et al.* 1991). Together the studies highlight the complex relationship between MTX, FAR and white matter changes as revealed by MRI.

Relationship between white matter abnormalities and age-at-diagnosis

Within the survivor groups, the presence of white matter changes was not related to age-at-diagnosis. In children treated with combined MTX + CI, younger age has been related to higher likelihood of LT changes (Paakko *et al.* 1992), particularly calcification (Riccardi *et al.* 1985; Chessells *et al.* 1990; Iuvone *et al.* 2002). However, this relationship is not always observed (Mulhern *et al.* 1992; Kingma *et al.* 1993; Cetingul *et al.* 1999) especially with respect to white matter changes (Iuvone *et al.* 2002). Similarly, the incidence of white matter changes (clinically defined as leukoencephalopathy) at autopsy was not significantly different between older and younger patients (all patients 6 – 54 months post treatment and received CI + IT MTX) (Price & Jamieson 1975). Together the results and the literature indicate the brain is vulnerable to gross long-term white matter changes at all ages.

Relationship between abnormalities and indices of function

The current study failed to identify a relationship between the white matter abnormalities observed and indices of cognitive function. This is consistent with the literature where long-term white matter changes were not related to function in children treated with chemotherapy-only (Harila-Saari *et al.* 1998; Kingma *et al.* 2001). For example, no difference was observed between children with normal vs. abnormal MRI scans and measures of IQ, memory and learning (RAVLT), attention (dot cancellation) (digit span), visual motor integration, motor function (Purdue pegboard), or subtests of the NEPSY (Harila-Saari *et al.* 1998; Kingma *et al.* 2001). Within the literature, however, there are single case exceptions. For example, persistent bilateral white matter changes were associated with a low VIQ in a child treated with HD MTX (Harila-Saari *et al.* 1998). Finally, while white matter changes have been correlated with cognitive function during treatment with MTX-only (Wilson *et al.* 1991; Paakko *et al.* 2000) white matter changes identified during the treatment period were not related to long-term measures of general function (IQ) and academic achievement (Ochs *et al.* 1991).

The lack of relationship between the abnormalities observed and indices of function is, however, inconsistent with the results of similar studies of children treated with combined MTX + CI (Brouwers *et al.* 1985; Brouwers & Poplack 1990; Chessells *et al.* 1990; Mulhern *et al.* 1992; Hertzberg *et al.* 1997; Iuvone *et al.* 2002)(but see also Jannoun 1983; Kingma *et al.* 1993). For example, a relationship has been demonstrated between children with abnormal scans and cognitive function following treatment with combined CI + MTX (Chessells *et al.* 1990; Mulhern *et al.* 1992). In

particular calcifications of the basal ganglia have been related to measures of verbal memory and learning (Brouwers *et al.* 1985; Brouwers & Poplack 1990). Similarly, in these children long-term white matter changes have been related to poor performance on the Culture free test, d2 concentration test, Freedom from distractibility index, and visual motor integration (Hertzberg *et al.* 1997; Iuvone *et al.* 2002). Long-term white matter changes accompanied by atrophy following combined MTX + CI have also been related to impaired performance of the picture completion and arithmetic subtests of the WISC (Hertzberg *et al.* 1997). It has also been reported that the children with the most extensive white matter changes following treatment with combined CI + MTX had the most symptoms (e.g. motor clumsiness, exaggerated deep tendon reflexes and impaired short-term auditory memory)(Paakko *et al.* 1992).

5.5 Summary

The number of children with brain abnormalities detected using conventional MRI methods was not significantly different between the survivor and sibling groups. The next chapter investigates brain integrity on a more subtle level. In particular, the investigation attempts to quantify common subtle changes that may not be visible using conventional MRI.

6 Voxel Based Morphometry

6.1 General Introduction

Many LT survivors of leukaemia suffer subtle cognitive and behavioural impairments (see Section 1.1.5 and Chapters 3-4). However, the pathological substrate and radiological expression of the long-term deficits observed in LT survivors is unclear (Section 1.1.6 and Chapter 5).

A limitation of previous research has been the reliance on conventional qualitative methods to identify subtle pathology in this population. Recently, new quantitative techniques have become available that are more sensitive to changes in brain composition. Indeed using an automated volumetric technique subtle volume changes of otherwise normal appearing white matter have been revealed in a similar population of LT survivors. Children in that study were treated with radiation and/or chemotherapy for paediatric brain tumours (Reddick *et al.* 2003). Furthermore, the subtle changes, in apparently normal white matter, were directly related to cognitive and behavioural function.

Since subtle brain changes are difficult to quantify by inspection and may not be revealed by conventional qualitative imaging (Ashburner & Friston 2000), it is important to investigate LT survivors of ALL with more quantitative techniques. Voxel-based morphometry (VBM) is an automated, *in vivo* technique that compares 3D-MRI images on a voxel-by-voxel basis with uniform sensitivity through out the entire brain. It allows regionally specific differences in brain tissue to be identified after more global differences in brain structure have been eliminated (Woermann *et al.* 1999; Ashburner & Friston 2000).

In this chapter, VBM was used to assess brain integrity and to identify group differences between the survivors and their siblings. An attempt was also made to relate the changes in brain structure revealed by VBM, to changes in behaviour as described in Chapter 3.

6.2 3D T1 weighted images

6.2.1 Introduction

VBM analyses of 3D, T1-weighted datasets have identified regional differences in brain structure in many clinical populations. For example, VBM has revealed regional changes in grey and/or white matter densities in epilepsy (Richardson *et al.* 1997; Woermann *et al.* 1999; Woermann *et al.* 2000); foetal alcohol syndrome (Sowell *et al.* 2001); child onset schizophrenia (Sowell *et al.* 2000), and ADHD (Overmeyer *et al.* 2001). In addition, VBM analyses have highlighted compositional changes in otherwise normal appearing brain tissue (Woermann *et al.* 1999). Structural changes identified in VBM analyses have also been correlated with clinical outcome. For example, grey and white matter densities have been correlated with syndrome scores in adults diagnosed with schizophrenia (Wright *et al.* 1995).

Recently, the VBM method for the analysis of 3D structural (3D FLASH) data has been tailored to search exclusively for bilateral brain abnormalities. Using this method, it has been possible to incorporate *a priori* information concerning the bilateral nature of the underlying pathology to increase the sensitivity of the VBM analyses. For example, bilateral abnormalities have been identified in children with hypoxic-ischemic damage to the hippocampus (Gadian *et al.* 2000), and individuals with an inherited disorder of speech and language (Belton *et al.* 2003).

Since selective functional impairments are often associated with bilateral rather than unilateral pathology in childhood (Vargha-Khadem *et al.* 1994), brain structure was assessed throughout the whole brain of the LT survivors and their siblings using VBM and the bilateral method. In addition, an attempt was made to relate brain integrity, to specific indices of cognitive function as described in Chapter 3.

6.2.2 Methods

6.2.2.1 Data acquisition

All subjects were scanned unседated using a 1.5T Siemens Vision scanner. 3D data sets of the whole head were acquired using a multi-slice T1 weighted FLASH (Fast Low Angle Shot) sequence (TR = 16.80 ms; TE = 5.70 m; flip angle = 21°). This sequence provides high spatial resolution and grey:white contrast. Data were then transferred to a separate workstation and reconstructed in the sagittal plane (voxel size 1.5 x 1.5 x 1.5 mm).

To identify bilateral pathology, data sets were processed and analysed using SPM99 (Wellcome Department of Imaging Neuroscience, London, UK) and the method described by Salmond et al (2000).

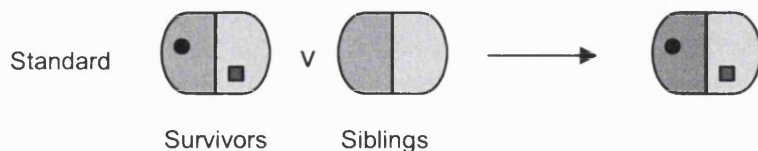
6.2.2.2 Data processing

Principles of the bilateral method are shown in Figure 6.1. Briefly, each data set was normalised to a symmetric template using the sum of squared differences approach (Friston *et al.* 1995; Ashburner & Friston 1999). A symmetric template was selected to reduce gross anatomical asymmetries and more closely co-localise homologous brain regions (Wright *et al.* 1995; Salmond *et al.* 2000). The number of discrete cosine transformations was 4 x 5 x 4 (4 in x, 5 in y and 4 in z).

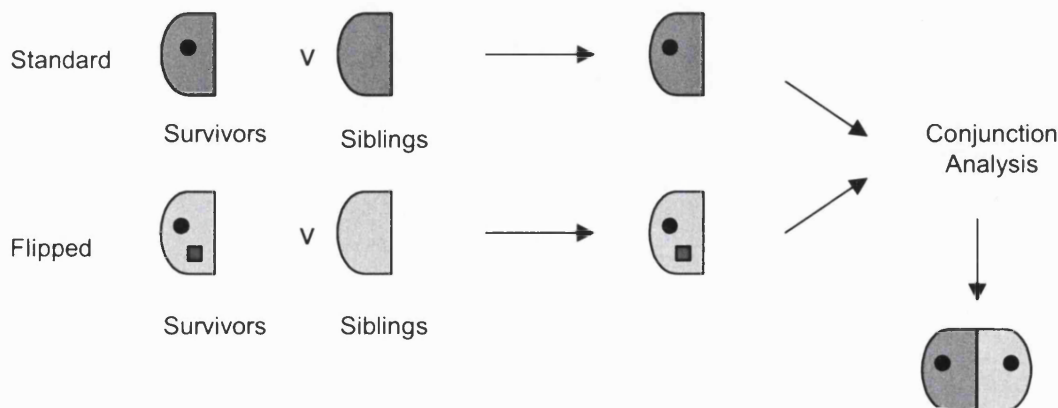
The spatially normalised data were then symmetrically segmented to produce continuous probability maps. The maps represented the probability that each voxel belonged to the white (or grey) matter segment based on its spatial location and signal intensity (Ashburner *et al.* 1997). A copy of each segmented white (and grey) matter image was also flipped in the interhemispheric plane.

Figure 6-1 Comparison of the unilateral and bilateral methods

A) Unilateral analysis



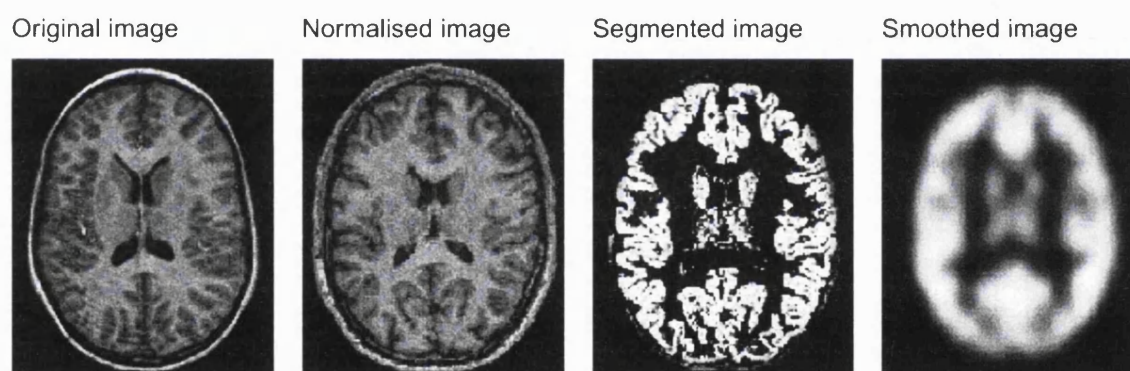
B) Bilateral analysis



Flipped and unflipped segmented white (and grey) matter images were then smoothed with an isotropic Gaussian kernel. The size of the kernel determined the extent of smoothing and in turn the spatial scale for statistical analyses. Segmented white matter images were smoothed to 4mm, 8mm, and 12mm to reflect the size of subcortical, periventricular, and deep white matter. Segmented grey matter images were smoothed to 8mm and 12mm, reflecting the cross sectional size of the subcortical nuclei and the cerebral cortex, respectively. Voxel values of the smoothed white (and grey) matter images represented the local amount of white (or grey) matter per unit volume, otherwise referred to as 'white (and grey) matter density' (Ashburner & Friston 2000).

The effect of normalisation, segmentation, and smoothing on the original data can be seen in Figure 6.2.

Figure 6-2 The post-processing steps.



6.2.2.3 Data analyses

Voxel-by-voxel conjunction analyses were conducted to investigate the similarities and differences between survivor and sibling groups. The analyses identified homologous regions where -

- (1) There were increases or decreases in white (or grey) matter that were common to both survivor groups (N=27),
- (2) There were increases or decreases in white (or grey) matter that differed between the IT (N=13) and HD (N=14) survivor groups

For consistency, additional conjunction analyses were conducted. These analyses identified homologous regions where -

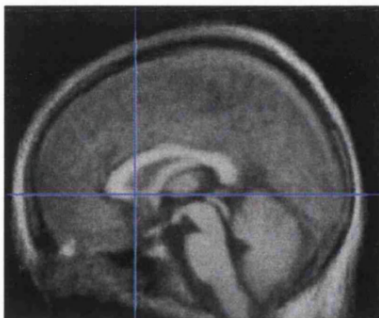
- (1) There were increases or decreases in white (or grey) matter in the HD survivor group (N=14) relative to their sibling group (N=6),
- (2) There were increases or decreases in white (or grey) matter in the IT survivor group (N=13) relative to their sibling group (N=7).

Each analysis was restricted to include only the voxels with an 80% or greater probability of being white (or grey) matter. This level was set to reduce the effect of partial volume. In addition, global white matter was treated as a confounding covariate. This ensured regional white matter density differences could be detected irrespective of differences in total white (or grey) matter. Furthermore, age-at-test was included as a covariate of no interest to account for developmental differences in anatomy.

Results of the conjunction analyses were investigated at two levels -

- (1) After correction for multiple comparisons across the whole brain
- (2) After correction limited to the region of interest (ROI) identified in our hypotheses. This region was defined as a box (60 mm X 60 mm X 90 mm) centred around (-30 45 15)¹⁰. The ROI encompassed all of the orbitofrontal and dorsolateral prefrontal cortex, the genu of the corpus callosum, and a large part of the anterior cingulate cortex (see Figure 6.3).

Figure 6-3 The ROI included all voxels anterior to the vertical limit displayed



¹⁰ It was only necessary to apply the ROI to one hemisphere since the analyses were bilateral.

To avoid false positives, only the regional differences surviving correction ($p < 0.05$) and not appearing at the midline have been reported (Salmond *et al.* 2000). In addition, regional differences approaching corrected significance ($0.05 < p < 0.10$) and not appearing at the midline are listed. The locations of the regional differences are given in mm; x defining the lateral displacement from the midline (right=positive), y defining the anterior-posterior position (anterior=positive), and z defining the vertical position (superior=positive).

Results have also been superimposed on the mean normalised image of all survivors and siblings to facilitate anatomical location. The images are displayed in neurological convention (left is left) at a threshold of $p < 0.005$ (uncorrected). Cross hairs indicate the location of the maximal regional difference peak.

6.2.3 Results

All data collected were successfully normalised, segmented, and smoothed and were therefore included in the conjunction analyses. The results of the segmentation, conjunction analyses, and correlations are presented below.

6.2.3.1 Data processing

Segmentation

The total number of voxels assigned to the white matter partition was related to the age-at-test of the subject ($r = 0.34$; $p = 0.03$)(Figure 6.4). There was a corresponding significant relationship between the total number of voxels allocated to the grey matter segment and the age-at-test of the subject ($r = -0.61$; $p < 0.001$)(Figure 6.5). Furthermore, there was a significant inverse relationship between the total number of grey matter voxels and the total number of white matter voxels identified in each subject ($r = -0.63$; $p < 0.001$)(Figure 6.6). Correlations between age-at-test and the number of voxels allocated to grey and white matter partitions were similar in survivor and sibling groups (see Table 6.1).

Table 6-1 Correlations between age-at-test and voxel allocation in each group

	No of voxels allocated to the white matter partition	No of voxels allocated to the grey matter partition	Relationship between no of voxels allocated to the white and grey matter partitions
Survivors	0.19 ($p = 0.35$)	-0.54 ($p = 0.004$)	-0.56 ($p = 0.002$)
Siblings	0.65 ($p = 0.02$)	-0.78 ($p = 0.002$)	-0.80 ($p = 0.001$)

Figure 6-4 No of voxels allocated to the white matter partition and age-at-test (all subject)

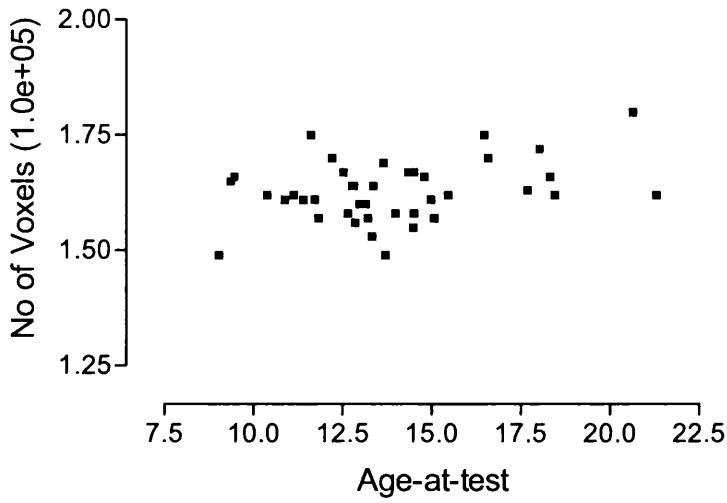


Figure 6-5 No of voxels assigned to the grey matter partition and age-at-test (all subjects)

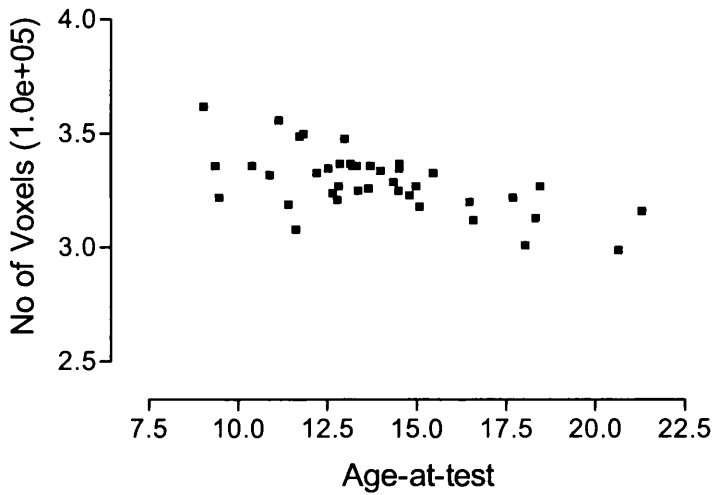
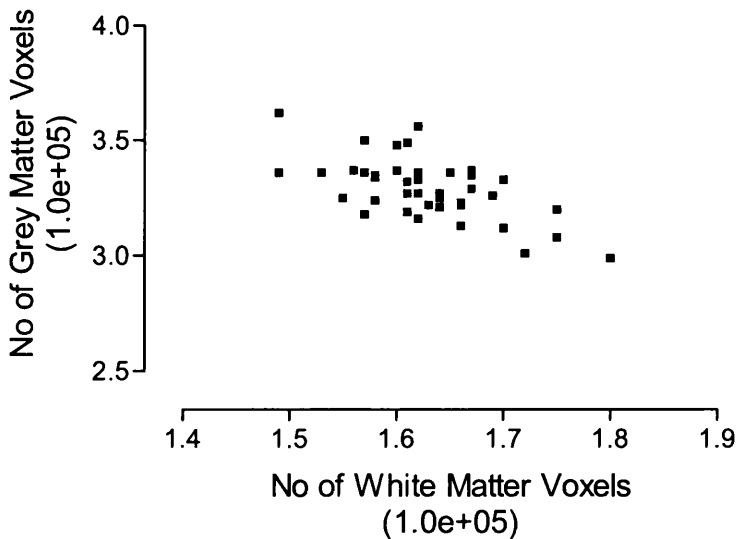


Figure 6-6 Relationship between grey and white matter voxels (all subjects)



6.2.3.2 Data analyses

Comparisons between the survivor and the sibling groups

Common differences in grey or white matter density were not detected in the survivor groups when compared to the sibling group at any level of smoothing.

Comparisons between HD and IT groups

Differences of grey or white matter density were not identified in the comparisons between the IT and HD groups at any level of smoothing.

Comparisons between HD and their sibling group

Comparisons between the HD group and their sibling group failed to detect regional increases or decreases of grey or white matter density at any level of smoothing.

Comparisons between IT and their sibling group

Similar comparisons between the IT group and their sibling group also failed to reveal regional increases or decreases of grey matter density at any level of smoothing. However, with respect to white matter, comparisons identified a significant increase in density at 4 mm of smoothing (Table 6.2). This increase was located on the lateral border between white and grey matter in the genu of the internal capsule ($z=4.84$; $p=0.04$)(Figures 6.7). In addition, there was an increase in white matter density in the corpus callosum (splenium) that approached significance ($z=4.66$; $p=0.09$)(Figure 6.8). No significant changes of white matter density were detected at other levels of smoothing.

Table 6-2 Bilateral increases of white matter density in IT group when compared the sibling group

Anatomical area	Comparison	Smoothing	Coordinates (mm)	z score	Corrected p value	Figure
Internal capsule (genu)	IT > Sibs	4 mm	-24 -2 9	4.84	0.04 *	6.7
Corpus callosum (splenium)	IT > Sibs	4 mm	-8 -24 26	4.66	0.09 *	6.8

* Corrected for multiple comparisons across the whole brain

Figure 6-7 Bilateral increase of white matter density in the internal capsule ($p < 0.05$)

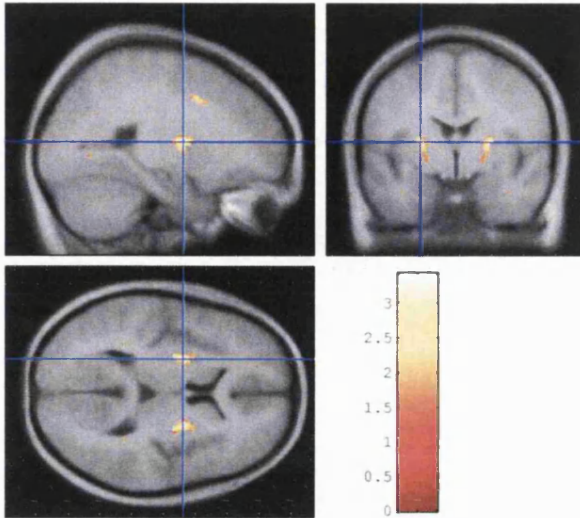
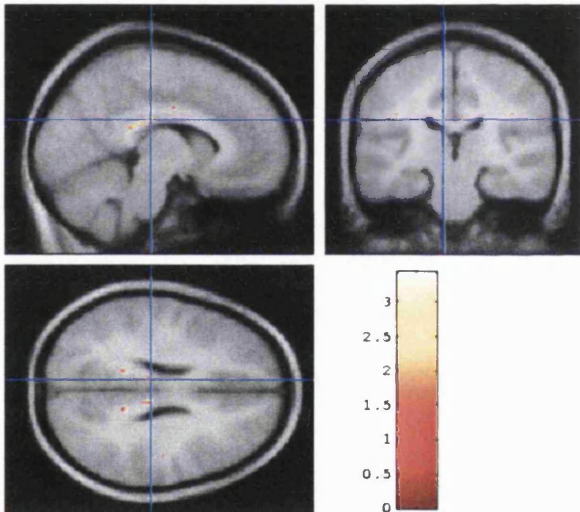


Figure 6-8 Bilateral increase of white matter density in the corpus callosum ($p = 0.09$)



6.2.3.3 Correlational analyses

A series of correlational analyses were conducted to examine the relationship between changes in white (or grey) matter density throughout the brain and indices of cognitive function. In particular, five indices of cognitive function were selected from the neuropsychological investigations detailed in Chapter 3. The indices differentiated the survivor group from the sibling group (i.e. visual discrimination), and IT group from the HD group (i.e. speeded motor response, first reversal, verbal fluency and extinction). Correlations were conducted separately for the sibling, IT, and HD survivor groups. Correlation analyses were unable to establish a relationship between white (or grey) matter density and indices of function in any group.

6.2.4 Discussion

The VBM analyses revealed few group differences between survivors and siblings. Specifically, regional increases in white matter density were only demonstrated between the IT group and their sibling group. While few differences were identified between groups this should not be interpreted as evidence against differences between survivors and their siblings. Group differences may not have been established for a number of reasons. For example, the analyses may not have been sensitive to the subtle level of change. Consistent with this, at autopsy children treated with MTX without CI and developing leukoencephalopathy show minimal decreases in total white matter volume. On a microscopic level, however, the same children show multiple small changes of white matter (e.g. patchy demyelination and necrotic lesions) (Liu *et al.* 1978). While children in our study did not develop encephalopathy the study indicates that even severe forms of white matter pathology associated with the treatment of ALL using MTX do not lead to significant changes in white matter volume. It is therefore possible that the technique employed in the current study did not have the sensitivity to reveal the pathology associated with the treatment of ALL.

In addition, age-at-test was included as a covariate of no interest in the analyses. The inclusion of this covariate has assumed a linear relationship between age-at-test and developmental changes in white (or grey) matter density. While the results of the segmentation support a linear relationship between age and changes in white and grey matter density on a global level, it is unlikely that this relationship is strictly linear and homogeneous across all areas of the brain (Winick *et al.* 1991). Therefore, in areas where the relationship with age is not linear, the degrees of freedom have unnecessarily been reduced by the inclusion of age as a covariate. As a consequence the chance of making a type II error has increased and it is possible subtle difference between groups have been masked.

Furthermore, the location of abnormalities may have varied between participants. Consistent with this, there was interindividual variation in the location of structural changes observed in the study participants using conventional MRI techniques (see Chapter 5).

Increases in white matter density

Typically, white matter pathology identified on VBM analyses of T1-weighted data is associated with a decrease in white matter density (presumably reflecting demyelination and/or axonal loss). The current study observed bilateral increases in white matter density of the internal capsule and corpus callosum within the IT group. An increase rather than a decrease may appear counterintuitive; however, using the present technique white matter density does not refer to cell packing but the amount of white matter labelled by the segmentation procedure per unit volume (Sowell *et al.* 1999).

White matter pathology and the internal capsule

In the current study, a significant bilateral increase in white matter density was observed in the IT group when compared to their sibling group. Specific white matter abnormalities of the internal capsule are not commonly reported in the LT survivors of leukaemia. Calcification of the left internal capsule following combined MTX + CI has been reported in one child five years post-diagnosis (Harila-Saari *et al.* 1998). While not specific to the internal capsule, subtle changes in surrounding areas have also been reported. For example, unilateral perfusion deficits have been identified in the striatum of three children treated with MTX (Harila-Saari *et al.* 1997). However, similar changes were not seen in other studies (Phillips *et al.* 1991; Kahkonen *et al.* 1999; Kahkonen *et al.* 2000).

White matter pathology and the corpus callosum

In the current study, a bilateral increase in white matter density approaching significance was identified in the corpus callosum of the IT group when compared to their sibling group. White matter changes of the corpus callosum are not commonly reported in the LT survivors of ALL. However, white matter changes of the corpus callosum have been described in one child treated with MTX-only. In addition, the white matter change was not seen at the end of treatment but first appeared in the longer-term (at the five-year follow-up)(Ohmoto *et al.* 1996).

Volumetric changes of the corpus callosum have also been observed in children treated for medulloblastoma (>1 year start of treatment) (Palmer *et al.* 2002). Specifically, these children demonstrated a reduction in corpus callosum volume across all subdivisions of the corpus callosum. Furthermore, the greatest volume reduction occurred in the posterior subdivisions (i.e. the splenium and isthmus). However, the children in that study were treated with more aggressive therapy

including surgical resection, craniospinal irradiation and chemotherapy. In addition, the posterior section of the corpus callosum received the greatest cumulative dose of CI, and CI has been associated with decreases in white matter volume in other studies (Reddick *et al.* 2000).

Correlations between white and grey matter densities and indices of function

In the current study, no correlation was established between white and grey matter density and indices of cognitive function. The lack of a significant correlation should not necessarily be interpreted as evidence against links between the white and grey matter density and the cognitive impairments identified in Chapter 3. A linear correlation may not have been established in the present study for a number of reasons, for example, as a consequence of insufficient variation in the cognitive scores and/or density estimates. Furthermore, the analyses conducted in the current study examined only linear relationships between variables. There is no *a priori* reason to indicate that the relationship between pathology and indices of function is necessarily linear and in fact the relationships may be non-linear.

6.2.5 Summary

In the current study, few differences in brain structure were identified between groups. In particular, bilateral white matter density increases were observed in the IT group when compared to the sibling group. These changes were significant in the internal capsule and approached significance in the corpus callosum. These results suggest that (1) the VBM analyses of 3D FLASH data were not sensitive to the small changes in morphometry associated with the treatment of ALL, (2) the pathology following the treatment of ALL with MTX does not lead to common LT structural changes, or (3) the nature of the pathology was not exclusively bilateral.

The following section describes a quantitative study using diffusion tensor images. This study provided information about brain structure using a different type of contrast (i.e. diffusion). The method was sensitive to changes in directionality (i.e. anisotropy) and was exquisitely sensitive to changes occurring in white matter.

6.3 Diffusion tensor imaging

6.3.1 Introduction

Diffusion is a physical process that involves the translational movement of molecules via random motions (Moseley *et al.* 1990). In a solution this process is influenced by molecular weight, intermolecular interactions (viscosity), and temperature. Within tissues the overall mobility of diffusing molecules is also influenced by the underlying cellular microstructure (Beaulieu 2002).

When diffusion is not restricted (e.g. within a tissue in which cellular barriers are not coherently orientated) diffusion occurs equally in all directions and is termed isotropic. In contrast, when diffusion is constrained and depends upon direction (e.g. in a tissue with highly oriented barriers) it is termed anisotropic diffusion (Moseley *et al.* 1990).

Using diffusion tensor imaging (DTI) the diffusion of water can be observed *in vivo* (Basser *et al.* 1994). As the rate and direction of diffusion is determined by local tissue composition, the characteristics of the diffusion tensor serve as an index of local tissue integrity (Eriksson *et al.* 2001). For example, the directionality of the diffusion tensor (i.e. anisotropy) provides an index of the geometric alignment and order of tissue microstructure (Le Bihan *et al.* 2001).

In children, anisotropy changes have been associated with pathology in periventricular leukomalacia (Huppi *et al.* 1998; Huppi *et al.* 2001; Miller *et al.* 2002), X-linked adrenoleukodystrophy (Ito *et al.* 2001; Eichler *et al.* 2002), Krabbe disease (Guo *et al.* 2001), and following treatment for medulloblastoma (Khong *et al.* 2003). Similarly, in adults regional changes in anisotropy have been observed in epilepsy (Eriksson *et al.* 2001; Wiesmann *et al.* 2003), stroke (Sorensen *et al.* 1999; Werring *et al.* 2000; Mukherjee *et al.* 2000), amyotrophic lateral sclerosis (Ellis *et al.* 1999), and multiple sclerosis (Tievsky *et al.* 1999; Werring *et al.* 1999; Bammer *et al.* 2000; Ciccarelli *et al.* 2001; Guo *et al.* 2002). Generally, pathology has been associated with a decrease in local anisotropy (i.e. reflecting a loss of cellular coherence) although increases in anisotropy are also reported, for example in the acute stages of stroke (Yang *et al.* 1999). In many cases, changes in anisotropy were identified in otherwise normal appearing tissue (Werring *et al.* 1999; Werring *et al.* 2000; Ciccarelli *et al.* 2001; Eriksson *et al.* 2001; Khong *et al.* 2003). In addition, decreases in anisotropy have also been related to clinical outcome (Ellis *et al.* 1999; Ciccarelli *et al.* 2001) and cognitive performance (Klingberg *et al.* 2000; O'Sullivan *et al.* 2001; Schmithorst *et al.* 2002).

In the present study, local anisotropy was estimated throughout the whole brain of the survivors and their siblings. Using voxel based-morphometry (VBM) regional differences in anisotropy were then identified between groups. Finally, an attempt was made to relate changes in anisotropy to indices of cognitive and behavioural function.

6.3.2 Methods

6.3.2.1 Data acquisition

Each child was scanned unседated using a 1.5T Siemens Vision scanner. DTI data of the whole brain were acquired using a twice-refocused diffusion-weighted spin echo echoplanar imaging (EPI) sequence (TE = 110 ms; No of contiguous slices = 40; Slice thickness = 3 mm). Data were collected in twenty non-collinear directions by applying a series of diffusion-encoding gradients ($b = 1000\text{s/mm}^2$). In addition, data were acquired without diffusion weighting ($b = 0$) at the beginning, middle, and end of the sequence. This sequence provided high grey:white contrast with reasonable anatomical resolution.

Raw data were then transferred to a separate workstation for post-processing. Details of the post-processing steps and the statistical analyses conducted are described below.

6.3.2.2 Data processing

Using in-house software the raw data for each subject were converted to 23 base images (voxel size = 1.5 x 1.5 x 3.0 mm, No of slices = 40). Base images were visually inspected for movement and CSF/arterial pulsation artefacts. Individual slices containing artefacts were removed. In addition, if a slice contained artefact in more than five of the 23 base images (22%) the slice was excluded from all base images for the subject. Similarly, if more than eight of the 40 slices (20%) within a base image contained artefacts the entire base image was discarded.

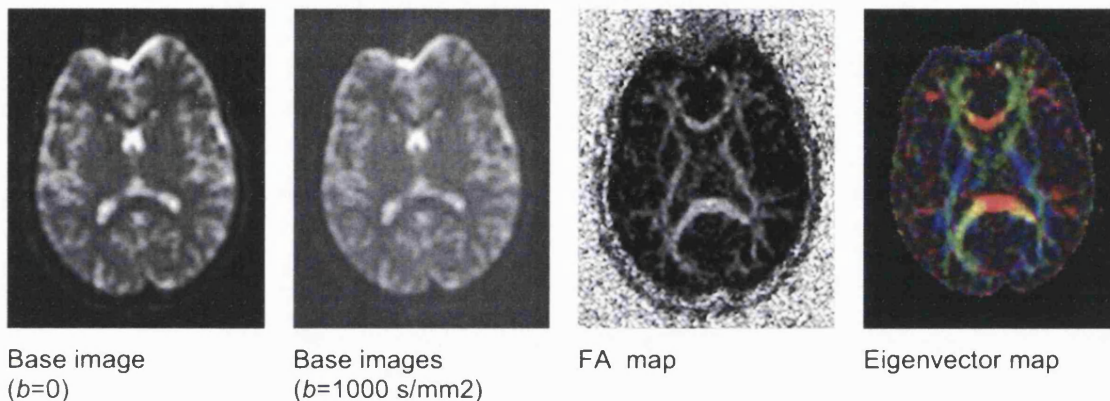
Following the removal of gross artefacts a fractional anisotropy (FA) map was generated for each subject. Within the FA map each voxel value represented the fraction of the magnitude of the diffusion tensor that was anisotropic. This index ranged from zero (where diffusion is equal in all directions) to one (a medium with maximum anisotropy)(Pierpaoli *et al.* 1996).

Before further processing FA maps were reviewed specifically for directional bias. This involved displaying the main direction of diffusion in each voxel i.e. the production of eigenvector maps (Le Bihan *et al.* 2001). Eigenvector maps were visually inspected

and a directional bias was suspected if areas of grey matter had a consistent colour. If a directional bias was identified in this procedure all DTI data collected from the subject (including the FA map) were excluded from further processing.

Figure 6.9 provides examples of the base images collected, and the fractional anisotropy (FA), and eigenvector maps generated for each subject. Colours displayed in the eigenvector map indicate the main direction of local diffusion in x (red), y (green) or z (blue).

Figure 6-9 Base images ($b=0$ and $b=1000 \text{ s/mm}^2$), fractional anisotropy (FA) map, and Eigenvector map at the level of the basal ganglia for a sibling control

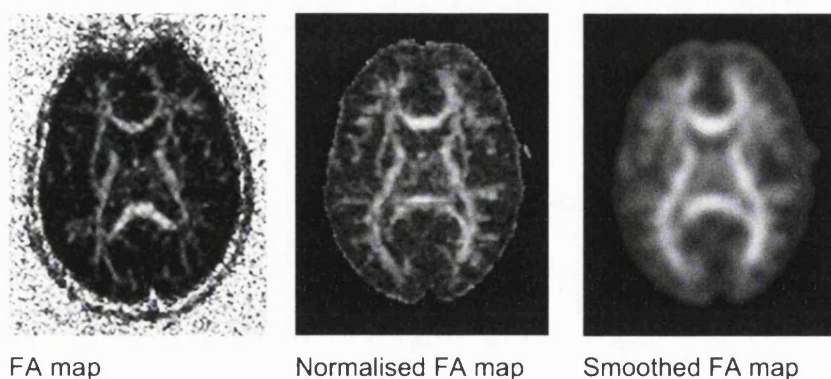


The $b=0$ images and the FA maps for each subject were then imported into SPM99. Using this software, data were processed further and analysed in accordance with method described by Eriksson et al (2001). The method was also similar to that described in Section 6.2.2 for the T1-weighted 3D FLASH data.

Base images ($b=0$) were spatially normalised to a standard EPI template. This involved a combination of linear and non-linear transformations, and accounted for global differences in brain position, orientation, and size (for further detail see section 6.2.2). The parameters generated in this process were used to normalise the FA maps.

Following normalisation unsegmented FA maps were smoothed with an isotropic Gaussian kernel. The size of the kernel determined the extent of smoothing and in turn the spatial scale for statistical analyses. FA maps were smoothed to 4mm, 8mm, and 12mm to reflect the typical dimensions of subcortical white matter, subcortical nuclei, and deep white matter respectively. The effect of normalisation and smoothing on an individual FA map can be seen in Figure 6.10.

Figure 6-10 Normalisation and smoothing of the fractional anisotropy (FA) map.



FA map

Normalised FA map

Smoothed FA map

6.3.2.3 Data analyses

To investigate the similarities and differences between survivor groups conjunction analyses were then performed on the smoothed FA maps. These analyses identified regions where -

- (1) There were increases or decreases in anisotropy that were common to both survivor groups (N=22)
- (2) There were increases and decrease in anisotropy that differed between the IT (N=10) and HD (N=12) survivor groups.

For consistency, statistical comparisons with standard contrasts were also conducted. These analyses identified regions where -

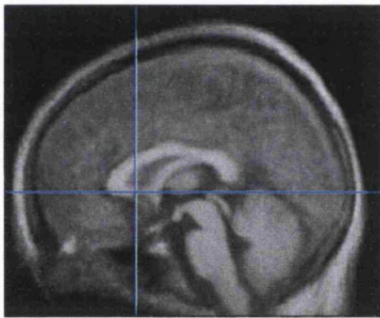
- (1) There were increases or decreases in anisotropy in the HD survivor group (N=12) relative to their sibling group (N=3)
- (2) There were increases or decreases in anisotropy in the IT survivor group (N=10) relative to their sibling group (N=4).

Each analysis was limited to included voxels with an intensity value exceeding 80% of the whole-brain FA mean intensity (i.e. the most anisotropic voxels). This restriction reduced the effect of partial volume on FA estimates in the analyses. In addition, global FA was treated as a confounding covariate. This ensured regional anisotropy differences could be detected irrespective of differences in total anisotropy. Furthermore, age-at-test was included as a covariate of no interest to account for developmental differences in anatomy.

Inferences from statistical parametric maps were made at two levels -

- (1) After correction for multiple comparisons across the whole brain ($p < 0.05$)
- (2) After correction limited to the regions of interest (ROI) identified in our hypotheses (i.e. the prefrontal cortices). ROI's were defined separately for each hemisphere. Each ROI was a box (60 mm X 60 mm X 90 mm) centred at (-30 40 15) or (30 40 15) and encompassed all of the orbitofrontal and dorsolateral prefrontal cortex, the genu of the corpus callosum and a large part of the anterior cingulate cortex in either the left or right hemisphere (see Figure 6.11). As ROI's were defined separately for each hemisphere inferences were made using a modified corrected significance level of $p < 0.025$.

Figure 6-11 The ROI included all voxels anterior to the vertical limit



Regional differences surviving whole brain ($p < 0.05$) or region of interest ($p < 0.025$) correction have been reported. In addition, regional differences approaching corrected significance ($p < 0.10$) are listed. The locations of the regional differences are given in mm; x defining the lateral displacement from the midline (right=positive), y defining the anterior-posterior position (anterior=positive), and z defining the vertical position (superior=positive).

Results have also been superimposed on the mean normalised FA map (all patients and siblings) to facilitate anatomical location. The images are displayed in neurological convention (left is left) at a threshold of $p < 0.005$ (uncorrected). Cross hairs indicate the location of the maximal regional difference peak.

6.3.3 Results

The quality of the raw data collected from each subject was evaluated during post-processing as described in Section 6.3.2. Details of the data excluded and the results of the statistical analyses are presented below.

6.3.3.1 Data processing

Approximately 2% (range: 0.3 – 13.9%) of data collected from each subject was rejected due to movement or CSF/arterial pulsation artefacts. The number of slices removed from any of the 23 base images was similar for each group (K-W $\chi^2=4.55$, $p=0.10$)(Table 6.3). A slice was not excluded from all base images for any subject. However, an entire base image was discarded for two subjects (1=HD survivor; 1=IT survivor) and three base images were rejected for one subject (HD survivor).

Table 6-3 Number of slices removed from any of the base images in each group

	IT survivors	HD survivors	Siblings
Mean	29.36 (3.19%)	27.21 (2.96%)	11.88 (1.29%)
SE	10.25	5.29	2.52
Minimum	3	4	4
Maximum (max 920)	128	71	22

A directional bias was identified in the eigenvector maps of three subjects (2=HD survivors; 1=IT survivor). The bias co-occurred in subjects where entire base images had been removed due to artefact. It was therefore most likely that the distortion was introduced during post-processing. The bias could not be corrected. All data (including FA maps) for the three subjects were therefore excluded from further analyses.

The removal of the three subjects and the failure to acquire DTI's in many controls (see Appendix C) did not significantly change the mean age-at-diagnosis, age-at-test or length of follow-up in each group. In addition, while the majority of siblings were girls, this failed to reach statistical significance between groups (Table 6.4).

Table 6-4 Characteristics of the children with usable DTI data sets

	Sex (M:F)	Age-at-diagnosis (years)	Age-at-test (years)	Length of follow-up (years)
HD survivors	7:5	4.66	13.35	8.70
IT survivors	5:5	5.54	14.17	8.63
Siblings	2:5		13.86	

6.3.3.2 Data analyses

Comparisons between survivor and the sibling groups

Common regional differences in anisotropy were identified in the survivor groups when compared to the sibling group (Table 6.5). Anisotropy decreases were revealed in the thalamus (dorsomedial nucleus right hemisphere), head of the caudate nucleus (left hemisphere) and the corona radiata (right hemisphere). The decrease in the thalamus was revealed at 4mm smoothing ($z=5.47$; $p=0.004$)(Figure 6.12) and approached corrected significance at 8mm smoothing ($z=4.72$; $p=0.08$)(Figure 6.13). The decreases in the caudate nucleus and corona radiata approached corrected significance at 12mm ($z=4.50$; $p=0.09$)(Figure 6.14) and 8mm ($z=4.52$; $p=0.05$)(Figure 6.15) smoothing respectively.

Table 6-5 Common anisotropy decreases in survivor groups when compared to sibling group

Anatomical area	Hemisphere	Comparison	Smoothing	Coordinates (mm)	z score	Corrected p value	Figure
Thalamus (dorsomedial nucleus)	Right	P < Cont	4 mm	6 -16 3	5.47	0.004*	6.12
Thalamus (dorsomedial nucleus)	Right	P < Cont	8 mm	2 -18 9	4.72	0.08*	6.13
Caudate nucleus (Head)	Left	P < Cont	12 mm	-8 6 9	4.50	0.09*	6.14
Corona radiata	Right	P < Cont	8 mm	14 24 42	4.52	0.05**	6.15

*Corrected for multiple comparisons across the whole brain (corrected significance $p<0.05$)

** Corrected for region of interest (corrected significance $p<0.025$)

Comparisons between the HD and IT groups

Significant differences in anisotropy could not be identified between the two survivor groups at any level of smoothing.

Comparisons between the HD and their sibling group

A decrease in anisotropy was identified in the comparisons between the HD survivor group and their sibling group at 12mm smoothing (Table 6.6). This regional decrease was located in the corona radiata (left hemisphere) and approached corrected significance ($z=4.38$; $p=0.05$) (Figure 6.16). Changes in anisotropy were not observed at remaining levels of smoothing.

Table 6-6 Regional anisotropy decrease in the HD group when compared to the sibling group

Anatomical area	Hemisphere	Comparison	Smoothing	Coordinates (mm)	z score	Corrected p value	Figure number
Corona radiata	Left	HD < Cont	12 mm	-24 12 42	4.38	0.05**	6.16

** Corrected for region of interest (corrected significance $p < 0.025$)

Comparisons between the IT and their sibling group

Comparisons between the IT survivor group and their sibling group failed to detect regional increases or decreases in anisotropy at any level of smoothing.

Figure 6-12 Regional anisotropy decrease in the right thalamus (4mm smoothing)

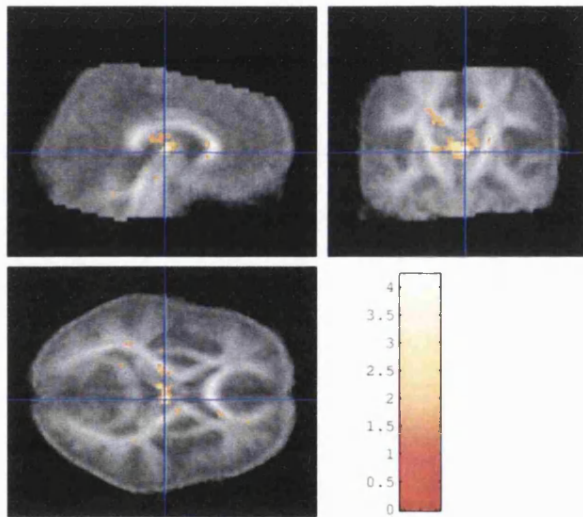


Figure 6-13 Regional anisotropy decrease in the right thalamus (8mm smoothing)

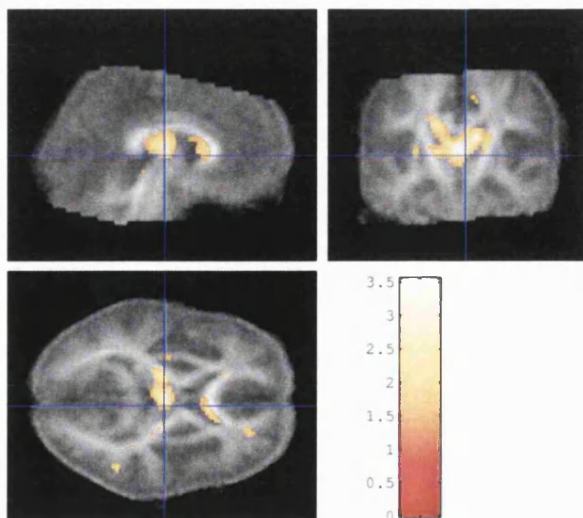


Figure 6-14 Regional anisotropy decrease in the left caudate nucleus

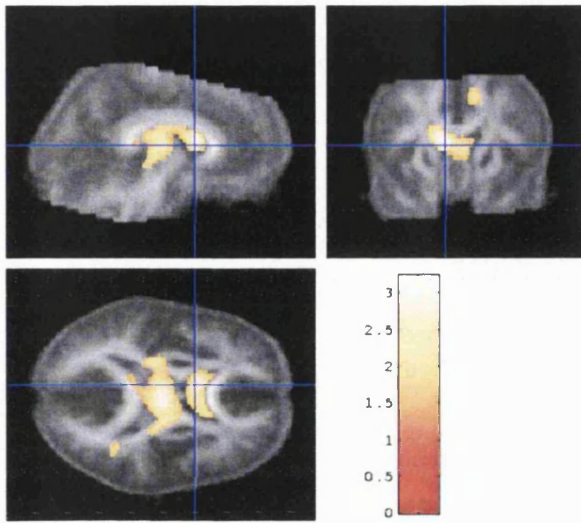


Figure 6-15 Regional anisotropy decrease in the right corona radiata

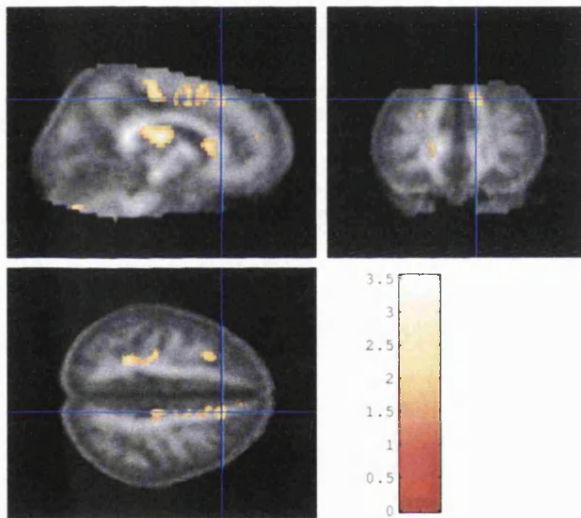
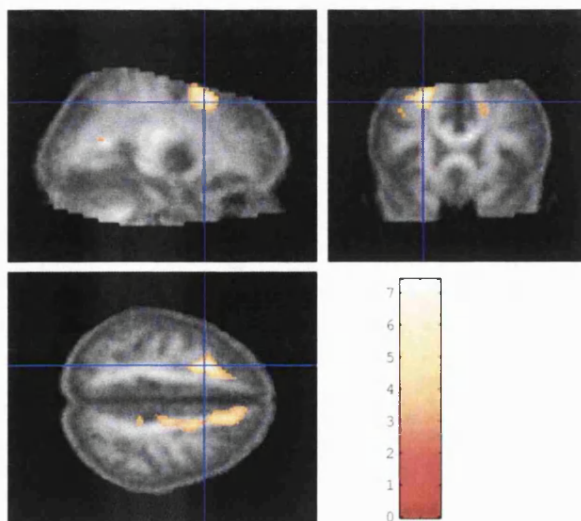


Figure 6-16 Regional anisotropy decrease in the left corona radiata



6.3.3.3 Individual anisotropy estimates

To further investigate the areas of abnormality identified in the VBM analyses smoothed individual anisotropy values were obtained from the left and right thalamus, caudate nucleus, and corona radiata. At each location the an asymmetry coefficient was calculated

$$\text{Asymmetry coefficient} = 2 (\text{Left} - \text{Right}) / (\text{Left} + \text{Right})$$

Results were also compared to smoothed individual anisotropy values obtained from areas where no significant differences in anisotropy were identified. These included bilateral regions of frontal and parietal periventricular white matter, the cerebellum, and the pons. At all locations the relationship between anisotropy and age-at-test was also explored.

Anisotropy in areas of abnormality as identified in the VBM analyses

The smoothed anisotropy values acquired for all survivors and siblings in the thalamus, caudate nucleus, and corona radiata are displayed in Figures 6.17 – 6.19. The degree of change in anisotropy at each anatomical location is shown in Tables 6.7.

For survivors, anisotropy was reduced by 6.5% and 24.9% in the left and right thalamus respectively. These results were accompanied by smaller anisotropy decreases in left (12.1%) and right (9%) caudate nucleus. While the homologous changes were not sufficient to reach statistical significance (see VBM results) nevertheless some degree of change appeared to occur bilaterally in the thalamus and caudate nucleus.

The anisotropy changes were also bilateral in the corona radiata (see VBM results). Within the right corona radiata anisotropy was reduced by 11.6% in survivors when compared to their siblings. A similar decrease of 9.2% was observed in the left corona radiata. As expected, based on the VBM results, the reduction in the left corona radiata was greater for the HD survivor group (13%) than the IT survivor group (4.6%)(Table 6.8). This pattern was observed for all locations except the left thalamus where the trend was reversed (i.e. the mean for IT survivor group was lower than the mean for HD survivor group). No statistical differences were found however between survivor groups (see VBM results).

Figure 6-17 Individual anisotropy values in the left and right thalamus

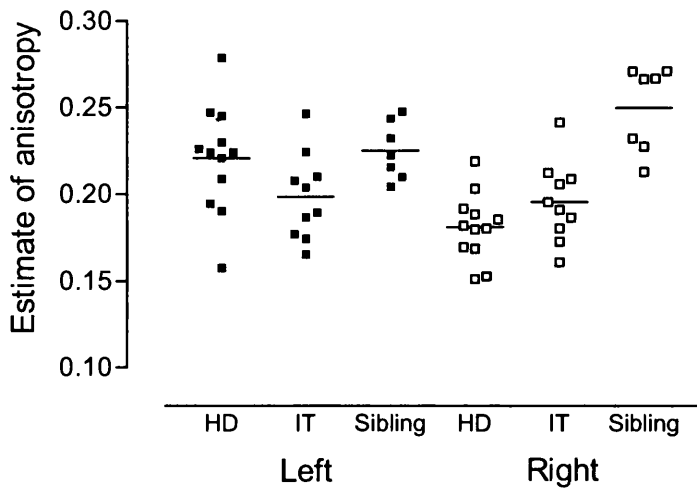


Figure 6-18 Individual anisotropy values in the left and right caudate nuclei

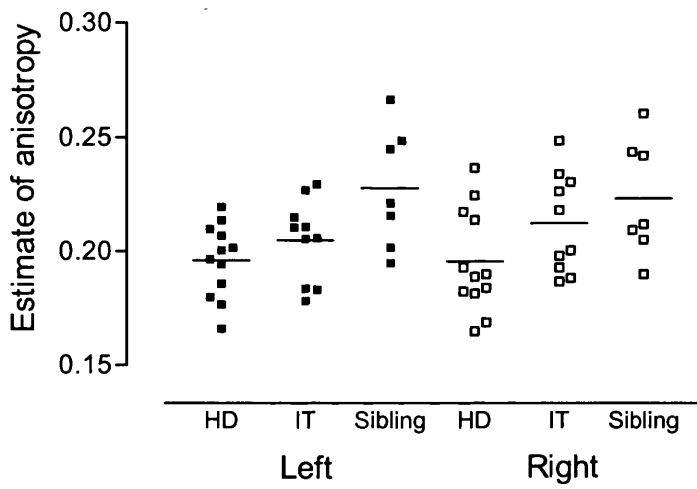
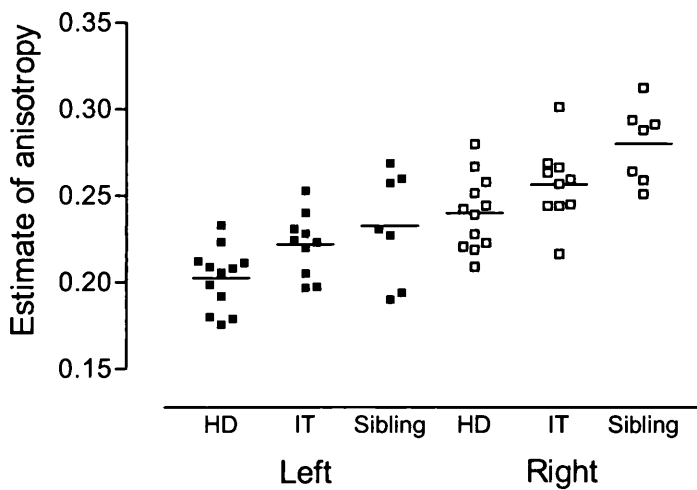


Figure 6-19 Individual anisotropy values in the left and right corona radiata



Within the sibling group, individual anisotropy values were similar between hemispheres for the caudate nucleus. However, anisotropy in the thalamus and corona radiata showed hemispheric asymmetry with individual anisotropy values greater in the right than left hemisphere (Table 6.9). This trend was observed within the survivor group for the caudate nucleus and corona radiata. Within the thalamus however the reverse trend was observed (i.e. left greater than right) presumably as a consequence of the much greater decrease in anisotropy on the right when compared to the left (Figures 6.20 – 6.22).

Table 6-7 Mean anisotropy for the survivor and sibling groups in areas of abnormality

	Survivors Mean FA (SD)	Siblings Mean FA (SD)	FA reduction in survivors (%)
Thalamus (L)	0.21 (0.03)	0.23 (0.02)	- 6.5
Thalamus (R)	0.19 (0.02)	0.25 (0.02)	- 24.9
Caudate (L)	0.20 (0.02)	0.23 (0.03)	- 12.1
Caudate (R)	0.20 (0.02)	0.22 (0.03)	- 9.0
Corona radiata (L)	0.21 (0.02)	0.23 (0.03)	- 9.2
Corona radiata (R)	0.25 (0.02)	0.28 (0.02)	- 11.6

Table 6-8 Mean anisotropy for the HD, IT, and sibling groups in areas of abnormality

	HD survivors Mean (SD)	IT survivors Mean (SD)	Siblings Mean (SD)	FA reduction HD survivors (%)	FA reduction IT survivors (%)
Thalamus (L)	0.22 (0.03)	0.20 (0.02)	0.23 (0.02)	- 2.0	- 11.8
Thalamus (R)	0.18 (0.02)	0.20 (0.02)	0.25 (0.02)	- 27.5	- 21.7
Caudate (L)	0.20 (0.02)	0.20 (0.02)	0.23 (0.03)	- 13.9	- 9.9
Caudate (R)	0.20 (0.02)	0.21 (0.02)	0.22 (0.03)	- 12.4	- 4.8
Corona radiata (L)	0.20 (0.02)	0.22 (0.02)	0.23 (0.03)	- 13.0	- 4.6
Corona radiata (R)	0.24 (0.02)	0.26 (0.02)	0.28 (0.02)	- 14.2	- 8.4

Table 6-9 Mean left and right anisotropy estimates in the sibling group

	Left Mean FA (SD)	Right Mean FA (SD)	t	p value
Thalamus	0.23 (0.02)	0.25 (0.02)	3.19	0.02
Caudate	0.23 (0.03)	0.22 (0.03)	-0.93	0.39
Corona radiata	0.23 (0.03)	0.28 (0.02)	5.30	0.002

Figure 6-20 Asymmetry coefficients in the thalamus

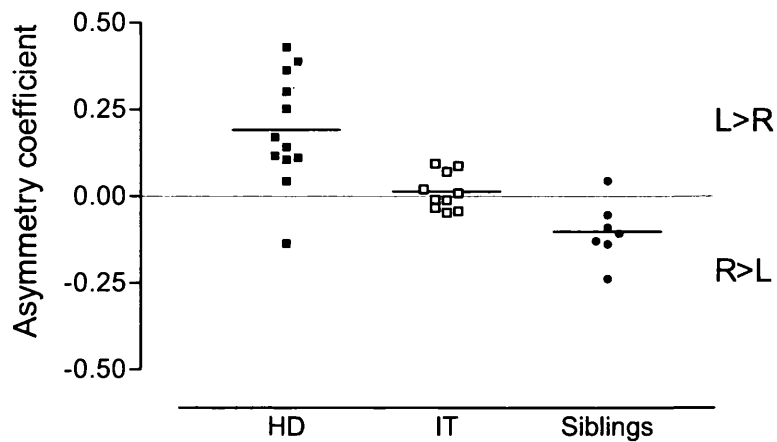


Figure 6-21 Asymmetry coefficients in the caudate nucleus

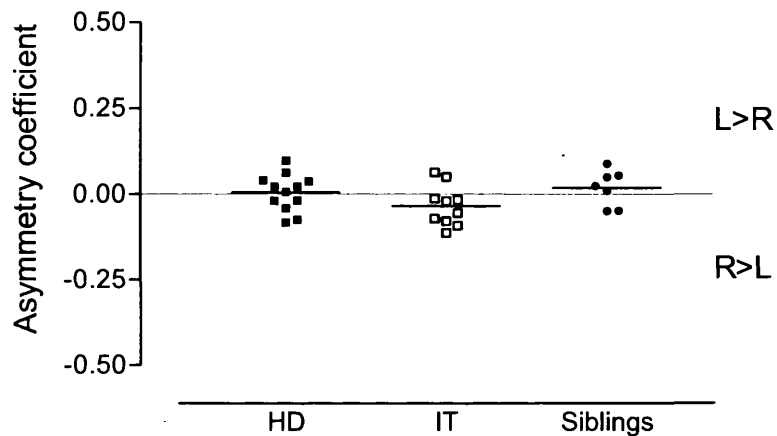
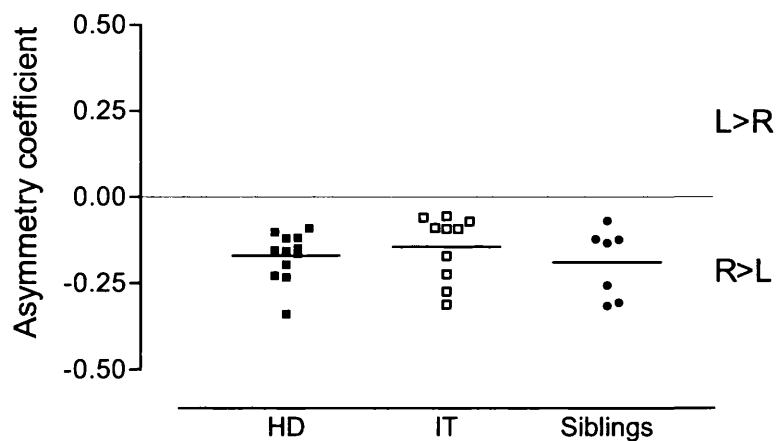


Figure 6-22 Asymmetry coefficients in the corona radiata (voxels not homologous)



Regions of interest where significant differences in anisotropy were not identified

Smoothed anisotropy values were acquired for homologous regions of the frontal and parietal periventricular white matter, the cerebellum, and the pons (Figure 6.23). These regions were similar to those selected by Khong et al 2003 (Figure 6.24).

As shown in Figures 6.25 - 6.28, the range of individual anisotropy values observed in areas where significant differences in anisotropy were not identified was similar for survivors and siblings. Similarly, the mean anisotropy values for survivor and sibling groups were not significantly different with the discrepancy less than 5.7% at all locations (Table 6.10). Mean anisotropy values in the frontal periventricular white matter, cerebellum, and pons were also similar to anisotropy estimates reported in another study (Khong et al. 2003). However, the mean anisotropy estimated for the parietal periventricular white matter in the current study, was less than that reported by Khong et al. (2003). In addition, individual anisotropy values did not show hemispheric asymmetry with similar left and right values observed in survivor and siblings groups (Table 6.11 – 6.12).

Figure 6-23 Regions of interest where significant differences in anisotropy were not identified.

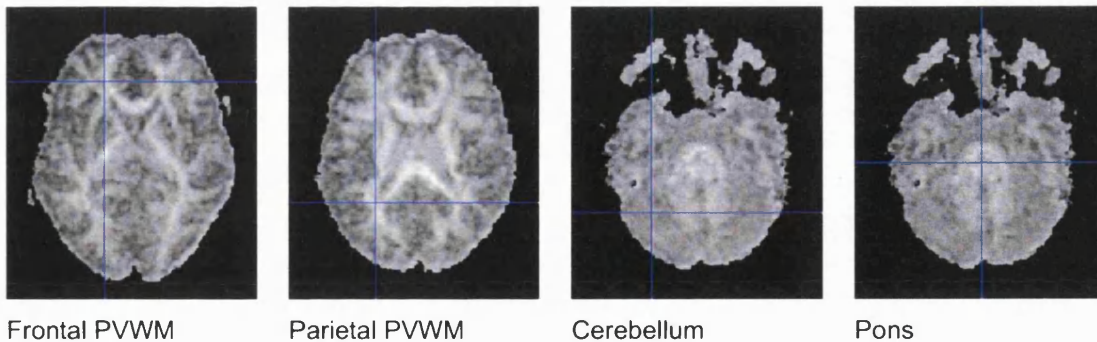


Figure 6-24 Axial T2- weighted images showing the regions of interest used by Khong et al 2003.

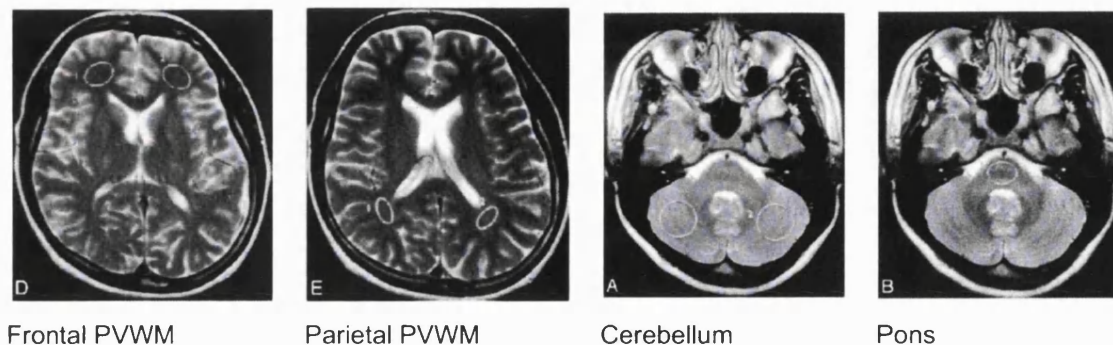


Figure 6-25 Individual anisotropy values in frontal periventricular white matter

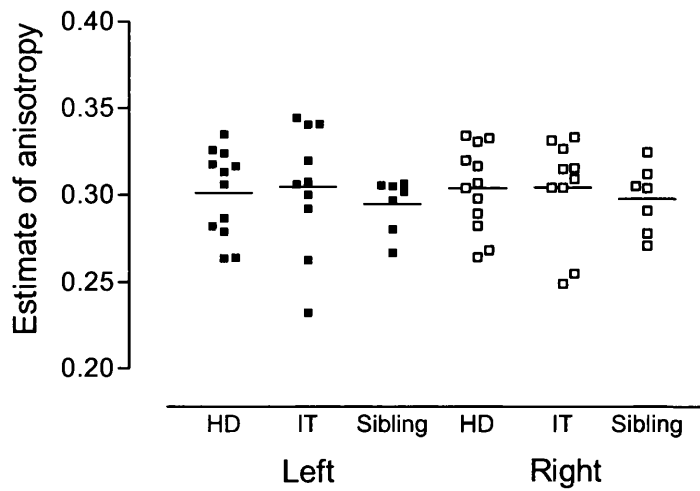


Figure 6-26 Individual anisotropy values in parietal periventricular white matter

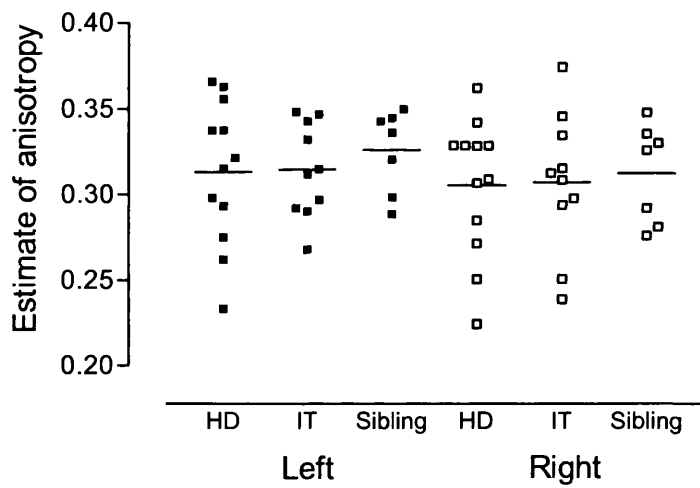


Figure 6-27 Individual anisotropy values in the cerebellum

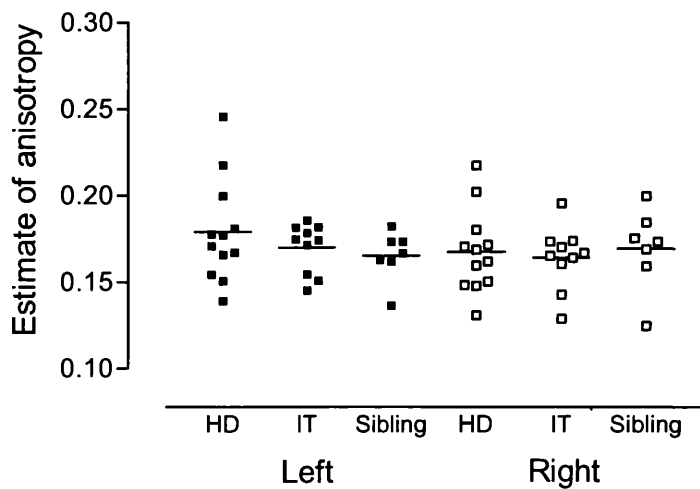


Figure 6-28 Individual anisotropy values in the pons

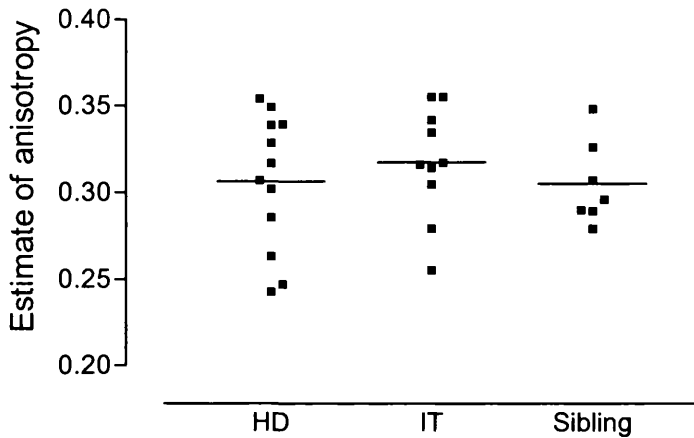


Table 6-10 Mean values for the survivor and sibling groups in areas where significant differences in anisotropy were not identified

	Survivors Mean FA (SD)	Siblings Mean FA (SD)	FA change in survivors (%)	Khong et al. 2003 controls (SD)
Frontal PVWM (L)	0.30 (0.03)	0.29 (0.02)	+ 2.8	0.30 (0.06)
Frontal PVWM (R)	0.30 (0.03)	0.30 (0.02)	+ 2.1	
Parietal PVWM (L)	0.31 (0.04)	0.33 (0.02)	- 3.7	0.41 (0.05)
Parietal PVWM (R)	0.31 (0.04)	0.31 (0.03)	- 2.1	
Cerebellum (L)	0.17 (0.02)	0.17 (0.01)	+ 5.7	0.17 (0.04)
Cerebellum (R)	0.17 (0.02)	0.17 (0.02)	- 2.0	
Pons	0.31 (0.04)	0.31 (0.02)	+ 2.0	0.35 (0.02)

PVWM periventricular white matter.

Table 6-11 Left and right mean anisotropy estimates in the survivor group

	Left Mean FA (SD)	Right Mean FA (SD)	t	p value
Frontal PVWM	0.30 (0.03)	0.30 (0.03)	-0.39	0.70
Parietal PVWM	0.31 (0.04)	0.31 (0.04)	1.19	0.25
Cerebellum	0.17 (0.02)	0.17 (0.02)	1.15	0.22

Table 6-12 Left and right mean anisotropy estimates in sibling group

	Left Mean FA (SD)	Right Mean FA (SD)	t	p value
Frontal PVWM	0.29 (0.02)	0.30 (0.02)	-0.66	0.53
Parietal PVWM	0.33 (0.02)	0.31 (0.03)	1.25	0.26
Cerebellum	0.17 (0.01)	0.17 (0.02)	-0.76	0.48

Relationship between individual anisotropy values and age-at-test

Age-at-test was significantly correlated with anisotropy in the caudate nucleus, corona radiata and right frontal periventricular white matter within survivor and/or sibling groups. Age-at-test was not related to anisotropy in the thalamus, parietal periventricular white matter, cerebellum, or pons in either group (Table 6.13).

Table 6-13 Relationship between anisotropy and age-at-test

	Survivors Pearson's r (p)	Siblings Pearson's r (p)
Thalamus (L)	-0.03 (p=0.91)	-0.50 (p=0.26)
Thalamus (R)	0.31 (p=0.16)	-0.20 (p=0.67)
Caudate (L)	0.48 (p=0.02)	0.68 (p=0.09)
Caudate (R)	0.52 (p=0.01)	0.65 (p=0.12)
Corona radiata (L)	0.24 (p=0.27)	0.75 (p=0.06)
Corona radiata (R)	0.06 (p=0.80)	0.84 (p=0.02)
Frontal PVWM (L)	0.23 (p=0.30)	0.04 (p=0.93)
Frontal PVWM (R)	0.45 (p=0.04)	-0.29 (p=0.52)
Parietal PVWM (L)	0.09 (p=0.68)	0.05 (p=0.91)
Parietal PVWM (R)	-0.01 (p=0.96)	0.21 (p=0.65)
Cerebellum (L)	0.07 (p=0.77)	0.54 (p=0.21)
Cerebellum (R)	0.08 (p=0.74)	0.58 (p=0.17)
Pons	0.32 (p=0.15)	-0.06 (p=0.89)

6.3.3.4 Homogeneity of pathology identified in survivors**Homogeneity of pathology with respect to sex**

To explore the homogeneity of the pathology identified within the survivor group, smoothed anisotropy values for the right thalamus, left caudate nucleus, left and right corona radiata were plotted separately for boys and girls. As shown in Figures 6.29 – 6.32, similar changes in anisotropy were observed in all survivors irrespective of sex or type of treatment received. Mean anisotropy values were also similar between boys and girls (Table 6.14). However, results indicated that the anisotropy decrease was greater for boys than girls in the thalamus ($t=-2.14$; $p=0.05$).

Figure 6-29 Individual anisotropy values for the boys and girls in the right thalamus

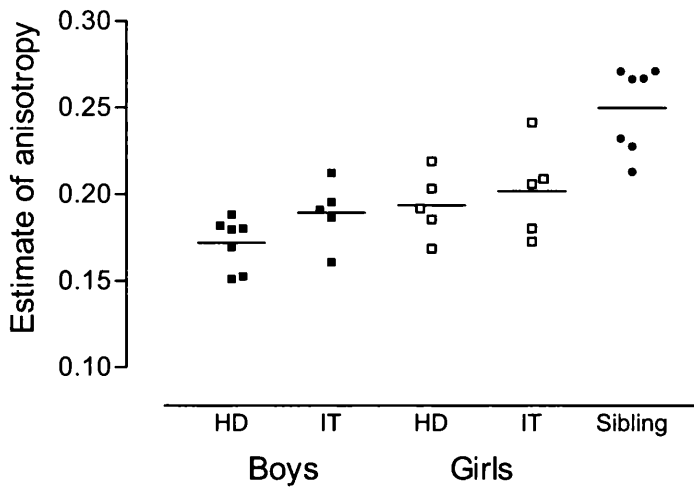


Figure 6-30 Individual anisotropy values for the boys and girls in the left caudate nucleus

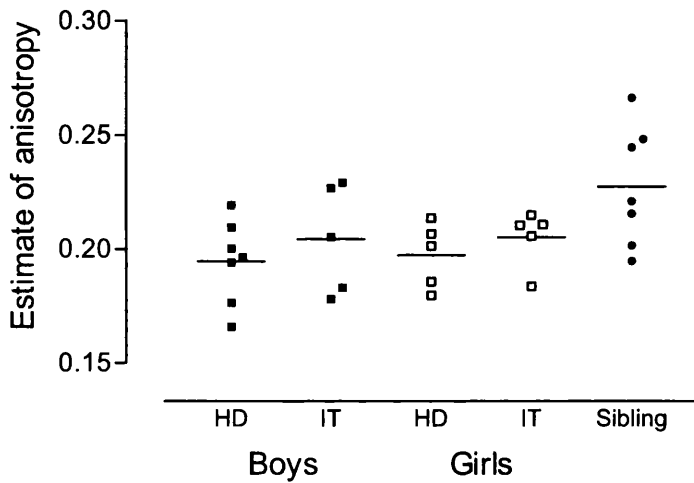


Figure 6-31 Individual anisotropy values for the boys and girls in the left corona radiata

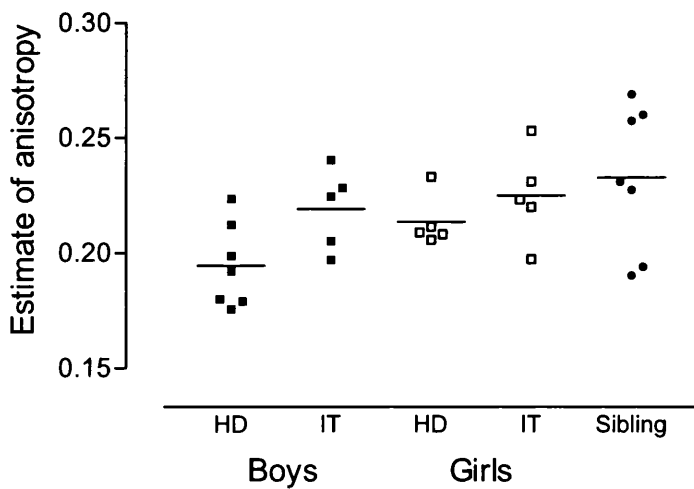
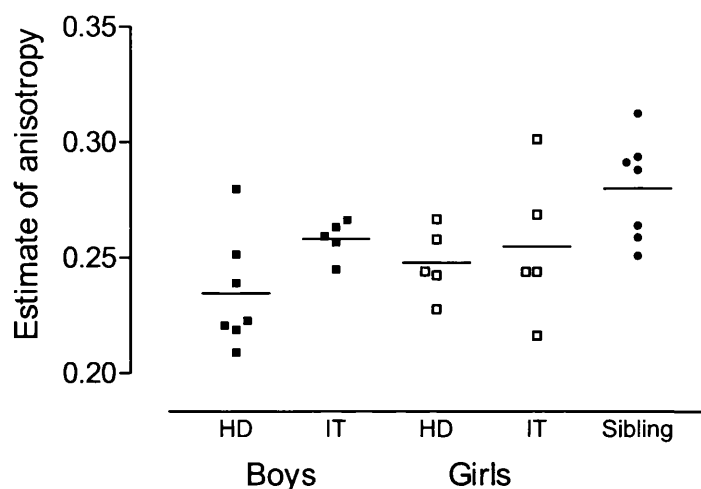


Figure 6-32 Individual anisotropy values for the boys and girls in the right corona radiata**Table 6-14 Mean anisotropy values for boys and girls (survivors only)**

	Boys Mean (SD)	Girls Mean (SD)	t	p value
Thalamus	0.18 (0.02)	0.20 (0.02)	-2.14	0.05
Caudate	0.20 (0.02)	0.20 (0.01)	-0.33	0.74
Left corona radiata	0.20 (0.02)	0.22 (0.02)	-1.76	0.09
Right corona radiata	0.24 (0.02)	0.25 (0.02)	-0.71	0.49

Homogeneity of pathology with respect to age-at-diagnosis

To explore the homogeneity of the pathology identified within survivors, smoothed anisotropy values for the right thalamus, left caudate nucleus, left and right corona radiata were plotted with respect to age-at-diagnosis. As shown in Figures 6.33 – 6.36, there was no clear relationship between anisotropy and age of diagnosis in the thalamus or corona radiata. Results indicated, however, that the change in anisotropy in the caudate nucleus was related to age-at-diagnosis (Table 6.15). While this correlation indicated that the anisotropy decrease was greater for children treated at a younger age than children treated at a later age, there were insufficient data to describe this relationship independently from age-at-test.

Figure 6-33 Age-at-diagnosis vs anisotropy in the right thalamus for all survivors

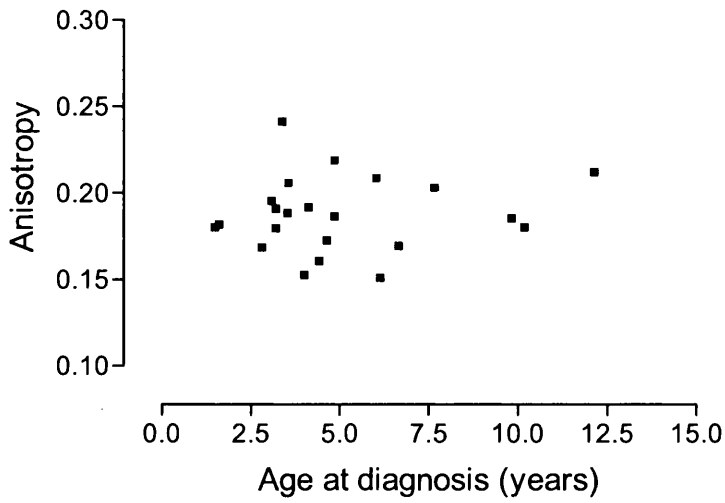


Figure 6-34 Age-at-diagnosis vs anisotropy in the left caudate nucleus for all survivors

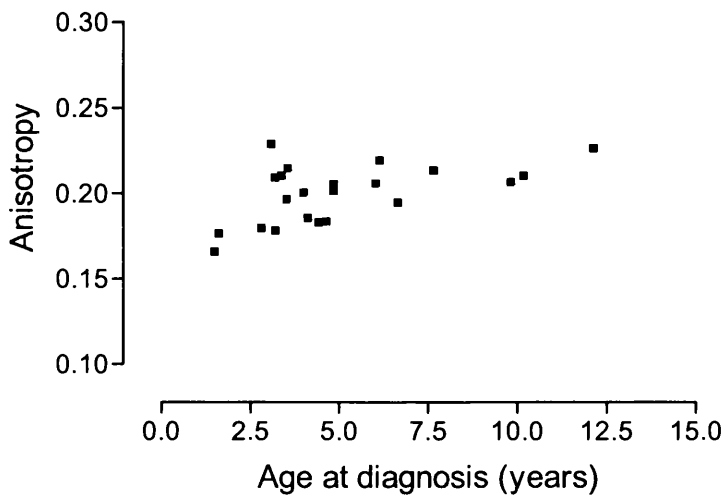


Figure 6-35 Age-at-diagnosis vs anisotropy in the left corona radiata for all survivors

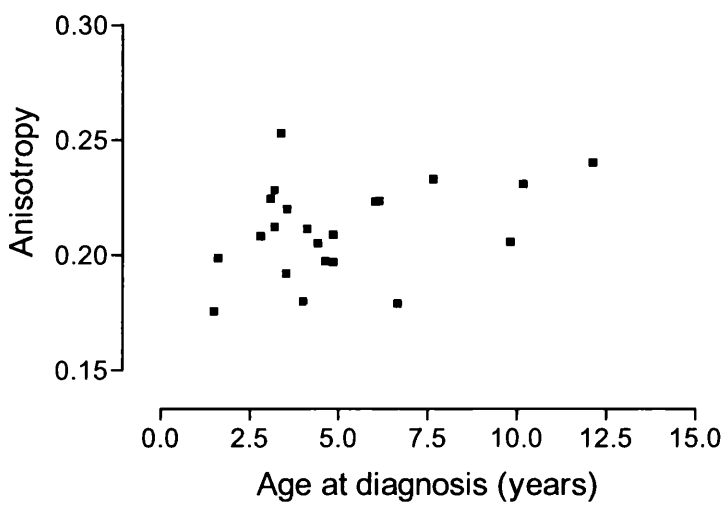
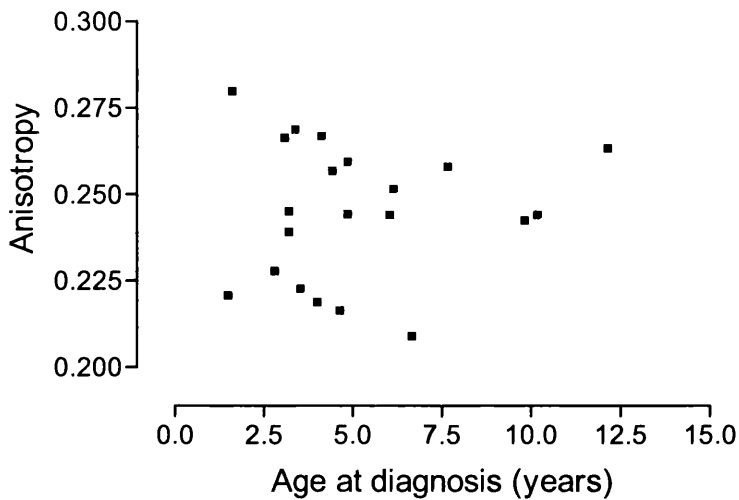


Figure 6-36 Age-at-diagnosis vs anisotropy in the right corona radiata for all survivors**Table 6-15 Relationship between anisotropy and age-at-diagnosis (survivors only)**

	r	p value
Thalamus	0.10	0.64
Caudate	0.54	0.01
Left corona radiata	0.36	0.10
Right corona radiata	-0.10	0.96

6.3.3.5 Functional significance of pathology

To investigate the significance of the changes observed in the thalamus, caudate nucleus, and corona radiata correlations were conducted between individual FA values at each location and indices of cognitive function. In particular, five indices of cognitive function were selected from the neuropsychological investigations detailed in Chapter 3. The indices differentiated survivors from their siblings (i.e. visual discrimination) and IT from HD survivors (i.e. speeded motor response, first reversal, verbal fluency, and extinction). Each measure was also included in the VBM analyses of the 3D FLASH data (see Section 6.2.3). Correlations were conducted separately for the siblings, IT, and HD survivors.

Correlation analyses were unable to establish a relationship between anisotropy in the right thalamus, left caudate nucleus or left and right corona radiata and indices of function in any group.

6.3.4 Discussion

Anisotropy estimates

Of the anatomical regions investigated in the present study, the highest mean anisotropy was observed in the parietal and frontal periventricular white matter, the pons, and the corona radiata. This was followed by mean anisotropy in the thalamus and caudate nucleus. The lowest mean anisotropy was seen in the cerebellum. For survivors and siblings, all individual anisotropy values were greater than zero at each anatomical location. Variation in anisotropy throughout the brain has also been observed in others studies. For example, anisotropy is usually highest in the major central white matter tracts (due to their coherent organisation) and progressively decreases in the tissues closer to the cortex, where fibres cross or fan out (Pierpaoli *et al.* 1996; Tievsky *et al.* 1999; Eriksson *et al.* 2001; Chepuri *et al.* 2002; McGraw *et al.* 2002). Differences in anisotropy also exist within grey matter. For example, both the thalamus and basal ganglia are more anisotropic than cortical grey matter (Shimony *et al.* 1999; Mukherjee *et al.* 2001).

As a consequence of the normal variation in anisotropy throughout the brain, a subtle lesion in an area where anisotropy is naturally low (e.g. grey matter or subcortical U fibres) may not have been detected in our analyses (Werring *et al.* 1999; Rugg-Gunn *et al.* 2001). This implies, therefore, that our results are biased to reflect the most abnormal changes, particularly in areas where anisotropy is naturally low.

Decreases in anisotropy

In the current study, VBM analyses revealed local decreases (rather than increases) in anisotropy between survivors and their siblings. This is consistent with previous studies associating pathology (and presumably a loss of cellular coherence) with a reduction in regional anisotropy. Within the survivors, the decreases in anisotropy ranged from 9.2% in the corona radiata to 24.9% in the thalamus. The degree of anisotropy reduction was similar to that identified in children treated for medulloblastoma where anisotropy was reduced by 12.4% to 19.0% (Khong *et al.* 2003). However, medulloblastoma survivors showed more widespread changes in anisotropy including decreases in the frontal and parietal periventricular white matter, the cerebellum and pons. Decreases in anisotropy were not observed in these areas in the current study. It is likely that this distinction reflects different disease processes and/or treatment protocols since children treated for medulloblastoma underwent tumour resection and craniospinal irradiation. In addition, the results suggest that the anisotropy changes

observed in ALL were not global but include at least the thalamus, caudate nucleus, and corona radiata.

Anisotropy of the thalamus

In the present study, quantitative DTI identified local changes in anisotropy between survivors and their siblings. In particular, survivors displayed a significant 24% decrease in anisotropy within the right dorsomedial nucleus of the thalamus. Additional investigations extended these findings to highlight a non-significant anisotropy decrease (6.5%) in the homologous region of the left thalamus. The anisotropy decrease in the thalamus was not significantly related to the type of treatment received. However, evidence suggested that the reduction in anisotropy of the right thalamus was greater for boys than girls.

Within the literature, gross structural abnormalities of the thalamus are uncommon in the long-term survivors of leukaemia. However, two studies employing other imaging techniques indicate that subtle abnormalities of the thalamus can be identified in this population.

A PET study has revealed a bilateral decrease in regional glucose utilisation of the thalamus in eight LT survivors of ALL (>5 years post treatment) when compared to healthy controls (Kahkonen *et al.* 1999). The decrease was not observed in frontal, parietal, temporal, or occipital cortex, the striatum, or white matter matrix. The survivors received IT + HD MTX as PCNS treatment with five survivors receiving additional CI.

A reduction in glucose uptake of the thalamus has also been reported in 12 survivors (>1 year post treatment) when compared to controls (Phillips *et al.* 1991). In this study survivors received combined therapy (either TIT (N=3) or MTX + CI (N=9)) as PCNS therapy. Similar decreases in glucose utilisation were not identified in other regions of interest throughout the brain.

Despite the majority of survivors receiving combined PCNS therapies, the results of each study suggest the thalamus is particularly vulnerable to insult in the treatment of leukaemia. In addition, the damage persists beyond the treatment period and can be detected in the longer-term. Furthermore, for the majority of patients in each study, visible abnormalities of the thalamus were not evident on clinical T2 weighted images ((6/8 (Kahkonen *et al.* 1999)) and 10/12 (Phillips *et al.* 1991)). Our results were similar with only one survivor having an obvious thalamic abnormality in routine clinical imaging (see Chapter 5). This indicates that the pathological process leading to a

decrease in glucose metabolism and/or anisotropy is subtle in nature and not sufficient to cause gross structural change.

The anisotropy reduction observed in survivors was greater in the right than left thalamus. This resulted in an apparent loss and in some cases reversal of the hemispheric asymmetry (i.e. right greater than left) seen in the siblings. In healthy adults (21-69 years), no significant differences were seen between FA estimates of the left and right thalamus (Abe *et al.* 2002). To our knowledge similar interhemispheric comparisons for FA of the thalamus have not been reported in healthy children.

A previous study has reported symmetrical changes in the thalamus immediately following the administration of HD MTX in children treated for ALL. In this study, while a diffuse and symmetrical decrease (19%) in cerebral glucose metabolism was reported across the whole brain, the second greatest decrease in metabolism was restricted to the thalamus. In that study, the reduction was similar in the right (25%) and left (23%) thalamus. Importantly, none of the children studied (N=8) developed neurological or CT abnormalities (Shishido *et al.* 1986).

The results also suggested that the decrease in anisotropy was greater for boys than girls. The interpretation of this finding, however, requires caution as the normal range of FA values for boys independently of girls was not established in the current study. This occurred as a consequence of the limited number of DTI's successfully collected in the sibling group. Therefore, it may be the case that girls naturally have higher anisotropy than boys in the thalamus. Consistent with this, the X chromosome has been identified as a factor determining the adult size of the thalamus (Murphy *et al.* 1993). In addition, the absence of the intermedia massa is more common in men than women (Nopoulos *et al.* 2001). Sex related factors could therefore affect determinants of anisotropy. To our knowledge sex related discrepancies of FA estimates in the thalamus have not been reported in children. It should be noted, however, anisotropy within the thalamus was not significantly different between healthy men and women (Abe *et al.* 2002).

In the present study, the greatest decrease in anisotropy was localised to the dorsomedial (DM) nuclei. The DM thalamus has extensive connections with the prefrontal cortex (Goldman-Rakic & Porrino 1985; Fuster 1989). However, this finding needs to be interpreted with caution since the use of VBM techniques with DTI data requires image smoothing. For example, in the present analysis the resolution of our images has been reduced to 4mm isotropically as a consequence of smoothing. In humans, the thalamus is approximately 30 (rostrocaudal) X 15 (width) x 15 (height) mm

(Barr 1974) and the DM nucleus is one of at least 14 different nuclei (Morel *et al.* 1997; Scannell *et al.* 1999). In the present analysis with 4mm smoothing, the thalamus has therefore been described in approximately 63 (7 x 3 x 3) voxels and the DM nucleus in relatively few voxels. Furthermore, the boundaries between nuclei are not well defined and therefore edge voxels are contaminated with other thalamic structures. As a consequence it is difficult to assess specific effects for the DM nucleus in relation to neighbouring thalamic nuclei and structures (e.g. the midline nuclei and internal lamina medialis (Herrero *et al.* 2002). Clarification of the thalamic nuclei involved in the LT survivors of ALL requires further investigation using alternative DTI methods. For example, a recent report has identified thalamic nuclei based on the orientation of corticothalamic/thalamocortical striations within each nucleus in healthy controls (Wiegell *et al.* 2003).

With this in mind, however, lesions of the DM nuclei have been associated with affective disturbances, and impairments of memory, and executive functions. More specifically, lesions of the thalamus that extend anteriorly to encompass the anterior nuclei and/or mammillo-thalamic tract (MTT) can result in the amnesic syndrome (i.e. impaired encoding and retrieval). Lesions sparing the MTT including lesions of the DM nucleus can result in other memory problems (for example impaired free recall with relatively intact recognition)(Ogawa *et al.* 1999). However, non-amnesic type memory problems are not always reported following discrete lesions of the DM nucleus and this may be related to lesions not involving a critical amount of DM (Kritchevsky *et al.* 1987). Furthermore, the problems may in part be related to poor attention and/or search strategies. EF problems have been reported following lesions involving the DM nucleus. These include symptoms consistent with the dysexecutive syndrome (Van der Werf *et al.* 2000), for example disturbances of executive abilities, poor attention, initiation, and temporal organisation of behaviours. However, it is not clear if discrete lesions of the DM nucleus are sufficient to produce these problems. It has been suggested that combined lesions of the DM and internal lamina medialis or midline nuclei contribute to these impairments (Van der Werf *et al.* 2000). Lesions of the DM nucleus have also been associated with disorders of affect. For example, bilateral lesions of the DM nuclei can result in apathy, indifference, and poor motivation (Engelborghs *et al.* 2000).

Difficulties with executive functions and/or memory are consistent with behavioural patterns observed in long-term survivors (see Section 1.1.5). In addition, changes in glucose metabolism within the thalamus have been associated with WISC-R/WAIS-R subtest scores in survivors of ALL (Phillips *et al.* 1991). In a similar study, thalamic

glucose metabolism was not, however, related to WISC-R index scores or measures of visual-motor integration, fine motor skills, memory, and executive function (Trail Making B) in LT survivors (Kahkonen *et al.* 1999). Lack of emotion, depression, and withdrawal are also reported in LT survivors of ALL (see Section 1.1.5).

Anisotropy of the caudate nucleus

Results of the current study also indicated that smaller bilateral anisotropy decreases in the head of the caudate nucleus (i.e. left caudate = 12.1%; right caudate = 9%). The anisotropy reduction in each area approached corrected significance when comparing all survivors and their siblings. Generally, the changes were not related to treatment type received. Results indicated that the decrease in anisotropy observed in the left caudate nucleus was greater for children treated at a younger age than children treated at an older age. However, this result is difficult to interpret, as the relationship could not be separated from age-at-test.

Moderate structural changes involving the caudate nucleus have been reported in the literature for LT survivors of leukaemia. For example, volumetric measurements of the head of the caudate nucleus were lower (but not significantly) in long-term survivors receiving TIT therapy (> 3 years post-diagnosis) and age-, sex- matched controls (Ciesielski *et al.* 1999). In addition, perfusion deficits have been identified in the striatum of three children treated with MTX (Harila-Saari *et al.* 1997) but similar changes were not seen in other studies (Phillips *et al.* 1991; Kahkonen *et al.* 1999; Kahkonen *et al.* 2000).

Discrete unilateral or bilateral lesions of the caudate nucleus can result in abulia (i.e. apathy, reduced initiative and spontaneity of thought)(Mendez *et al.* 1989), and in rare cases disinhibition and obsessive-compulsive behaviour (Bhatia & Marsden 1994; Fitzgerald *et al.* 2000). Pathology of the caudate nucleus can also be associated with poor attention (Howes & Boller 1975). However, lesions are unlikely to result in motor impairment unless they extend to adjacent areas (e.g. lentiform nucleus and/or internal capsule)(Bhatia & Marsden 1994). In addition, experimental studies highlight the role of the caudate in new learning. In particular, lesions including the tail of the caudate nucleus impair simple discrimination learning in monkeys. Similarly, lesions of the ventral caudate have also been shown to impair visual discrimination reversal (Divac *et al.* 1967).

Disorders of attention and affect are consistent with the problems reported in the LT survivors of ALL (see Section 1.1.5). In addition, the impairment in discrimination learning observed in the survivor groups when compared to the sibling group is

consistent with pathology of the caudate nucleus. Moreover, poor verbal fluency in the HD group may also be related to pathology of the caudate nucleus (see Chapter 3).

Anisotropy of the corona radiata

Results of the current study highlighted a smaller bilateral anisotropy decrease in the corona radiata (i.e. left caudate = 12.1%; right caudate = 9%; left corona radiata = 9.2%; right corona radiata = 11.6%). The anisotropy reduction in each area approached significance after small volume correction (rather than whole brain correction) when comparing all survivors and their siblings.

Specific white matter changes of the corona radiata have been not previously been identified in the LT survivors of ALL. However, deep white matter changes (Iuvone *et al.* 2002) and diffuse white matter changes (Laitt *et al.* 1995) following combined MTX + CI have been reported. White matter changes in the corona radiata and centrum semiovale have also been noted in the short term after the administration of MTX. These may be asymptomatic (Asato *et al.* 1992) or associated with encephalopathy (Colosimo *et al.* 1994). Generally these abnormalities resolve with time and are not detected with conventional methods in the longer term (Asato *et al.* 1992).

In the current study, anisotropy of the corona radiata showed hemispheric asymmetry with right hemisphere values greater than left. While asymmetry has not been specifically reported to our knowledge for the corona radiata a similar right greater than left anisotropy asymmetry has previously been reported for frontal white matter in children and adults (Klingberg *et al.* 1999). In addition, estimates of anisotropy based on three orthogonal directions show greater alignment in the right than left anterior limb of the internal capsule (subjects aged 18-44 years)(Peled *et al.* 1998).

The relative degree of asymmetry in this study, however, cannot be evaluated since the voxels chosen from the left and right corona radiata were not homologous. In particular, voxels of the corona radiata were selected based on the VBM analyses and the location of the left voxel was in fact more posterior than the right. As white matter of the corona radiata is not homogenous with respect to direction (i.e. fanning out and/or crossing) it is likely that anisotropy throughout this area is variable. For example, a voxel sampling the intersection of radiations of the corona radiata may even appear isotropic (Pierpaoli *et al.* 1996).

The reduction in anisotropy in the left corona radiata was also more apparent in children treated with HD MTX. This result is contrary to our prediction based on the

CSF study (see Section 1.5). The HD group did not however significantly differ from the IT survivors at this location.

Lesions of the corona radiata affect descending corticospinal and corticobulbar systems. They can result in loss of fine motor movement, weakness or paralysis of distal motor movements (e.g. Babinski signs or spasticity (i.e. increased muscle tone and exaggerated deep tendon reflexes). In adults, lesions of the corona radiata do not always lead to predictable patterns of impairment due to the diffuse distribution of descending pathways (Misra & Kalita 1997). Developmental studies indicate that motor skills dependent upon the corticospinal system (e.g fine finger movements) undergo a relatively protracted rate of refinement over childhood (Lawrence & Hopkins 1976)(Forssberg *et al.* 1991). This indicates these systems may be particularly vulnerable following damage of the corona radiata. Poor fine manual movements including handwriting have been described in the LT survivors of ALL (see Section 1.1.5). In addition, deep white matter pathology has been correlated with visual-motor integration in LT survivors treated with combined MTX + CI (Iuvone *et al.* 2002)

Anisotropy and indices of behaviour

In the present study, the functional significance of the changes observed in the thalamus, caudate, and corona radiata could not be determined. Several other studies have found relationships between regional FA and measures of cognitive function (de Groot *et al.* 2000; Klingberg *et al.* 2000; Sullivan *et al.* 2001; Schmithorst *et al.* 2002). For example, a significant correlation was established between FA and IQ bilaterally in the anterior portion of the inferior longitudinal fasciculi in children aged 5-18 years (Schmithorst *et al.* 2002). A similar association has also been identified in adults between reading, nonverbal IQ, and white matter FA of the temporo-parietal region (below the perisylvian region)(Klingberg *et al.* 2000).

However, not all studies have found correlations between FA and indices of function (O'Sullivan *et al.* 2001; Rovaris *et al.* 2002; Yoshiura *et al.* 2002). For example, an association between verbal fluency (semantic fluency) scores and average lesion FA was not established in patients with relapsing remitting multiple sclerosis (Rovaris *et al.* 2002). Scores on another test of executive function (trail making) were also not associated with white matter FA in elderly volunteers (O'Sullivan *et al.* 2001).

In the present study, the lack of a significant correlation should therefore not necessarily be interpreted as evidence against links between the pathology detected and cognitive impairments identified in Chapter 3. A linear correlation may not have been established in the present study for a number of reasons, for example as a

consequence of insufficient variation in the cognitive scores and/or anisotropy estimates. Furthermore, the relationship between pathology and indices of function may also be non-linear.

6.3.5 Summary

VBM analyses of the DTI data, identified common differences in anisotropy within the survivor groups when compared to the sibling group. In particular, there were significant decreases in anisotropy of the right thalamus and decreases approaching significance in the left caudate and right corona radiata. There was also evidence to suggest that these changes may have been bilateral. This was supported in the HD group, where there was evidence of a decrease in anisotropy approaching significance of the left corona radiata. However, the bilateral nature of the pathology in the remaining areas requires clarification.

Further investigations indicated that the changes in anisotropy observed within the survivor groups were not global, apparently sparing the frontal and parietal periventricular white matter, the cerebellum, and the pons. In addition, the data suggested that the decrease in anisotropy of the thalamus was greater in boys than girls, and, the decrease in anisotropy identified in the caudate nucleus may have been related to age-at-diagnosis.

6.4 General summary

Previous imaging studies have relied on conventional methods to identify pathology in the LT survivors of ALL. Using these methods the small number of studies attempting to quantify pathology have employed grading scales (e.g. mild, moderate and severe)(Gangji *et al.* 1980; Wilson *et al.* 1991; Asato *et al.* 1992; Mahoney *et al.* 1998). However, there are several limitations of this approach. For example, subtle changes are unlikely to be detected, the degree and extent of abnormalities cannot be assessed, and subjective scales do not easily lend themselves to inter-institutional comparison (Reddick *et al.* 2002). Furthermore, in many instances the studies have focussed on predetermined regions of interest (e.g. white matter).

VBM can now provide an objective way of identifying brain abnormalities that may be too subtle to detect on visual inspection of MR images. While a region of interest can be accommodated with this approach, it is not essential thereby ensuring assessment of the whole brain with uniform sensitivity.

In the present study, VBM analyses revealed significant differences in both groups of survivors. In particular, a significant common decrease in anisotropy was observed in the right thalamus of the survivor groups when compared to the sibling group. Further investigations demonstrated moderate common decreases in anisotropy within the left thalamus, right corona radiata and bilaterally in the caudate nucleus within the survivor groups. While VBM analyses were unable to identify significant differences between survivor groups treated with alternative PCNS therapies, results indicated that children randomised to receive HD MTX displayed an additional decrease in anisotropy within the left corona radiata. In contrast, children randomised to receive IT MTX demonstrated bilateral increases of white matter density in the internal capsule and corpus callosum.

Importantly, the VBM analyses revealed changes common to survivors that were not identified using conventional imaging techniques (see Chapter 5) thereby highlighting the advantage of this automated technique to identify common pathology in this population. Furthermore, VBM analyses of 3D FLASH and DTI data sets were sensitive to different pathologies. For example, VBM analyses of the 3D FLASH data revealed group changes in compact white matter structures (e.g. the internal capsule and corpus callosum) whereas the DTI analyses demonstrated group changes in the subcortical nuclei (e.g. the thalamus and caudate nucleus) and non-compact white matter (e.g. the corona radiata).

Part 4: Discussion

7 General Discussion

This chapter begins with a review of the thesis objectives. The results of the neuropsychological and neuroimaging investigations are then summarised. This is followed by an evaluation of the model of pathology proposed to account for the LT problems identified in this population. Finally, directions for future studies are highlighted.

7.1 Review of the thesis objectives

The recent introduction of PCNS therapy for the prevention of CNS relapse in children diagnosed with ALL has significantly improved the chances of LT survival. However, PCNS therapies are neurotoxic and have occasionally been associated with clinical neurotoxic syndromes. More commonly, LT survivors suffer subtle cognitive and behavioural impairments, and these problems may also be related to PCNS therapy. Nevertheless, the exact nature of the deficits and underlying pathology giving rise to the impairments has not been identified.

The aim of this thesis was to describe the LT outcome of the average child treated with current chemotherapy-based PCNS therapies for standard-risk ALL. In particular, an attempt was made to investigate the role of PCNS therapy in determining the LT outcome. In addition, the study attempted to relate the cognitive outcome to underlying brain pathology.

A model of pathology (see Chapter 1) was hypothesised based on the biochemical actions of the main component of current PCNS treatments, methotrexate (MTX). This model proposed that the administration of MTX not only prevents CNS relapse, but also causes the accumulation of oxidised folates, thereby inhibiting the activity of the enzyme methylene tetrahydrofolate reductase (MTHFR). This secondary action can be associated with subclinical demyelination, where the degree of demyelination is related to the type of PCNS treatment administered (i.e. IT worse than HD). In addition, the period of subclinical demyelination sustained during treatment may be responsible for some of the impairments described in the LT the survivors of ALL. Furthermore, the extent of demyelination may be related to the degree of impairments observed.

The model was extended further to suggest that brain areas developing at the highest rate at the time of insult would show the greatest impact of MTX treatment. For children treated with MTX in early to late childhood (a period associated with a high diagnosis of ALL) these areas would include the frontal lobes and the corpus callosum.

The model was consistent with experimental studies of MTX, and CSF and neuroimaging studies conducted in children during treatment for ALL. Support for a developmental hypothesis was also consistent with at least two observations in the LT ALL literature. Firstly, the cognitive impairments observed in the survivors of ALL emerge with time. Secondly, reports suggest that the deficits are greatest in children treated at a younger rather than older age.

In order to test the model, 32 LT survivors (> 6 years post-diagnosis) and 14 siblings participated in the current research. All survivors had been randomised to receive either (1) HD MTX + IT MTX + FAR, or (2) IT MTX-only as PCNS therapy. In addition, all survivors received treatment without developing complications (i.e. neurotoxic syndromes or relapse).

Participants completed a battery of tailored neuropsychological tests to assess the integrity of the frontal lobes and corpus callosum. In addition, a combination of conventional and modern MRI techniques was employed to assess brain structure in these children. Finally, attempts were made to relate functional changes to the pathology identified.

7.2 Review of the findings

7.2.1 Neuropsychological results

Chapters 2-4 describe detailed neuropsychological investigations that were conducted in the LT survivors and their siblings. These included an assessment of general functions over the first five years post-diagnosis, and a detailed LT evaluation of functions believed to be associated with the integrity of the frontal lobes and corpus callosum. The results of the current investigations indicated that there were few significant differences between groups on the battery of neuropsychological tests administered (see Table 7.1).

Table 7-1 Summary of neuropsychological findings

Diagnosis	3 years post-diagnosis	5 years post-diagnosis	> 6 years post-diagnosis
Survivors < Siblings			
p<0.05	Verbal delayed recall (p=0.03)		6 elements organisation (p=0.003) Visual discrimination learning (p=0.05)
p<0.10			
HD < IT			
p<0.05			Verbal fluency (p=0.01) Speeded motor response (p=0.02)
IT < HD			
p<0.05			First reversal (p=0.04)
p<0.09			Extinction (p=0.06)

7.2.1.1 Survivor group vs sibling group

At diagnosis there were no significant differences between groups on estimates of intelligence, language, or fine motor function. Over the first 5 years post-diagnosis, a transient change in verbal memory was identified in the survivor groups when compared to the sibling group. However, at five years post-diagnosis this change had apparently resolved. In addition, at five years post-diagnosis, there were no significant differences between survivor and sibling groups on estimates of intelligence, memory and verbal learning, language, visual perception, academic achievement, or fine motor function.

Assessment of executive functions and bimanual coordination at the final follow-up suggested that the performance of the survivor groups was similar to the performance of the sibling group. In particular, the survivor groups showed only subtle differences in organisation and visual discrimination learning when compared to the sibling group.

7.2.1.2 HD group vs IT group

At the longest follow-up, there was also evidence of subtle differences between the survivor groups treated with alternative PCNS therapies. Specifically, the HD group were impaired on the tests of verbal fluency and speeded motor response when compared to the IT group. In contrast, the IT group showed poor stimulus-reward learning (i.e. reversal and extinction) when compared to the HD group.

Despite these mild differences, poor performance in the survivor groups was unlikely to be related to pathology of the frontal lobes or corpus callosum.

7.2.2 Neuroimaging results

Using conventional neuroradiological assessment (Chapter 5), the number of children with abnormal scans was not significantly different between the survivor and sibling groups. On a qualitative level, however, the changes when identified in the survivor group occurred in white matter. Changes in white matter were not identified in any of the siblings. This result suggested that white matter was sensitive to the diagnosis and/or treatment of ALL. Despite this, common, location-specific pathology was not established within the survivor groups.

VBM analyses were then conducted to assess brain integrity on a more subtle level and to allow inter-group, quantitative comparisons (Chapter 6). This included the VBM analysis of 3D T1-weighted datasets and of fractional anisotropy maps generated from diffusion tensor images. The results of these investigations indicated that there were subtle differences between the survivor and siblings groups in terms of brain structure (Table 7.2). Importantly, no significant differences were observed between the HD and IT survivor groups. In addition, common discrete pathology of the frontal lobes was not observed in any group.

Table 7-2 Summary of the neuroradiological findings

	Increases in white matter density	Decreases in anisotropy
Survivors < Siblings		
p<0.05		Thalamus (R) (p=0.004) Corona radiata (R) (p=0.05) (SVC)
p<0.10		Caudate (L) (p=0.09)
HD < Siblings		
p<0.05		Corona radiata (L) (p=0.05) (SVC)
IT < Siblings		
p<0.05	Internal capsule (Bilat)(p=0.04)	
p<0.10	Corpus callosum (Bilat)(p=0.09)	

(R) = right hemisphere; (L) = left hemisphere, Bilat = bilateral; SVC = small volume correction.

7.2.2.1 Survivor group vs sibling group

A significant decrease in diffusion anisotropy was observed in the right thalamus when comparing the survivor group to the sibling group. In addition, there were decreases approaching significance seen in the left caudate nucleus and the right corona radiata.

7.2.2.2 HD group vs sibling group

The HD group also showed an anisotropy decrease approaching corrected significance in the left corona radiata when compared to their sibling group.

7.2.2.3 IT group vs sibling group

A significant bilateral increase in white matter density was identified in the internal capsule in the IT group when compared to their sibling group. In addition, a bilateral increase in white matter density was seen in the corpus callosum that approached corrected significance.

7.2.3 Relationship between neuropsychological and neuroimaging findings

The results of the current study indicated that the LT outcome of children treated for standard-risk ALL with current MTX-based PCNS therapies (i.e. no cranial irradiation) was complicated by minimal cognitive and subtle neuroradiological abnormalities.

In particular, the LT outcome (irrespective of the type of PCNS therapy administered) was associated with mild impairments in visual discrimination learning and organisation. In addition, the LT survivor groups showed common, subtle pathology of the thalamus (RH), caudate nucleus (LH), and corona radiata (RH) when compared to the sibling group.

Additional subtle problems were seen in the survivor groups that may have been related to the type of PCNS therapy received. For example, additional LT problems with stimulus-reward learning were identified in the IT group. These problems were accompanied by subtle bilateral white matter changes of the internal capsule and corpus callosum. In contrast, the HD group showed additional impairments in tasks of speeded motor response and verbal fluency. These impairments were accompanied by additional subtle pathology of the corona radiata (LH).

Significant relationships between the mild impairments and pathology identified were not established. In addition, there was little evidence to suggest that pathology of the frontal lobes and/or corpus callosum was responsible for the impairments observed.

7.3 Can the proposed model account for the findings?

The proposed model highlighted the vulnerability of white matter to MTX-based PCNS therapy. Based on CSF studies and reports of children recovering from ADEM, the model also predicted that the level of demyelination during treatment would be related to the extent of LT pathology and severity of impairments observed (i.e. IT worse than HD). In addition, the inhibition of MTFHR activity was the mechanism resulting in this pathology. Finally, the model predicted areas developing at the highest rate at the time of insult would be most vulnerable to the effects of MTX therapy. In childhood, these areas included the frontal lobes and corpus callosum.

7.3.1 Unique differences identified between the survivor groups (HD vs IT group)

7.3.1.1 White matter pathology

While the imaging analyses failed to reveal significant changes in brain structure between the HD and IT groups, separate analyses indicated there were subtle differences between each of the survivor groups when compared to their sibling group. In particular, the internal capsule and corpus callosum were vulnerable within the IT group. The corona radiata were vulnerable in the HD group (see Chapter 6). In addition, visual inspection of the clinical scans (see Chapter 5) suggested that the different types of white matter abnormalities were associated with the type of treatments (e.g. the IT group showed bilateral diffuse loss of white matter; the HD group showed old white matter injuries (e.g. gliotic scars)).

Together, these findings indicate that the LT neuroradiological features of ALL may have been dependent upon the type of PCNS therapy received. In addition, the results suggested that white matter was sensitive to the type of PCNS therapy received. Specifically, the IT group showed more LT changes in white matter (i.e. including internal capsule and corpus callosum) than the HD group. Furthermore, the changes observed in the IT group were identified on a structural level (i.e. increases in white matter density), whereas the changes in the HD group occurred on a more subtle level (i.e. decreases in diffusion anisotropy in the corona radiata).

This aspect of the results provides modest supports the proposed model, where white matter is especially vulnerable to MTX-based PCNS treatment, and the IT group has more extensive LT pathology.

7.3.1.2 Neuropsychological impairments

The neuropsychological results provided weak support for distinct cognitive profiles in the HD and the IT groups. However, despite more extensive white matter pathology in the IT group, there was no clear evidence that the general level of neuropsychological impairment was greater in the IT group than the HD group (e.g. there was no significant difference between survivor groups on tests of intelligence, attention, language, memory, visual perception, fine motor function, attainments, or behaviour ratings)

7.3.1.3 The profile of abnormalities and MTHFR deficiency

The areas differentially affected in the survivor groups included the internal capsule, corpus callosum, and corona radiata. This profile of abnormalities is consistent with the areas of pathology observed in MTHFR deficiency (see Section 1.4.2). MTHFR deficiency presenting in childhood is characterised by demyelination of subcortical and central cerebral white matter, the corpus callosum, fornix, optic nerves and tracts, and the spinal cord. These findings suggest that the inhibition of MTHFR by MTX is one possible mechanism for the differential pathology identified in each survivor group.

The results do not exclude, however, alternative mechanisms of pathology by MTX, for example the indirect effect of MTX on neurotransmitter systems (e.g. via the inhibition of dihydropteridine reductase (DHPR) and the recycling of biopterins) and the effects on the vascular system (e.g. promotion of vascular disease as a consequence of the accumulation of homocysteine)(Quinn & Kamen 1996). It would be advantageous to compare the current survivor groups to a group of children with childhood onset, well controlled MTHFR deficiency to address this question further.

7.3.1.4 Developmental aspects of the model

The model tested in the current thesis also predicted that brain areas developing at the highest rate at the time of insult would show the greatest impact of MTX treatment. For children treated with MTX in early to late childhood (a period associated with a high diagnosis of ALL) these areas would include the frontal lobes and the corpus callosum.

However, the neuropsychological investigations revealed little support for the selective vulnerability of frontal lobes or corpus callosum in these children. Similarly, the neuroimaging investigations failed to reveal common, discrete pathology of the frontal lobes. The analyses did, however, reveal evidence of an increase in white matter density of the posterior corpus callosum in the IT group.

Preferential sites of injury

The difference between the profile of abnormalities identified and the pathology predicted may reflect the irregular distribution of MTX throughout the CNS. For example, following lumbar injection in monkeys (*macaca fascicularis*) MTX levels were greater in the cerebellum and brain stem than the cerebral hemispheres (Yen *et al.* 1978).

In addition, while HD MTX infusion in rats produced a global reduction in cerebral glucose metabolism that was reversed following the administration of high dose leucovorin (FAR), regionally specific changes were observed (Phillips *et al.* 1989). In particular, regional decreases in metabolism were seen in auditory cortex, inferior colliculus, internal capsule, cerebellar white matter, and posterior lateral thalamic nucleus that were greater than the global changes. Furthermore, the reductions in the internal capsule, auditory cortex, and inferior colliculus were closely related to MTX dose effect (i.e. balance between MTX dose + leucovorin dose given).

These findings suggest that the administration of MTX and/or the combination with FAR may preferentially affect brain areas. Thus the frontal lobes and/or corpus callosum may be less affected relative to other brain areas.

Age at diagnosis

The current results also show modest support for a relationship between age-at-diagnosis and LT outcome. In particular, a significant correlation was revealed between age-at-diagnosis and the decrease in anisotropy in the caudate nucleus in the survivor groups. In addition, a relationship between age-at-diagnosis and performance on the test of speeded motor response task was seen in the HD group. However, in the current study each relationship was confounded by the effects of age-at-test. The relationship between age-at-test and each of these variables, therefore, requires further investigation.

The use of normed neuropsychological tests spanning the entire age range (7- 21 years) would help in this endeavour. Furthermore, 3DFLASH and DTI data from children developing normally (birth – 18 years) would allow the effects of age on white/grey matter density and anisotropy throughout childhood to be modelled in the analyses. Alternatively, selection of a sample of children with a smaller age range at test would help to minimise the effects of normal development on cognition and brain structure.

The inconsistent relationship between age-at-diagnosis and LT outcome is incompatible with a developmental hypothesis. However, age-at-diagnosis may become an important factor in determining cognitive function in adult life. For example, pathology sustained in childhood may limit the rate and level of development of function (Dennis 1989). Follow-up assessment of these children as adults would help to quantify the relationship between age-at-diagnosis and LT outcome.

7.3.2 Common differences in the survivor groups (Survivor vs sibling group)

The current investigations also revealed significant common differences between the LT survivor groups, and the sibling group. These results indicate that the diagnosis and treatment of ALL in childhood is associated with LT pathology irrespective of the type of PCNS therapy received.

Common differences in the survivor groups can be interpreted, within the proposed model, if a MTX threshold hypothesis is considered. For example, the common pathology observed between survivor groups may be related to the administration of MTX irrespective of the dose.

The relationship between total MTX dose and the presence of gross white matter pathology is unclear within the literature. For example, visible white matter changes were detected on MRI in 3/7 children diagnosed with standard-risk ALL and treated with HD MTX ($8 \times 1-5 \text{ g/m}^2$) + IT MTX ($13 \times 12 \text{ g/m}^2$) + FAR. In contrast, visible white matter changes were not seen in children (N=7) diagnosed with intermediate-risk ALL and treated with higher dose HD MTX ($9 \times 5 \text{ g/m}^2$) + IT MTX ($13 \times 12 \text{ g/m}^2$) + FAR (Paakko *et al.* 2000).

However, the common differences may also be related to a number of other factors and/or their combination common to all survivors participating in the current study. These factors include: (1) the disease process; (2) the administration of any type of PCNS therapy, (3) the effects of other components of treatment, or (4) a genetic component common to ALL and brain development.

7.3.2.1 The disease process

Similarities between the survivor groups may be related to the disease process. For example, the leukocyte count at diagnosis (in children with high-, medium-, or low-risk ALL and no evidence of CNS disease) has been related to glucose metabolism in the striatum and the cortex in LT survivors of ALL treated with HD MTX (Kahkonen *et al.* 2000). In addition, the leukocyte count at diagnosis was inversely correlated with LT fine motor function (Purdue peg board). Other factors, for example, amount of MTX received or age-at-diagnosis were unable to account for this association. As the authors highlight, while these findings require replication in a larger sample and do not account for factors such as leukocyte characteristics (e.g. molecular genetics), the results nevertheless suggest the severity of the disease at diagnosis may have some effect on LT outcome.

Furthermore, while the children in our study had no evidence of CNS involvement at diagnosis (i.e. no blast cells detected in CSF) the breakdown of the blast cells may initiate an immune response within the CNS. For example, the breakdown of blast cells releases the inflammatory cytokines including tumour necrosis factor- α and interleukin- 1β (Kurzrock *et al.* 1993). It is important to note, however, MRI abnormalities identified during the treatment period in ALL do not commonly show contrast enhancement indicating a CNS immune response is unlikely (Asato *et al.* 1992).

7.3.2.2 The administration of any type of PCNS therapy

The profile of common abnormalities identified in the LT survivors included the thalamus, caudate nucleus, and corona radiata. Additional investigations suggested that the profile was regionally specific apparently sparing, for example, the pons, cerebellum and periventricular white matter. It is possible that this profile of abnormalities was related to the use of any PCNS therapy.

The profile of abnormalities identified in the present study differed from the pathology associated with the delayed neurotoxic syndromes (e.g. mineralising microangiopathy or progressive leukoencephalopathy) occasionally seen in the LT survivors of ALL and associated with CNS directed therapies (see Appendix A). For example, mineralising microangiopathy most often involves calcification of the lentiform nucleus, dentate nuclei of the cerebellum, and cerebral cortex (Flament-Durand *et al.* 1975). In this syndrome the thalamus, caudate nucleus, and internal capsule are spared (Price & Birdwell 1978). Similarly, pathology in progressive leukoencephalopathy is most commonly observed in the periventricular white matter (specifically around the frontal and occipital horns) and centrum semiovale, areas apparently not involved in the present study. These findings indicate that the common pathology seen in the survivor group was not related to a delayed clinical neurotoxic syndrome associated with CNS-directed therapy.

7.3.2.3 The effects of other components of treatment

The additional components of treatment unchanged within the therapy protocols may also be responsible for the common differences. For example, other components of treatment are associated with their own neurotoxicities, for example, vincristine (i.e. neuropathy) and L-asparaginase (i.e. intracranial haemorrhage)(Keime-Guibert *et al.* 1998; Bargallo *et al.* 2000). In addition, steroids may be associated with their own effects. For example, prolonged glucocorticoid exposure reduces hippocampal neuron number in rats (Sapolsky 1985). Similarly, the administration of steroids can be related

to poor memory (Waber *et al.* 2000). For example, memory deficits have been documented in children treated with prednisone for asthma (Bender *et al.* 1988; Bender *et al.* 1991).

The combination of steroids and MTX may also aggravate neurotoxic effects (Osterlundh *et al.* 1999). For example, in the rat the combination of MTX and prednisolone was associated with more effects on behaviour than any either drug alone (Mullenix *et al.* 1994).

7.3.2.4 A genetic component common to ALL and brain development.

Finally, current theories suggest a genetic predisposing cause leading to the development of ALL in children. It is possible that the same genetic factors may also alter brain development such that the degree and/or extent of pathology revealed in the current study reflects abnormal brain development prior to treatment. While an attempt has been made in the current study to control for genetic components of brain development by comparing ALL survivors to their siblings, this question cannot be addressed without pre-diagnostic scans.

7.3.3 Summary

Support for the model tested in this thesis was mixed. In particular, there was evidence indicating that white matter was vulnerable to LT changes and that the level of white matter involved may have been related to the type of PCNS therapy received. In addition, analyses indicated that the extent of white matter pathology was greater in the IT group than the HD group. However, there was no relationship between the extent of pathology and the general level of cognitive impairment. Furthermore, while the inhibition of MTHFR activity was a possible explanation of the pathology, alternative mechanisms could not be excluded. Moreover, there was limited support for the specific vulnerability of the frontal lobes and corpus callosum. In addition, the unclear relationship between age-at-diagnosis and the results did not provide support for a developmental hypothesis. Finally, changes common to both survivor groups were identified. These changes could not clearly be unaccounted for by the model proposed.

7.4 Directions for future research

Based on the findings of the current study, there are a number of recommendations for future research.

Firstly there was a degree of heterogeneity in the study participants. This was highlighted in the neuropsychological assessments conducted over the first five years post-diagnosis in the survivors. In particular, there was evidence to suggest that changes in verbal IQ over the first five years post-diagnosis were clinically significant (i.e. a decrease of at least 15 IQ points) in girls but not boys. Similarly, declines in PIQ over this period were greater in children with higher estimates of PIQ at diagnosis. In addition, the clinical radiological scans suggested heterogeneity in the location and extent of gross pathology within the survivor groups. These findings suggest a level of inter-individual vulnerability to treatment effects in ALL. Consistent with this, individual differences have been observed in disorders of folate metabolism and individual variation in MTX neurotoxicity seen in children treated for ALL (Lampkin *et al.* 1967; Liu *et al.* 1978; Chiusolo *et al.* 2002; Taub *et al.* 2002). It would be advantageous therefore to explore the universality of the pattern of pathology identified in future studies.

The current study was also unable to describe a significant relationship between cognitive function and the pathology identified. Functional imaging studies of this population may help to identify the neural correlates of cognition in this population. In addition, the use of advanced fibre tracking techniques would permit detailed investigations of the thalamic, caudate, and cortical connections.

In addition, the impact of the abnormalities identified on subsequent brain development was not evaluated in the current study. For example, due the extensive connections between thalamus, caudate, and frontal lobes (Alexander *et al.* 1986; Wise *et al.* 1996), abnormalities identified in the thalamus and caudate may lead to subtle changes in the frontal lobes during a later stage of development. Alternatively, given time the change may resolve. As neuropathological studies are unlikely within a growing population of survivors that are treated without complications, these issues may be addressed with *in vivo* longitudinal MRI studies beginning at diagnosis.

Furthermore, the current study has not controlled for the effect of emotional and psychosocial factors on neuropsychological function (e.g. experience of life threatening illness)(Hill *et al.* 1998). For example, stress during development has been associated in monkey with a decrease in cortical dendrite branching (Chugani *et al.* 2001). Therefore, it would be advantageous to compare the survivor group under study to children treated for other cancers, without PCNS therapy. These comparisons would help to tease apart the psychosocial impact and the direct effects of PCNS treatment on LT outcome.

Also, the current investigations do not account for the amount of schooling missed by the survivors. Studies of absence from school indicate that children miss around 75 days of school in the first year post diagnosis (42% of the school year). After the first year and until the third years they continued to miss an average of 20 days of school (Stehbens *et al.* 1983; Lansky *et al.* 1984). Experience is known to change brain development. For example, rats placed in complex environments instead of standard laboratory cages show large changes in dendritic length and synapse number (primary visual and somatosensory cortex)(Greenough *et al.* 1985; Beaulieu & Colonnier 1989). Therefore, in future studies it would be useful to account for the possible effects of school missed. However, it should also be noted that absenteeism did not significantly correlate with academic measures in children treated with MTX + CI (Taylor *et al.* 1987). In addition, the effects of missed schooling would be expected to result in underachievement in all subjects at school. Results in Chapter 2, indicate that while poor mathematical skills are noted in these children reading and spelling skills were IQ-appropriate.

Finally, an advantage of the current study has been the use of highly selective criteria to recruit participants (e.g. all standard-risk, no complications of treatment, no relapse, randomised for treatment, etc.). This affords greater statistical power despite the relatively small group sizes by controlling for many confounding factors. However, as a consequence, the results of the current study are limited in their generalisability. The findings are therefore of limited relevance to children treated with more aggressive PCNS therapies, for example MTX + TIT or MTX + CI, or children developing complications of treatment, for example clinical neurotoxic syndromes or relapse. Moreover, the results relate to children diagnosed with standard-risk ALL between the ages of 18 months and 15 years. The relevance of the findings to infants and adults diagnosed with ALL is somewhat limited. Replication of the current study in the wider population of survivors of ALL is therefore warranted.

7.5 Concluding remarks

The LT outcome of childhood ALL was associated with minimal cognitive deficits and subtle pathology. The methods used in the current research were unable to establish a clear relationship between the mild cognitive deficits and the subtle pathology identified. In addition, there was only modest support for the proposed model of pathology. Nevertheless, the mild level of impairment associated with the diagnosis and treatment of this once uniformly fatal disease was remarkable.

Appendix A – Clinical neurotoxic syndromes

Acute, sub-acute, and delayed neurotoxic syndromes have been associated with the administration of MTX in the treatment of ALL (see Table A.1). A description of each syndrome is provided below.

Table A-1 Clinical neurotoxic syndromes

Clinical syndrome	Onset	Duration	Outcome	Related to Treatment
Acute				
Aseptic meningitis	2-4 hrs	12-72 hrs	Reversible*	IT MTX IT Cytarabine
Myelopathy	Hrs – 2 weeks	Variable	Variable	IT MTX**
Sub-acute				
Encephalopathy	< 48 hrs		Reversible	HD MTX
Stroke-like episodes	3-10 days	48-72 hrs	Reversible	HD MTX L-asparaginase
Delayed				
Mineralising microangiopathy	> 3 months	Permanent	Permanent	MTX + CI*** HD MTX + TIT CI only***
Progressive leukoencephalopathy	> 3 months	Permanent	Permanent* (may stabilise or progress to death)	MTX + CI**** HD MTX + TIT ** CI only

Hrs = hours; IT = intrathecal; MTX = methotrexate; CI = cranial irradiation

* Steroids may help to relieve symptoms

** Related to the frequency of administration

*** Related to the dose of CI administered (worse if higher doses used (i.e. > 20 Gy)

**** Related to the order of administration (worse if MTX follows CI)

Acute syndromes

Acute syndromes develop within hours of administration and include chemical (aseptic) meningitis and myelopathy.

Chemical meningitis is characterised by CSF pleocytosis and relatively mild symptoms (eg headache, fever, nausea, lethargy)(Naiman *et al.* 1970; Rosner *et al.* 1970; Mott *et al.* 1972; Kubo *et al.* 1992). Myelopathy is characterised by weakness and pain in the limbs, sensory abnormalities/loss, paraplegia, and cranial nerve palsies (Pasquinucci *et al.* 1970; Baum *et al.* 1971; Luddy & Gilman 1973; Garcia-Tena *et al.* 1995; Massenkeil *et al.* 1998; Sterman & Schaumburg 2000). Generally, myelopathy affects the legs,

although other extremities can be involved (Martino *et al.* 1984; Massenkeil *et al.* 1998). The acute syndromes are related to the intrathecal administration of chemotherapeutic agents and the peak concentration of MTX in CSF (Poplack 1984). These syndromes generally resolve without direct treatment (steroids sometimes given) within days (Massenkeil *et al.* 1998). However, more advanced myelopathy may result in permanent quadriplegia (Garcia-Tena *et al.* 1995) or death (Skullerud & Halvorsen 1978; der Weid *et al.* 1991). The frequency of chemical meningitis is approximately 15% (Jaffe *et al.* 1985) while myelopathy is very rare (Garcia-Tena *et al.* 1995) in children treated for ALL.

Sub-acute syndromes

The sub-acute clinical syndromes develop within days to weeks after therapy and include encephalopathy and stroke-like episodes.

Sub-acute encephalopathy is characterised by seizures, mental changes (eg confusion, lethargy, stupor, somnolence (Price & Jamieson 1975; Rubinstein *et al.* 1975; Ito *et al.* 1991; Kubo *et al.* 1992) and focal neurological deficits (eg cortical blindness (Kubo *et al.* 1992), aphasia (Massenkeil *et al.* 1998), slurred speech (Ito *et al.* 1991; Kubo *et al.* 1992), paresis/hemiparesis (Crosley *et al.* 1978), and ataxia (Rubinstein *et al.* 1975). Symptoms may be accompanied by EEG abnormalities (Rubinstein *et al.* 1975; Kubo *et al.* 1992) and T2 hyperintensities, particularly in periventricular white matter (e.g. frontal horns), corona radiata or centrum semiovale (Colosimo *et al.* 1994). Symptoms generally resolve (Osterlundh *et al.* 1997) and white matter changes spontaneously recover (Massenkeil *et al.* 1998) or decrease in size (Ito *et al.* 1991) with time.

Stroke-like episodes are characterised by focal seizures, unilateral paresthesia, weakness and disorientation, aphasia, and dysarthria (Bleyer 1981; Yim *et al.* 1991; Winick *et al.* 1992). These episodes are related to the use of HD MTX and symptoms are similar to episodes seen in approximately 4% of children treated with higher doses of MTX for osteosarcoma (Rosen *et al.* 1979; Ochs 1989). Generally, symptoms spontaneously resolve (Yim *et al.* 1991; Osterlundh *et al.* 1997). However, the ischemic insults to cortical white matter may persist on MRI at follow up (Yim *et al.* 1991). The frequency of stroke-like episodes is rare (Yim *et al.* 1991).

Delayed syndromes

Delayed clinical neurotoxic syndromes develop months to years after treatment and include mineralising microangiopathy and progressive (or necrotising) leukoencephalopathy.

Mineralising microangiopathy is a distinct pathological process involving calcification of the microvasculature of the CNS particularly in small vessels. It usually involves the basal ganglia (particularly putamen), dentate nuclei of the cerebellum, and occasionally cerebral cortex (Flament-Durand *et al.* 1975). The caudate nuclei, thalami, and internal capsules are generally spared (Price & Birdwell 1978). Mineralising microangiopathy is identified mainly in children treated with combined MTX (systemic or IT MTX) and CI (Shanley 1995; Rabin *et al.* 1996), particularly in children treated under the age of 10 years (Price & Birdwell 1978; Colosimo *et al.* 1994). The order of administration of treatment has also been associated with an increased risk of developing mineralising microangiopathy (e.g. when MTX follows CI)(Flament-Durand *et al.* 1975). However, mineralising microangiopathy has been described in patients treated with CI only (in higher doses than used in ALL)(Price & Birdwell 1978) and in children treated with combined HD MTX and TIT(Lovblad *et al.* 1998). Mineralising microangiopathy has not been reported in children treated with IT MTX-only (Price & Jamieson 1975).

Clinical symptoms and signs of basal ganglia dysfunction are usually absent. However, cognitive impairment (e.g. low IQ and learning disabilities) and progressive neurological problems (e.g. ataxia, visual disturbance, seizures) are more common (Shanley 1995). Mineralising microangiopathy is often accompanied by progressive leukoencephalopathy (e.g. case 1; (Ito *et al.* 1991))(Lovblad *et al.* 1998). At 9 months post-treatment, the frequency of mineralising microangiopathy in children receiving combined radiation and chemotherapy is approximately 25-30% (Valk & van der Knapp 1992).

Progressive (or necrotising) leukoencephalopathy is a severe form of delayed neurotoxic syndrome. The pathology usually involves periventricular white matter (particularly of frontal horns)(Ito *et al.* 1991) and centrum semiovale (Flament-Durand *et al.* 1975). It may also involve cortical U fibres of both hemispheres (Liu *et al.* 1978; Spencer 1998)(but see also (Matsumoto *et al.* 1995; and case 1 (Laxmi *et al.* 1996)). Cortical grey matter (Norrell *et al.* 1974;Price & Jamieson 1975;Liu *et al.* 1978; Bissett *et al.* 1991), the basal ganglia (Liu *et al.* 1978; Matsumoto *et al.* 1995), and brain stem (Norrell *et al.* 1974) are notably spared in the process and there are often no gross changes to cerebellum, pons, or medulla (Liu *et al.* 1978). Lesions do not usually show

contrast enhancement (Asato *et al.* 1992). Increases in glucose metabolism have also been observed in the basal parts of frontal lobes, occipital lobes and in the basal ganglia in progressive leukoencephalopathy (Shishido *et al.* 1986). Progressive leukoencephalopathy has been seen in children treated for ALL with combined MTX + CI (Ito *et al.* 1991; Matsumoto *et al.* 1995; Chen *et al.* 1996), particularly when MTX follows CI (Price & Jamieson 1975)), and in children treated with HD MTX (Wilson *et al.* 1991; Laxmi *et al.* 1996). It is more common in children receiving combined CI + MTX at a younger (<6 years at diagnosis) rather than older age (Matsumoto *et al.* 1995; Colosimo *et al.* 1994).

The main clinical symptoms associated with progressive leukoencephalopathy are (1) severe cognitive deterioration/dementia (e.g. decreased vocabulary, memory loss, disorientation, poor handwriting, coordination and concentration (Ito *et al.* 1991; Spencer 1998), (2) gait disorders ranging from mild retropulsion to severe ataxia (Keime-Guibert *et al.* 1998), (3) seizures, and (4) mental changes (e.g. apathy, aggressiveness, depression, irritability, confusion, somnolence and slurred speech) (Ito *et al.* 1991; Spencer 1998). Symptoms may stabilise with some recovery when MTX is withheld or following the administration of FAR (Kay *et al.* 1972). However, symptoms may progress to severe and permanent mental retardation (case 1 (Ito *et al.* 1991)) or may result in death (case 1 (Kay *et al.* 1972))(case 1 (Laxmi *et al.* 1996)). Symptoms may be accompanied by EEG abnormalities (e.g. asymmetry in the distribution of the excessive slow wave activity over various regions to indicate patchy involvement of the cerebral hemispheres). The EEG abnormalities improve slowly with time and may take months to resolve (case 3 and 4)(Kay *et al.* 1972).

Neuropathological studies in leukoencephalopathy show variation between patients (Liu *et al.* 1978). Generally, on a macroscopic level the brain and spinal cord are grossly normal (Liu *et al.* 1978). However, extensive white matter changes are noted, with white matter (e.g. of the periventricular region and centrum semiovale) having a grey/chalky white colour (Bissett *et al.* 1991). Despite this, the changes lead to only a slight reduction in total white matter volume (Liu *et al.* 1978). Histologically, the white matter shows patchy demyelination with discrete multifocal non-inflammatory coagulative necrotic lesions (Rubinstein *et al.* 1975; Crosley *et al.* 1978; Liu *et al.* 1978) with little evidence of perivascular distribution (Norrell *et al.* 1974; Rubinstein *et al.* 1975). Early in the disease process the lesions contain swollen and fragmented axons (particularly in centrum semiovale) (Norrell *et al.* 1974; Rubinstein *et al.* 1975; Liu *et al.* 1978). In more advanced disease (more severe lesions), there is total loss of myelin, degeneration of oligodendrocytes (Price & Jamieson 1975; Rubinstein *et al.* 1975),

gliosis (Liu *et al.* 1978), and occasionally calcification (Rubinstein *et al.* 1975; Liu *et al.* 1978; Moore *et al.* 2002). In the later stages of the disease the pathology can include subcortical U fibres and spinal cord and is similar to sub-acute combine degeneration of the cord (SACD)(Crosley *et al.* 1978). Vascular changes (e.g. thickening, hyalinization, fibrinoid necrosis of vessel walls) are rarely present (Rubinstein *et al.* 1975). The white matter surrounding lesions may show spongiosis and diffuse reactive astrocytosis (Norrell *et al.* 1974; Flament-Durand *et al.* 1975; Rubinstein *et al.* 1975; Crosley *et al.* 1978; Liu *et al.* 1978). The frequency of leukoencephalopathy has been estimated to be 1% in children treated for ALL with combined MTX + CI (Liu *et al.* 1978).

Appendix B – The study participants and the wider UKALL XI cohort

Table B-1 Comparisons between the study participants and the wider UKALL XI cohort at 5 years post-diagnosis – HD groups

	Current sample (N=16) (\bar{x} ±SEM)	Wider UKALL XI cohort (N=101) (\bar{x} ±SEM)	t	p value
Verbal IQ	106.21 (3.32)	98.19 (1.41)	2.02	0.05
Non-verbal IQ	110.71 (4.77)	98.54 (1.48)	2.81	0.01
Verbal comprehension	105.93 (3.42)	97.85 (1.43)	2.02	0.05
Perceptual organisation	109.64 (4.53)	97.85 (1.46)	2.79	0.01
Freedom from distractibility	100.43 (3.81)	97.68 (1.42)	0.68	0.50
Processing speed	107.00 (4.99)	102.09 (1.57)	1.07	0.29
Receptive language	111.54 (4.62)	98.53 (1.34)	3.17	<0.01
Fine motor (dominant)	-0.89 (0.28)	-0.75 (0.12)	-0.42	0.68
Fine motor (non-dominant)	-0.67 (0.28)	-0.51 (0.10)	-0.54	0.59
Verbal immediate memory	0.12 (0.21)	-0.21 (0.11)	1.11	0.27
Verbal delay memory	0.21 (0.38)	-0.26 (0.10)	1.50	0.14
Visual immediate memory	0.01 (0.27)	0.08 (0.10)	-0.24	0.81
Visual delay memory	0.41 (0.24)	-0.08 (0.11)	1.58	0.12
Paired associate learning	-0.42 (0.34)	-0.52 (0.11)	0.32	0.75
Visual perception	18.09 (1.99)	18.41 (0.68)	-0.17	0.87
Reading	104.00 (4.23)	97.93 (1.62)	1.28	0.20
Spelling	105.00 (3.78)	96.95 (1.68)	1.65	0.10
Reading comprehension	99.50 (4.43)	94.39 (1.55)	1.08	0.29
Mathematical reasoning	101.36 (4.76)	98.20 (1.36)	0.77	0.45
Numerical operations	102.08 (3.15)	101.49 (1.77)	0.12	0.91

Table B-2 Comparisons between the study participants and the wider UKALL XI cohort at 5 years post-diagnosis – IT groups

	Current sample (N=16) (\bar{x} ±SEM)	Wider UKALL XI cohort (N=91) (\bar{x} ±SEM)	t	p value
Verbal IQ	102.07 (4.35)	100.16 (1.78)	0.40	0.69
Non-verbal IQ	108.86 (4.69)	100.38 (2.02)	1.56	0.12
Verbal comprehension	102.08 (4.39)	99.62 (1.69)	0.51	0.61
Perceptual organisation	108.00 (4.82)	99.74 (2.02)	1.47	0.14
Freedom from distractibility	100.69 (5.57)	98.58 (1.62)	0.45	0.66
Processing speed	107.00 (5.14)	101.89 (1.87)	0.97	0.34
Receptive language	101.79 (3.84)	98.87 (1.61)	0.68	0.50
Verbal immediate memory	-0.36 (0.20)	-0.25 (0.12)	-0.35	0.73
Verbal delay memory	-0.37 (0.20)	-0.34 (0.11)	-0.08	0.94
Visual immediate memory	0.15 (0.15)	-0.01 (0.12)	0.50	0.62
Visual delay memory	-0.03 (0.24)	-0.07 (0.12)	0.13	0.90
Paired associate learning	-0.84 (0.36)	-0.36 (0.11)	-1.49	0.14
Visual perception	19.11 (2.23)	18.48 (0.81)	0.26	0.80
Fine motor (dominant)	-1.18 (0.20)	-0.74 (0.13)	-1.31	0.20
Fine motor (non-dominant)	-1.02 (0.34)	-0.60 (0.12)	-1.39	0.17
Reading	96.18 (4.86)	101.16 (1.66)	-1.07	0.29
Spelling	95.91 (3.72)	100.07 (1.71)	-0.89	0.37
Reading comprehension	88.91 (9.79)	96.57 (1.60)	-1.39	0.17
Mathematical reasoning	102.45 (5.45)	102.35 (1.73)	0.02	0.98
Numerical operations	104.50 (5.33)	103.95 (1.85)	0.10	0.92

Table B-3 Comparisons between the study participants and the wider UKALL XI cohort at 5 years post-diagnosis – Sibling groups

	Current sample (N=14) ($\bar{x}\pm$ SEM)	Wider UKALL XI cohort (N=121) ($\bar{x}\pm$ SEM)	t	p value
Verbal IQ	105.77 (4.43)	103.16 (1.27)	0.63	0.53
Non-verbal IQ	113.31 (5.26)	106.61 (1.44)	1.42	0.16
Verbal comprehension	102.00 (5.34)	101.87 (1.37)	0.03	0.98
Perceptual organisation	108.46 (5.31)	104.78 (1.54)	0.72	0.47
Freedom from distractibility	105.82 (3.56)	102.84 (1.43)	0.65	0.52
Processing speed	116.44 (4.98)	107.20 (1.58)	1.66	0.10
Receptive language	106.83 (3.92)	101.49 (1.21)	1.35	0.18
Verbal immediate memory	-0.40 (0.32)	-0.20 (0.10)	-0.59	0.55
Verbal delay memory	-0.19 (0.27)	-0.14 (0.10)	-0.15	0.88
Visual immediate memory	0.08 (0.33)	0.37 (0.08)	-1.67	0.10
Visual delay memory	-0.06 (0.32)	0.13 (0.09)	0.61	0.55
Paired associate learning	-0.55 (0.36)	-0.18 (0.09)	-1.19	0.24
Visual perception	17.33 (2.66)	21.13 (0.60)	-1.40	0.19
Fine motor (dominant)	-1.42 (0.21)	-0.97 (0.12)	-1.14	0.26
Fine motor (non-dominant)	-1.17 (0.17)	-0.75 (0.11)	-1.19	0.24
Reading	105.80 (3.27)	104.40 (1.46)	0.32	0.75
Spelling	102.00 (3.96)	103.04 (1.44)	-0.23	0.82
Reading comprehension	103.22 (4.33)	98.20 (1.37)	1.13	0.26
Mathematical reasoning	111.50 (2.75)	104.62 (1.48)	1.56	0.12
Numerical operations	113.27 (5.24)	108.44 (2.10)	0.73	0.47

Table B-4 Children showing a decrease of 15 or more VIQ points between diagnosis and 5 years – the larger UKALL XI cohort

Group	Sex	Age at diagnosis	VIQ diagnosis	VIQ 5 year	VIQ Change
HD	M	6.92	99	84	-15
HD	F	8.92	104	89	-15
IT	F	5.75	115	100	-15
HD	F	4.17	99	83	-16
IT	F	3.33	126	109	-17
IT	F	2.75	85	67	-18
Siblings	M	-	102	84	-18
Siblings	M	-	93	74	-19
Siblings	M	-	105	86	-19
IT	M	5.08	106	84	-22
HD	M	6.58	107	84	-23
IT	F	8.00	111	88	-23
Siblings	F	-	116	80	-36

Table B-5 Children showing a decrease of 15 or more PIQ points between diagnosis and 5 years – the larger UKALL XI cohort

Group	Sex	Age at diagnosis	PIQ diagnosis	PIQ 5 year	PIQ Change
HD	F	4.17	109	94	-15
HD	M	6.92	101	86	-15
IT	F	2.75	116	101	-15
IT	M	5.83	88	73	-15
HD	M	3.92	112	96	-16
HD	M	6.25	113	96	-17
IT	F	4.92	120	103	-17
Siblings	F	-	116	99	-17
HD	M	6.58	109	91	-18
IT	M	2.75	102	84	-18
IT	F	3.33	108	90	-18
Siblings	M	-	100	82	-18
HD	F	3.25	86	66	-20
IT	M	3.42	120	99	-21
HD	F	2.75	104	81	-23
IT	F	2.75	101	77	-24
HD	M	2.83	115	90	-25
IT	M	4.25	111	86	-25
IT	F	5.75	113	88	-25
HD	M	6.00	95	69	-26
HD	M	4.83	131	104	-27
IT	F	6.92	118	90	-28
HD	F	4.50	127	96	-31
IT	M	5.08	116	84	-32

Table B-6 Percentage of children with achievement scores within the clinically significant range – the larger UKALL XI cohort

	HD (%)	IT (%)	Siblings (%)
Reading	11/84 (13%)	4/73 (5%)	6/86 (7%)
Spelling	11/84 (13%)	6/73 (8%)	7/84 (8%)
Reading comprehension	17/83 (20%)	16/72 (22%)	21/85 (25%)
Maths reasoning	9/84 (11%)	7/71 (10%)	7/84 (8%)
Numerical operations	9/84 (11%)	3/72 (4%)	10/84 (12%)

Appendix C – Missing data

Table C-1 Missing data – Neuropsychological studies

Assessment	Group	Reason
SES	IT	Not provided by parent
Whole LT Neuropsychological protocol	IT	Refused
Selective attention	HD	Not saved
Speeded motor response	HD	Not saved
CBCL	HD	Not returned by parents
CBCL	IT	Incomplete
DEX questionnaire	HD	Not returned by parents
DEX questionnaire	HD	Added late to protocol
DEX questionnaire	IT	Added late to protocol
DEX questionnaire	IT	Added late to protocol
DEX questionnaire	IT	Added late to protocol
DEX questionnaire	IT	Added late to protocol
DEX questionnaire	IT	Added late to protocol
DEX questionnaire	Siblings	Incomplete
DEX questionnaire	Siblings	Added late to protocol
DEX questionnaire	Siblings	Added late to protocol
DEX questionnaire	Siblings	Added late to protocol
WCST	HD	Ran out of time
WCST	HD	Ran out of time
WCST	HD	Ran out of time
WCST	HD	Ran out of time
WCST	Siblings	Ran out of time
WCST	Siblings	Ran out of time
SWM	IT	Not saved
IDED Level 9 – ED reversal	IT	Only achieved level 8/9 levels
	IT	Only achieved level 8/9 levels
Extinction learning	HD	Failed discrimination stage
	HD	Failed discrimination stage

Table C-2 Missing data – Neuroimaging studies

Assessment	Group	Reason
Whole MR protocol	HD	Unable to tolerate the scanning environment
	IT	Unable to tolerate the scanning environment
	IT	Did not wish to participate
	Siblings	Recent metal plate inserted into body
Clinical scans	HD	Unable to acquire scan
	Siblings	Unable to acquire scan
3D FLASH	HD	Unable to acquire scan
	IT	Data collection failed (Braces)
DTI	HD	Movement or CSF/arterial pulsation artefact
	HD	Movement or CSF/arterial pulsation artefact
	HD	Unable to acquire scan
	IT	Movement or CSF/arterial pulsation artefact
	IT	Data collection failed (Braces)
	IT	Data collection failed (Braces)
	IT	Data collection failed (Braces)
	Siblings	Data collection failed (Braces)
	Siblings	Data collection failed (Braces)
	Siblings	Unable to acquire scan
	Siblings	Unable to acquire scan
	Siblings	Incomplete data set collected (16/23 images archived)
	Siblings	Computer error

Appendix D – Information Sheets and Consent forms

Letter of invitation

Dear,

Thank you for participating in the UKALL XI neuropsychology study. The project has been very successful with over 900 children and families becoming involved. Currently we are analysing all of the information collected and compiling our final results.

We are also beginning another important stage of this study. This stage is for children who have already completed three neuropsychology assessments and we are writing to ask if you would like for your child to be involved. In this next stage we are trying to link variations in the cognitive and behavioural outcome (e.g. IQ, attention, memory, reading, maths) to changes in the brain.

To do this we would like your child to attend another outpatient appointment. At this appointment your child will be asked to do some more paper and pencil tests and to play some computer games. We would also like for your child to have a magnetic resonance scan. We have attached more information about these tests to this letter.

The appointment will take place at Great Ormond Street Hospital in London. If you wish, we can help you to arrange transport for your visit to us. We can also reimburse all of your travel expenses and any incidental expenses that you may incur during your visit.

The information collected in this stage of the study will be very important in finding the best treatment for children with leukaemia in the future. The information will also ensure that your child's progress continues to be monitored and we can provide you with a summary of your child's results.

If you would like for your child to be involved in this next stage, please complete the enclosed consent form. We would like to reiterate that you are under no obligation to participate and even if you send the consent forms back, you are free to withdraw from our study at any time.

If you have any questions about the tests or what is involved, please do not hesitate to contact Leasha Lillywhite on (020) 7837 7618 extension 2934.

Yours sincerely,

Prof Faraneh Vargha-Khadem
Consultant Neuropsychologist

Leasha Lillywhite
Neuropsychology Team

Parent Information Sheet

We are asking for your permission to include your child in this project.

1. Title of the project

The neuropsychological outcome of children treated for acute lymphoblastic leukaemia.

2. Aims of the project

To identify changes in the brain that may be associated with different treatments for ALL, and to link these changes to cognitive and behavioural results.

3. Why is this project important?

In the past some children have been known to suffer behavioural and cognitive impairments following treatment for leukaemia. It is not clear whether this is due to the type of treatment received or the disease itself.

4. How are we going to do this?

We will use all of the information gathered from the UKALL IX study so far. In addition, we would like to collect more information from children who have already completed three neuropsychological assessments.

To obtain the extra information, your child will be given some more pencil and paper tests, which may involve doing maths problems, reading words or looking at patterns and pictures. Your child will also be asked to play some computer games. We will always explain to your child what we will be doing and what we are trying to find out before starting each test.

This testing will take approximately 3 hours and will be carried out by a member of the neuropsychology team working under the direction of Professor Vargha-Khadem.

Your child will also undergo a scan. This scanner uses a magnetic field and radio waves to show us the detailed structure of your child's brain. To get this picture, your child will need to lie still on a bed inside a cylindrical magnet for approximately 45 minutes. Children can listen to CD's during this time and your child can bring along their favourite CD to listen to if they wish.

5. Are there any risks or discomforts for my child?

There are no risks involved as far as we know. If your child stays awake during the scan (many children fall asleep!), he/she may hear a thumping noise created by movements inside the magnet. Also a child may become uncomfortable lying in a confined space and will be asked not to move his/her head. If your child does not like the feeling of being confined for too long, or does not like the noise, he/she can ring a bell and the staff will immediately take him/her out of the scanner.

6. What are the benefits for me?

This research may not bring any benefits to you or your child. However, your child's involvement may help children diagnosed with leukaemia in the future. If you wish, we will help you to arrange transport for your visit to us. We will reimburse all of your travel expenses and any incidental expenses you may incur during your visit. We will also schedule the appointment at a time that is most convenient to you and your child.

7. Who will be able to see my child's test results?

The research people working on this project and also one person from the Ethics Committee at the Great Ormond Street Hospital.

8. Does my child have to take part in the study?

No. Taking part in this study is completely voluntary and will not affect any benefits that you or your child are entitled to. You may also withdraw your child at any stage.

9. Who do I speak to if problems arise?

If you have any complaints about the study or the way in which this study is being run, please in the first instance discuss them with the researcher. If the problems continue, or you wish to comment in another way, please contact the Chairman of the Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or if urgent by telephone on (0207) 242 9789 ext 2620 and the Committee administration will put you in contact with him.

10. How do I contact the researcher?

Faraneh Vargha-Khadem
Developmental Cognitive Neuroscience Unit
The Wolfson Centre
Mecklenburgh Square
London WC1N 2AP
Telephone: (0171) 837 7618 ext 2980.

Child Information Sheet

We are asking for your permission to include you in this project. We would like you to do some tests with us to see how you are getting on. It is important that you read and understand this letter before you agree to help us.

1. Title of the project

The neuropsychological outcome of children treated for acute lymphoblastic leukaemia.

2. Aims of the project

We are trying to find a treatment that will help all children with leukaemia to survive and will also help each child do well at school

3. Why is this project important?

In the past some children ended up not doing very well at school after having treatment for leukaemia, and we don't know if this is because of the treatment they had for leukaemia or because of the leukaemia itself.

4. How are we going to do this?

We will use all of the results from the tests that you did last time you came and saw us and we will ask for you to do some new things. We would like you to do some pencil and paper tasks and some puzzles. We would also like for you to play some computer games. This will take about 3 hours.

Then we would like to get a picture of your brain. To do this you will need to lie very still on a bed inside a scanner that looks a bit like a very large tube of polo mints. This will take about 45 minutes but you can have a break if you want during this time. You can also bring along your favourite CD to listen to during this time.

5. Will this hurt me?

As far as we know, there will be know harm to you. You may feel a bit uncomfortable because of the small space inside the scanner and it may be a bit noisy. If you don't like it inside the scanner or you do not like the noise, you can ring a bell inside the scanner and we will immediately get you out. You won't have to go back in the scanner unless you want to.

6. What are the benefits for me?

This research may not help you directly. However, being involved may help children diagnosed with leukaemia in the future.

7. Who will be able to see my test results?

The research people working on this project and also one person from the Ethics Committee at the Great Ormond Street Hospital.

8. Do I have to take part in the study?

No. Taking part in this study is up to you and your family and will not affect any benefits that you are entitled to. You may also change your mind at any stage.

9. Who do I speak to if problems arise?

If you have any complaints about the study or the way in which this study is being run, please in the first instance discuss them with the researcher. If the problems continue, or you wish to tell somebody else, you can contact the Chairman of the Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, or if urgent by telephone on (0207) 242 9789 ext 2620 and the Committee administration will put you in contact with him.

10. How do I contact the researcher?

Faraneh Vargha-Khadem

Developmental Cognitive Neuroscience Unit

The Wolfson Centre

Mecklenburgh Square

London WC1N 2AP

Telephone: (0171) 837 7618 ext 2934.

Consent form for parents

**Great Ormond Street Hospital for Children NHS Trust and
Institute of Child Health Research Ethics Committee**

**Consent form for PARENTS OR GUARDIANS of Children Participating in Research
Studies**

Title: The neuropsychological outcome of children treated for acute lymphoblastic leukaemia.

NOTES FOR PARENTS OR GUARDIANS:

1. Your child has been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.
2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.
3. If you decide, now or at any other stage, that you do not wish for your child to participate in the research project, that is entirely your right, and if your child is a patient it will not in any way prejudice any present or future treatment.
4. You will be given an information sheet that describes the research project. This information sheet is for you to keep and refer to. Please read it carefully.
5. If you have any complaints about the way in which the research project has been or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in an other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, or if urgent, by telephone on 020 7905 2620 and the Committee administration will put you in contact with him.

CONSENT

I/We _____, being the
parent(s)/guardian(s) of _____ agree that the
Research Project named above has been explained to me to my/our satisfaction and I/We give
our permission for our child to take part in this study. I/We have read both the notes written
above and the Information Sheet provided, and understand what the research study involves.

SIGNED [Parents(s) or Guardians(s)]

DATE

TELEPHONE NO.

SIGNED [Researcher]

DATE

Consent form for young adults 16 years or older**Great Ormond Street Hospital for Children NHS Trust and
Institute of Child Health Research Ethics Committee****Consent form for Young Adults Participating in Research Studies**

Title: The neuropsychological outcome of children treated for acute lymphoblastic leukaemia.

NOTES FOR YOUNG ADULTS:

1. You have been asked to take part in a research study. The person organising that study is responsible for explaining the project to you before you give consent.
2. Please ask the researcher any questions you may have about this project, before you decide whether you wish to participate.
3. If you decide, now or at any other stage, that you do not wish to participate in the research project, that is entirely your right, and if you are a patient it will not in any way prejudice any present or future treatment.
4. You will be given an information sheet that describes the research project. This information sheet is for you to keep and refer to. Please read it carefully.
5. If you have any complaints about the way in which the research project has been or is being conducted, please, in the first instance, discuss them with the researcher. If the problems are not resolved, or you wish to comment in an other way, please contact the Chairman of the Research Ethics Committee, by post via the Research and Development Office, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, or if urgent, by telephone on 020 7905 2620 and the Committee administration will put you in contact with him.

CONSENT

I _____ agree that the Research Project named above has been explained to me to my satisfaction and I give my permission to take part in this study. I have read both the notes written above and the Information Sheet provided, and understand what the research study involves.

SIGNED

DATE

TELEPHONE NO.

SIGNED [Researcher]

DATE

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