Perinatal Factors and Umbilical Cord Characteristics in Determining Handedness: Results from the Belgian East Flanders Prospective Twin Survey

by

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Abstract

Aims: The objective was to determine if perinatal factors and umbilical cord characteristics showed any association with left-handedness in a set of twin subjects.

Subjects: Twins born from 1977 to 1991 and registered in the ongoing East Flanders Prospective Twin Survey (EFPTS) were studied.

Methods: Handedness as the outcome measure was assessed by the Strien (Preference score) questionnaire and the Bishop's card-reaching test (Performance score). Perinatal and umbilical cord characteristics were compared in left and right-handed subjects, adjusting for twin clustering in the data. Random effects logistic modelling was used to predict the odds of being left-handed.

Findings: Out of the 15 factors tested, only 2 had statistically significant relationships with left-handedness when assessed by Strien questionnaire.

Left-handedness was less common in older fathers (odds ratio (OR) per year increase in age=0.95 (0.91-1.00) and in non-primiparous births (OR=0.68 (0.48-0.97)) Left-handedness was also less common in subjects with undefined and mixed umbilical cord windings (OR= 0.61 (0.37-1.00) when compared to clockwise windings), though the factor as a whole was not significant (p=0.13).

Bishop's test did not confirm these findings.

Conclusion: Perinatal factors studied were not found to be significantly associated with left-handedness.

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List of Abbreviations:

EFPTS: East Flanders Prospective Twin survey

MZ: monozygotic

MCMZ: mono-chorionic mono zygotic

MCMA: mono-chorionic mono-amniotic

MCDA: mono-chorionic di-amniotic

DZ: dizygotic

DCDZ: dichorionic dizygotic

DCDA: di-chorionic di-amniotic

QHP task: quantification of hand preference test

IVF: in vitro fertilisation

RBS: Reported birth stress

ROA: right occipito- anterior

LOA: left occipito- anterior

List of Abbreviations: continued.

Types of Twins explained:

Type of twin	Description	Day of splitting
DCDA (from two fertilised ova. Genetically different)	Twins have two separate chorions and amniotic sacs	3rd day after fertilisation
MCDA (from single fertilised ovum)	Have two amniotic sacs but share the same placenta	4-8 days after fertilisation
MCMA ((from single fertilised ovum)	Share single amniotic sac and single placenta	9th day after fertilisation

Glossary of terms:

Sinistrality: condition of being left-handed.

Dextrality: condition of right-handed.

Laterality: preference in using one side of the body over the other.

Handedness (hand preference): tendency to use one hand over the other.

Zygote: a fertilised ovum

Monozygotic (identical): develops from one zygote that splits and forms two embryos.

Dizygotic (non-identical): develops from two separate eggs that are fertilised by two separate sperm.

Zygosity: degree of identity in the genome of twins.

Chorion: one of the membranes that exist during pregnancy between the developing foetus and the mother. The chorionic villi emerge from the chorion, invade the endometrium and allow the transfer of nutrients from maternal blood to foetal blood.

Concordance: the presence of the same trait in both the members of a pair of twins.

Discordance: the difference in a trait observed between the members of a pair of twins.

Mirror-image twins: seen in identical twins and have small mirror image differences but are actually genetically identical. This means they have the exact same DNA.

Ontogeny: The origin and development of an individual organism from embryo to adult.

Chapter 1: INTRODUCTION

1.1: Background

The study will focus on handedness and perinatal factors in twins. The term handedness refers to which hand (right or left) is dominant in a range of manual activities, such as writing, brushing or throwing a ball.

This study will employ handedness scores from two validated tests (Strien questionnaire-Preference test [1] and Bishop's Card reaching-Performance test [2]) . Details of the validation methods of both the tests are explained in section 1.4. The evolutionary background, theories and factors which could influence handedness and the different methods of measuring handedness are also covered.

The Perinatal period (according to tenth revision of the International statistical classification of diseases and related health problems (WHO, 1992)) refers to the period from 22 completed weeks of gestation (the time when birthweight is normally 500 grams) to 7 completed days after birth [3]. The perinatal factors used in this study are father's age, mother's age, parity of the mother, gestational age, birth weight, presenting position, delivery mode of the twins and the type of conception of the twins. In addition, umbilical cord features such as cord length, cord windings and cord knots are also included.

A detailed description of twin studies is given in section 1.3.3 and a summary of findings from studies on handedness in twins in section 1.3.4. Detailed explanation about the East Flanders Prospective Twin Survey (EFPTS), from which the perinatal factors were collected, is given in Section 1.2.

1.1.1: What is handedness?

This section aims to explain what handedness means. The neurological aspects and the implications of handedness are covered in the subsequent sections.

Lateralisation is the preference that most humans show for using one side of the body rather than the other. Although this preference can extend to feet, eyes and ears, handedness is the most explored variable. Handedness is one example of many forms of behavioural lateralisation seen in humans. In a broad classification, handedness would be right, left or mixed handed. Handedness refers to the preferential use of right or left hand to carry out a range of manual activities.

1.1.2: Neurological aspects of handedness

In this section, I have discussed the role of brain and cerebral hemispheres in causing a preference in handedness.

Handedness is thought to be an index of cerebral hemispheric asymmetries that underlie complex human cognition as well as asymmetries of the motor cortex [4, 5]. The human brain is a paired organ; it is composed of two halves, called the cerebral hemispheres. The term brain lateralisation refers to the fact that the two halves of cerebral hemispheres have their own functional specialisations. From a neuropsychological perspective, lateralisation in the form of hand or foot preference remains the best behavioural predictor of cerebral lateralisation. Left hemisphere language dominance is reported in approximately 95% of right-handers and 70% of left-handers [6]. Behavioural laterality has also been found to predict emotional lateralisation [7]. Orton first proposed that specific impairments of language and literacy were caused by confused cerebral dominance and regarded lack of lateralisation as a cause of developmental speech, language and reading problems [8]. Recently, brain imaging studies have provided some evidence of atypical morphological asymmetries in language and reading impaired children [9].

As well as controlling motor functions (e.g. muscle movements, reflexes) the cerebral cortex is responsible for sensing and interpreting input from various sources (hearing touch and vision) and maintaining the cognitive function (thinking, perceiving and understanding language) as well. Brain asymmetries also extend to the cognitive functions of the cerebral cortex. The left hemisphere specialises in analytical thought and linear reasoning functions of language such as grammar and word production. The right hemisphere sees the images in the imagination, have visions, dreams and is responsible for moments of revelation and creativity.

In the vertebrate nervous system the nerve connections between body and brain cross over and are in general linked to the opposite-side hemisphere of the brain. Thus the right occipital cortex receives input from the left visual field and the right motor cortex controls movement of the left hand. The areas of cortex assigned to various body parts are proportional not to their size, but rather to the complexity of the movements that they can perform. Hence, the areas for the hand and face are especially large compared with those for the rest of the body. This is not surprising, because the speed and dexterity of human hand and mouth movements are precisely what give us two of our most distinctly human faculties: the ability to use tools and the ability to speak. A diagram showing proportional representations of the body parts in the motor cortex is given in Fig.2.4.

The division of labour by the two cerebral hemispheres was once thought to be uniquely human, but the origin of the brain asymmetries linked to speech, right-handedness, facial recognition and the processing of spatial relations can be traced to brain asymmetries in early vertebrates as well. Fishes, reptiles and amphibians tend preferentially to strike at prey on their right side under the guidance of their right eye and left hemisphere [(10)].

The correlation between human hand preference and brain lateralisation is complex and has been debated to have a relationship with human mental development. Hence, handedness could be used as a proxy for cerebral lateralisation and this could reveal mechanisms of the underlying pathology of problems related to brain development.

Analysis of intra-hemispheric relationships between functions suggests that there may be a specific neurobiology to the inter-relationships between and among cognitive functions, handedness and intra-hemisphere localisation and of the function [(11)]. In humans, the most obvious functional specialisation is speech and language abilities. In the mid 1800s, Paul Broca (a french neurosurgeon) identified a particular area of the left hemisphere that plays a primary role in speech production. Broca also suggested that a person's handedness was opposite from the specialised hemisphere (so, a right-handed person has a left-hemispheric language specialisation) [(12)]. But a majority of left-handers also seem to have a left-hemispheric brain specialisation for language abilities.

Evolution of human speech has been associated with gesture and thus brain lateralisation for speech may be responsible for asymmetric hand use [(13)]. There seems to be a close, but imperfect tie between brain asymmetry, language and handedness[(14)]. Reliable differences in brain lateralisation between left and right-handers have been suggested [(15)]. Hence, handedness could be used as a marker for brain laterality [(16)].

The primary historical reason that the hand-brain link was considered important and became a generally accepted methodology was because for nearly a century, it was the only hint that a neurosurgeon had prior to surgery as to which hemisphere was specialised for language. Clinicians used handedness as a marker for brain lateralisation until the Wada (sodium amytal) test was introduced in 1960, in which unilateral injection of sodium amytal in to an internal carotid artery was done to produce transient hemiparesis (weakness) of the contralateral limbs [17].

Since this is an invasive drug testing method, still researchers found it useful to study patterns of brain asymmetry by using a person's handedness as a marker for brain lateralisation. Determining the hand preferences of a subject is important to many psychologists and clinical neurologists because hand preference is considered a marker for cerebral hemispheric dominance for speech and language [18].

1.1.3: Epidemiology and evolution of handedness

90% of humans are right handed, which leaves the remaining 10% to be left-handed [19]. It would be of interest to explore how the handedness trait has evolved over time and also the geographical spread of this trait in different populations.

1.1.3(a): Evolutionary background of handedness

Behavioural laterality has been demonstrated first between 9 and 10 weeks of gestational age from ultrasound studies. At 9–10 weeks, when the foetus begins to exhibit single arm movements, 75% exhibited a greater number of right arm movements, 12.5 per cent a greater number of left arm movements, and 12.5 per cent an equal number of left and right arm movements [20]. From 15 weeks of gestation, the foetus exhibits a preference for sucking its right thumb, and the sucking behaviour at foetal state is related to hand preference at a later age of 10–12 years [21, 22].

The origin of population bias toward right handedness remains obscure. If we have to understand the evolution of human handedness, two main questions need to be raised. (i) Which early life environmental conditions may influence to be left-handed? and (ii) Which developmental mechanisms might be inducing a switch in hand preference? [23]. Left-handedness was historically considered as an anomalous or pathological case, thus ignoring the relatively high proportion of left-handers within human populations.

Analysis of archaeological samples from skeletons, stone tools and various other artefacts were used to infer handedness in ancient humans [24]. Prevalence of right hand dominance in Neanderthal skeleton samples (dating from approx. 35 000 BP), has been observed by studying arm bone length [25]. Dental marks have also been used to infer hand use for cutting food with a stone tool. According to this, handedness polymorphism existed in Neanderthals [26, 27]. Again, for this task, right-handers outnumbered left-handers.

A significant polymorphism of hand use has been shown during prehistoric and historic times, with an overall dominance of right-handers. This polymorphism seems to have persisted over time, suggesting that selection may play an important role in the persistence of this diversity.

1.1.3(b): Geographical variation of handedness

Raymond and Pontier reviewed 81 studies on handedness that examined throwing or hammering in 14 countries in America, Africa, Europe, Asia and Australia. A range of 5–25.9% was reported by them to be left-handed, suggesting an important geographical variation in hand preference [28]. Similar variations have been observed by Perelle and Ehrman, for writing hand preference in a survey of 12,000 subjects from 17 countries. About 2.5–12.8% were left-handed for writing [29] . In an internet based study by the BBC, seven ethnic groups were asked which hand they preferred for writing and based on 2,55 ,100 responses, 7–11.8% were found to be left-handed [30].

The frequency of left-handedness thus seems to be variable among human populations, left-handers being always at a lower frequency than right-handers. Moreover, in most populations studied, the proportion of left-handers among women was lower than in men [reviewed in (28)], suggesting an important influence of sex in the determination of hand preference.

1.1.4: The implications of handedness

Apart from academic interest, the topic of handedness may have survival implications. This seems like a radical hypothesis and this is a point I go on to discuss in the following section.

1.1.4(a): Handedness and survival

Many researchers have studied the distribution of handedness in the population as a function of the age and have noted a decrease in percentages of left-handedness in the older age groups [31-34]. The proportion of left-handed subjects decreases, from around 15% (< than 20 years of age), to 5% (= 50 years), and to virtually 0% (= & > 80 years of age). This finding has been replicated in more studies [35-38].

This decrease in the number of left-handed individuals in the population as a function of age is puzzling. Coren and Halpern had explained this, based on a suggestion that it could be grouped under two categories as modification and elimination. Modification suggests that left-handers learn to become more right-handed, over a period of time, perhaps in response to overt pressures from the environment. In contrast, the elimination explanation suggests that left-handers are no longer found in the older age groups because of the death of these individuals. Most tools and equipment, furniture and even traffic patterns are structured for the convenience of the right-handed majority. In such a right-sided world left-handers may be at a greater risk of accidents [39].

Halpern and Coren have set an empirical basis for believing that left-handedness is associated with reduced longevity [40]. Though mortality data by handedness is rarely available, Halpern and Coren managed to investigate this as such. Reliable hand-use statistics and date of birth and death records were available in archival records for professional baseball players. The subjects they chose were the baseball players listed in The Baseball Encyclopaedia. The dates of birth and death and throwing and batting hand were reported (N = 2,271). The authors have shown the mean age of death for strong right-handers as 64.64 years (N = 1,472) and mean age of death for strong left-handers as 63.97 years (N = 236). This shows a difference of slightly over 8 months in favour of right-handers. They have listed three possible causes which would have caused this value. The range of life span in the sample is very large (with age of death varying from 20 to 109 years). The distribution of age of death has a marked deviation from the normal, with a pronounced positive skew, and there is a large disparity in the two sample sizes. Examining the age of survival would probably give a better picture. The oldest surviving left-handed subject was 91 years of age, whereas the oldest right-hander was 109. It confirmed that the groups differed in survival, with the right-handers more likely to survive to old age ($p < 0.001$) [41].

This finding is controversial and not universal. In a follow-up study of Danish twins (118 opposite-handed twin pairs) born between 1900 and 1910, there was no evidence of differential survival between right-handed and non-right handed individuals [42].

Cerhan et al prospectively studied 39,691 women aged 55-69 years from the Iowa Women's Health Study through 5 years of mortality follow-up. No increase in mortality risk for left-handed women as compared to right-handed women (also adjusted for body mass index, body fat distribution, smoking and education) was found [43].

Aggleton et al tested whether left-handedness was associated with a change in longevity in cricketers born between 1840 and 1960. Regression analyses of 5960 cricket players from British Isles (right, n=5041; l, n=1132) born showed no significant relation between mortality and left-handedness (p=0.3). Left-handedness was however associated with an increased likelihood of death from unnatural causes (p=0.03) and the authors speculated that this effect was especially related to deaths during warfare [44].

<u>1.1.4(b): Left-handedness as a beneficial trait</u>

Some workers have suggested that left-handers are more intelligent than right-handers because of different abilities. At the top end of the intellectual spectrum, they do better [45]. Left-handed people are more creative, more likely to notice the size, shape and form of things. Left-handers have more power of perception as compared to right-handers [46].

The Corpus Callosum connects the left and right cerebral hemispheres and facilitates interhemispheric communication. Non-right-handers have been shown to have a better interhemispheric transfer and a larger Corpus Callosum. This has been reported to be associated with superior verbal fluency and advantages in memory. The incidence of left-handers have been found to be very high in some categories as artists, musicians and mathematicians [47, 48].

Creativity has also been reported to be linked with left-handedness [49]. It has been shown to be linked more specifically in men [50]. The proportion of left-handers also appeared to be greater in gifted children (IQ>131) than in non-gifted children. Handedness distribution of a group of 578 gifted elementary school children (IQ=132) was compared to the handedness distribution of 391 non-gifted children (IQ<132) and found the gifted group to be significantly less right-handed than the non-gifted peers [51] . A few studies have considered that left-handers could have special talents that could lead to benefits, such as enhanced musical or mathematical capacities [44, 52-54]. All these advantages may play a significant role in the social status of left-handers.

1.1.4(c): Left-handedness as a detrimental trait

Some researchers have postulated that left-handedness is a variant which could result from early-life brain damage [55]. Some studies have shown that left-handedness is associated with poorer health or reduced longevity [39, 44]. Left-handedness has been reported to be common in some disorders which presumably might reflect developmental abnormality. These include neural tube defects, autism, psychopathy, cleft palate syndrome, stuttering and schizophrenia [56-58].

It has been observed that left handed children experience problems early in life, because they have not yet fully adapted to being in a right-handed world, and once they adapt, they do better [59]. Left-handedness has been reported to be common in disorders that presumably reflect developmental abnormality. It has been found to have twofold increase in the frequency of left-handers with central nervous system disorders (e.g. schizophrenia, epilepsy, mental retardation or learning disabilities). They claimed that early brain insult may cause the individual to switch to the opposite hand for unimanual activities [(60)].

The behaviour of the left-handers and their interaction with the environment might place them at a higher risk than their right-handed peers. This is reflected in the numerous reports that left-handed individuals are more clumsy, with suggestions that they may be more accident prone [(61)].

An unflattering and somewhat extreme portrayal of this comes from Burt who noted that:

> "Not infrequently the left-handed child shows widespread difficulties in almost every form of finer muscular coordination…they shuffle and shamble, they flounder about like seals out of water. Awkward in the house, and clumsy in their games, they are fumblers and bunglers at whatever they do (p. 287) [(62)] ".

This reputation for clumsiness seems to be responsible for the difficulties that left-handers have in a right-sided world. Left-handers were at increased risk of accident-related injuries [(63)]. In the study by Halpern and Coren, left-handers were more than five times likely to die of accident-related injury than were right-handers [(40)] .

The preceding section suggests that the left-handed phenotype may be associated with reduced survival fitness in surroundings in which the constructed environment favours the comfort and safety of the right-handed majority. Alternatively, it is possible that the reduced survival ability of left-handers is not due to handedness and its associated behaviours alone, but rather due to the factors that underlie the appearance of left-versus right-handedness. In this sense, left-handedness would simply serve as a marker for other factors that affect longevity.

One theoretical possibility is that left-handedness is a marker for a particular genotype which is associated with reduced longevity. Empirically, however, the idea of left-handedness as a genetic marker does not seem to hold up well. Since the early 1900s, many articles have evaluated handedness patterns as a function of familial relationship in the hope of determining the nature of any possible genetic contribution to the handedness phenotype. Despite large samples, most studies have concluded that there is no compelling evidence to support the notion that right- and left-handedness is under simple genetic control [64-67].

It is evident that there are some insufficiencies in the simple genetic explanation for handedness. The alternative suggestion is that, natural left-handedness could be caused by factors that are associated with the intrauterine environment, such as foetal position during gestation. It has been demonstrated that children born from the right occiput anterior position, are more likely to be left-handed than children born from the more common left occiput anterior position [68, 69].

The theoretically important suggestion is that, for natural right-handers, the presence of some forms of trauma or pathology may cause shifts away from dextrality. This occurs because the physiological structures that support dextrality, such as contra lateral neural control mechanisms and hemispheric specialization processes, or the normally expected cerebral asymmetries can be altered by neurological insult, either directly, or through secondary effects resulting from a disruption or alteration of the usual maturational processes. However, there seems to be some consensus that the perinatal environment is of great importance, and it is this period that has received the most attention in the study.

As left-handedness may be caused by pathological factors, sinistrality becomes a statistical marker for the possible existence of some form of neural pathology or developmental abnormality. It is then logical to suggest that it is the same pathology that caused the left-handedness, which might be the causal mechanism for the shorter life span observed in left-handers. This may operate by reducing physiological fitness through direct or secondary mechanisms.

1.1.4 (d): Developmental aspects of left-handedness

In the following section, I have tried to explain the developmental aspects of left-handedness. Two main factors were identified as neuropathological and maturational and these are explained further.

1.1.4(d- i): Neuropathology and left-handedness

Some forms of neuropathologies, abnormalities, damage, or lesions might be incurred during a stressful birth. This could possibly result in a shift in handedness. One approach begins its speculation by using handedness and other measures of lateral preference (foot, eye, and ear) as an index of brain organization. It is well known that motor control is mediated by mechanisms in the contralateral cerebral hemisphere [(70)]. For the right-hander, motor control of the dominant hand resides in the left-hemisphere. The specific linkage between lateral preference and stress is based upon earlier suggestions that the left cerebral hemisphere is more subject to damage than the right hemisphere of the brain [(71-73)]. Because of the contralateral control of the limbs, such damage to the left hemisphere would be expected to result in hypofunction of the right hand. This can cause a naturally right-handed individual to develop a left-handed preference.

The Apgar scoring system is a comprehensive screening tool to evaluate a newborn's condition at birth [74]. Low Apgar scores have been shown to be associated with hypoxia and increased incidence of neurological abnormality [75]. Schwartz reported that left-handedness was associated with lower Apgar scores at birth [76]. Regardless of the specific mechanism, however, the general notion of a shift in handedness toward sinistrality is supported by Liederman. After reviewing the extensive literature, he concurred that the left hemisphere is more vulnerable to damage than the right [77]. Hence, it seems that left-handedness could be a marker for mild or otherwise difficult to detect instances of neuropathology.

The link between left-handedness and neuropathology could be explained by the possible role of hormonal factors associated with the intrauterine environment. This theory is based on the presumption that the prenatal sex hormones exert powerful influences on the central nervous system of developing organisms [78]. These hormones direct and reflect the sexual differentiation of a foetus. Disruption to the normal neural development could be due to the circulating high levels of testosterone (or progesterone) during foetal development. The heightened sensitivity to these prenatal sex hormones cause a number of physiological changes, and also result in an increased likelihood of sinistrality [78, 79].

There are two sources of prenatal testosterone. The maternally produced testosterone comes from the maternal ovaries, adrenals, and other structures such as fat and, for male fetuses, testosterone is produced by their own developing testes. Thus, males are exposed to higher levels of prenatal testosterone. On the basis of this theory, numerous studies have found a higher proportion of sinistrality in males than in females (61, 80, 81). Females have been shown to be more strongly right-handed and more consistently right-sided (82).

1.1.4(d-ii): Maturational factors and left-handedness

An interesting alternative to the hypothesis that it is neuropathological damage caused by birth-related stressors that causes left-handedness is based on the possibility that the effect of the stressor is to alter the normal maturational pattern antenatally, hence resulting in left-handedness.

Maturational factors seem to be evident in early childhood manifestations of manual dominance. It has been agreed that this childhood trend toward increasing right-handedness is a secondary consequence of the normal maturational processes. It has been suggested that both cerebral laterality and handedness are under the control of a maturational gradient (83-85). According to this view, an asymmetry in growth rate manifests itself in more rapid development on the left side of the human brain. Because of the contralateral neural link between brain and limb control, this maturational gradient could result in the emergence of a preference for the right side. Other researchers have suggested that each part of the cortex possesses its own developmental time table, and that it is differences in the rate and duration of the growth that produce the structural asymmetries in the cortex that support normal right-handedness (78, 86).

Additional evidence that left-handedness may be associated with a maturational lag has come from some other clinical groups. There is evidence that males with 47, XXY Klinefelter's Syndrome have slower than normal maturational growth rates postpartum[87, 88]. It is possible to speculate that such an atypical or immature physiological system might lack the vitality of the normally developed system, hence decreasing the viability of the individual.

1.1.4 (d-iii): Pathological left-handedness:

The existence of associations between left-handedness and various health problems have often led to a distinction being made between pathological left-handedness, which would arise from developmental stresses and familial left-handedness, which would be due to genotype [89].

Satz et al have represented a model that describes how left-handedness may develop in more than one way [60]. They suggested that for the majority of left-handers, left-handedness is determined by genetic and/or environmental factors. For a smaller group though, left-handedness reflects neurological trauma and is reflected as pathological left-handedness. They have explained that this group includes natural right-handers for whom an early left-hemisphere injury causes motor impairment of the contra-lateral hand and thereby leads to a shift in hand preference.

1.1.4(e): Health categories/immune problems:

The hand preference of one over the other (left over the right) could also contribute to a range of health and immune problems. I have explored this in the following section.

The idea that a variety of developmental and health disorders are related to hand preference was given a theoretical underpinning by G/B/G theory (Geschwind/B: Geschwind and Behan, G/G: Geschwind and Galaburda) [79, 86]. The general underlying rationale of the theory was that left-handedness is a marker for "something not quite right". G/B/G theory proposed that testosterone, the male sex hormone delays the maturation of parts of the left hemisphere, resulting in the corresponding regions of the right hemisphere and those unaffected regions on the left developing more rapidly. As a result of depressed left hemispheric growth, verbal skills were reduced, increasing the risk of developmental language disorder; while rapid development of the right hemisphere will enhance those skills traditionally thought to be enriched in the right hemisphere, i.e. visual-spatial skills and mathematical ability. The resultant superiority of the right hemisphere may lead to right hemispheric motor control and left-handedness. In addition, they further speculated that testosterone may affect the maturation of thymus, resulting in an increased risk of developing immune disorders in late childhood and beyond.

An additional view of the sex hormone hypothesis is that other susceptible organs in the developing foetus are also affected by high testosterone and progesterone levels. One such organ is the thymus gland, which is an essential component of the developing immune system. Geschwind et al. have suggested that testosterone and progesterone diminish the size of the thymus gland during development (78). Several studies have documented similar finding (90, 91). The simultaneous effect of testosterone on the development of the left hemisphere and the thymus and other organs could have an effect on the greater incidence of immune disorders among left-handed individuals. The first evidence for this relationship was noted by Geschwind and Behan (79). They showed that autoimmune diseases involving the intestinal tract and the thyroid gland and atopic diseases (allergies, asthma, eczema, and hay fever) are seen more frequently in left-handers than right-handers. These results have been essentially confirmed in several subsequent studies (92, 93).

In humans, immune disorders can be expressed in a number of ways. One manifestation involves the lymphocytes mounting an attack on the body's own cells, resulting in autoallergy or autoimmunity. Diseases such as ulcerative colitis, ileitis, myasthenia gravis, and Hashimoto's thyroiditis would fall under this category. In a series of studies, Geschwind and Behan showed that individuals suffering from such diseases had an elevated incidence of left-handedness (79). This supports the hypothesized relationship between sinistrality and immune disorders. This was confirmed in a study of individuals suffering from Crohn's disease and ulcerative colitis. These are inflammatory bowel diseases that are often assumed to be of autoimmune origin. They reported that although only about 12% of their control sample was left-handed, among the Crohn's disease and colitis sufferers the percentage of left-handers was around 27% (94).

Immune disorders can also manifest themselves when defensive reactions occur to harmless substances – i.e. allergy. Smith showed a higher incidence of left-handedness in people who suffer from eczema, asthma, rhinitis and urticaria (93). The increasing age and assaults on the immune system could be associated not only with reduced physiological endurance, but also a lowered resistance to physiological assault. This in turn will result in premature deaths in left-handers. This hypothesis is supported from studies that have looked at other diseases such as early-onset insulin-dependent diabetes and early-onset breast cancer. Both have been found to be more prevalent in left-handers (93, 95). Ramadhani, M.K in a large prospective cohort study provide evidence for a substantially increased breast cancer risk among left-handed women. The connection between hand preference and breast cancer risk may lie in a common origin of intrauterine hormonal exposure (96).

These studies suggest that handedness, far from being simply a developmental variant, may have significant health implications. Therefore uncovering the factors which determine handedness is an important task.

1.1.5: Causative factors of handedness

Much work has focussed on the factors which cause handedness and the variation in exhibiting this trait between humans. The environmental and genetic factors have been considered (97).

The environmental factors may be important in the prenatal and the postnatal periods. In the perinatal period, the human brain is susceptible to adverse environmental influences such as hypoxia. Birth trauma and prematurity which may result in brain hypoxia are proposed to cause left-handedness. In addition, after birth (postnatal period), the social environment (cultural, educational, physical) may alter the existing innate handedness (96).

1.1.5(a): Environmental factors

There are many theories as to why handedness exists. This would range from cultural to more biological explanations. One historical theory suggests that ancient warriors who held their swords in their right hands and their shields in the left were more likely to survive (because the shields covered their hearts). According to this theory, right-handedness became the norm over time by natural selection (98).

Structural asymmetries of the brain appear in utero and are statistically related to hand preference (99). The prenatal factors are the birth risk factors associated with pregnancy. It is likely that handedness can be influenced by intrauterine and postnatal factors.

Some of the prenatal factors which have been explored previously include birth order, parity, presenting position of the foetus at birth, mode of delivery, maternal age, paternal age and season of birth [68, 100-102]. Badian showed that the birth order effects on handedness were stronger for males, and could be observed at all maternal ages [101]. The birth presentation of the child did not have an effect on laterality [103].

It has been postulated by Bakan that left handedness might be a result of stress during pregnancy or delivery. He suggested that this could be a result of left hemispheric pyramidal motor dysfunction following perinatal hypoxia. This hypoxia lead to brain damage and this can lead to a switch in hand preference from the right to the left side [104]. However, the hypothesis that birth stress is a potential cause of left handedness has not been supported in other studies [105-108]. Such inconsistencies in epidemiological studies could be contributed by the difference in the assessment of handedness and also retrospective collection of birth stress factors by the subjects and their mothers. This would introduce an element of subjectivity and also the reliability of the data based on their memory becomes questionable.

Of the prenatal factors which have been studied previously, birth weight is important as it has been shown to influence the cognitive development of the foetus [109]. Low birth weight is associated with perinatal complications, neurological problems and a number of adult pathologies [110]. There is evidence for an excess of left-handedness among extremely low birth weight babies [111, 112]. Low birth weight could play a key role in the health problems associated with left-handedness. The mechanism that has been proposed most frequently to explain an association between left-handedness and low birth weight involves early brain damage [113].

The prenatal environment is essential for both the physical and the mental development of the foetus and this is compromised in twins as they share their pre-natal environment. Twins whether monozygotic or dizygotic are much more crowded inutero and foetal movements are more restricted when compared to the single born child. It has been suggested that twinning and left handedness share causal elements, some of which may be heritable (114).

There is strong evidence that prenatal testosterone contributes to brain organization. One theory is that high levels of prenatal testosterone results in a higher incidence of left-handedness. This could account for the increased incidence of left-handedness in male twins (78). This theory has however been contradicted in a study by Elkadi and others, which found no differences between the opposite and same-sex twins in the measures of the strength of hand preference and the incidence of sinistrality (115). But subjects for this study were selected in an unusual and seemingly inappropriate way to enhance the proportion of left-handers in the study. Tested subjects from half of the twin pairs were selected from a larger sample using the criteria that one twin within each pair indicated a left hand preference. Such selection makes generalisation to comparison of handedness within a population-based sample of same- and opposite-sex female twins uncertain.

Also, what is important is that we look into the factors which determine the intra-uterine environment in twins and one of them has been shown to be chorionicity (116). The other factor which contributes to intra-uterine existence is the umbilical cord (117). Abnormalities in the umbilical cord could result in unfavourable outcome after delivery. These include: abnormalities in length and knots which results in reduced food supply (118-120). Any compromise in the intra-uterine environment would be detrimental to the physical or mental development of the foetus. About 1 percent of babies are born with one or more knots in the umbilical cord [121] . Knots occur most often when the umbilical cord is too long and in identical-twin pregnancies. The relationship between the umbilical cord knots and handedness, if any would have clinical significance and would be complementary in understanding this complex phenomenon. The postulation that left twisting in the umbilical cord might be related to handedness was suggested by Lacro et al. It was not supported by their data later [122] .

1.1.5(b): Genetic factors

The notion of a genetic basis for handedness has waxed and waned and controversy remains on the relative contribution of environmental and genetic factors to variations in handedness [123, 124].

In order to understand the genetic contribution, I have explained handedness due to familial contribution (the role of families) and also have included a genetic model to explain handedness.

1.1.5 (b-i): Handedness in families

The study of handedness in families is a first attempt to characterise the mechanism involved, since it allows assessment of the transmission of this trait across generations.

Two right-handed parents produce fewer left-handed offspring than parents with any other handedness combination and two left-handed parents produce the highest proportion of left-handed children, i.e. approximately 30–40% [125, 126]. This suggests that hand preference could be transmitted by parents to their children, either at a genetic or learning level.

There is a higher prevalence of left-handedness in children of right-handed men and left-handed women (RxL- right x left mating) than left-handed men and right-handed women (LxR- left x right matings) thus suggesting stronger maternal effects on offspring handedness [64, 126, 127]. Such a finding could result from a sex-linked genetic effect, or from a greater social influence likely to be exerted by the mother on the child. The fact that handedness runs in families is not convincing evidence of a genetic component, since parents also transmit a particular environment to their offspring.

The transmission of genes can be distinguished from the transmission of environment by means of adoption studies. Carter-Saltzman examined the effect of biological and socio-cultural effects on handedness. He compared handedness between biological and adoptive families. Complete handedness information was available from 286 adoptive families and 205 biological families. Children were between 16 and 22 years of age. Hand preference in older subjects who were 16 years of age or older was assessed by Oldfield's handedness inventory. Children between 4 and 16 years of age were assessed individually with a handedness kit that required them to act out each item on the handedness inventory. A handedness index score was computed by dividing the difference between right and left responses by the total number of responses and multiplying the ratio by 100. All subjects whose handedness index scores were at or below 0 were classified as left-handed and those who scored above 0 as right-handed. Parental handedness was significantly related to offspring handedness distributions within the biological ($p<.05$) but not the adoptive sample. This suggests that effects of shared biological heritage are more powerful determinants of hand preference than any of the socio-cultural factors [128].

1.1.5 (b-ii): Genetic models of handedness

Models of handedness generally assume a genetic basis to both laterality and hemispheric asymmetry. Causal models involving a single gene with major effects have been proposed, most influentially Annett's 'right shift theory' and McManus' model, which are based on a single hypothesised gene with two alleles (rs+/rs-: rs+ have a strong liability to right-handedness and rs- lack such a liability and have greater probability to be left-handed) [126, 129].

Analysis of hand preference and hand use on 1818 nuclear Hawaiian families failed to fit any fully genetic model of handedness and suggested that handedness phenotypic variation could be 10–20% explained by genetic causes and 80–90% by environmental causes. [130]. Nevertheless, there are a number of observed associations that are difficult to accommodate within any simple genetic model. The failure of these genetic models to fit the data indicates that the genetic determination of handedness is not simple and may imply several genes or other unidentified factors.

Genomic regions identified to be linked to handedness differed among studies, probably due to differences in the measurement of handedness [131-136]. This suggests that several genes could influence handedness. Therefore, large studies with better genome coverage are needed to clearly identify the genes implied in relative hand skills and hand preferences.

1.1.5 (c): Cultural and social pressure

There are strong cultural pressures against left-handedness in many societies. Such pressures, although relaxing to a certain extent in contemporary western societies, still remain.

Hertz has quoted that:

> "One of the signs which distinguish a well-brought up child is that the left hand has become incapable of any independent action" [137] (p. 5).

Parsons has quoted:

> "Left-handedness is cured among pupils." It describes the pressure placed on students (particularly in their writing behaviours) and states "An intensive campaign to cure left-handedness among pupils in local schools has resulted in a reduction from 250 to 66 since 1919" [138].

Porac et al. found that a high proportion of initially left-handed individuals (approximately 71%) have been subjected to social pressure from parents, teachers, or others to shift their handedness from left to right [139]. If all of these were successful, it would result in a shift of about 8% in the population toward dextrality. However, such a shift in the pattern is not observed. For females, the reported success rate in shifting from left- to right-handedness is 2.1%, whereas for males the success rate is only 1.5% of the population. This represents individuals that have been shifted from left- to right-handedness. This value is close to the 3.5% successful change of handedness reported by Leiber et al [140] . It has been shown that attempts to change hand usage are usually quite specific to a single activity and, even when successful, do not tend to produce a generalized effect [37, 61, 141] .

Kloppel et al examined 34 people who were forced to become right-handers between the ages of 22-59. Brain scans were performed using MRI. Their hypothesis was that if handedness was purely genetic based then even forced right-handers should display brain scans similar to left-handers. But on the contrary, forced right-handers displayed brain development similar to native right-handers. This tells us that environment does play a role in the development of handedness [142].

To summarise, both environmental and genetic factors play a role in determining handedness. More precisely, the gene-environment interaction aided by cultural and social factors seem to have an influence in causing the hand preference in an individual.

1.1.6: What are Twin studies?

Twin studies have been very useful in assessing the relative contribution of genetic and environmental factors to complex phenotypes [143]. Monozygotic (MZ) twins originate from the same zygote and so share the same genes, while dizygotic (DZ) twins are genetically as similar as siblings. By comparing the extent to which individuals within MZ and DZ twin pairs exhibit the same trait (are concordant), the extent to which genes and the environment influence that trait can be assessed. This is called the heritability (h2) of the trait. This can be defined as the extent to which genetic individual differences contribute to individual differences in observed behaviour. More about the contributions of twin studies is given in section 1.3.3. Furthermore, it has been suggested that the increased intra-uterine and higher perinatal risks experienced by twins lead to an unusually high rate of pathological left-handedness which arise from developmental stresses [39, 144]. This makes twins particularly suitable subjects for studies of perinatal factors and handedness.

Twin studies of handedness provide compelling evidence against a simple, strong genetic determinant for handedness. If there is a genetic component in handedness, monozygotic twins would be more likely to have concordant handedness than dizygotic twins. However, numerous twin studies have shown no significant difference in handedness between the monozygotic and dizygotic twin pairs [70, 145-149]. This suggests that variations among individuals in their phenotypic handedness cannot be explained by simple genetic mechanisms. If handedness itself does not seem to be transmitted genetically, then it certainly makes the argument that left-handedness is simply an indicator of some inherited factor that is associated with increased biological risk.

1.1.7: Measurement of Handedness: Preference and Performance

To determine the causes of handedness, it is first necessary to define or measure handedness. There are two conventional ways of describing the handedness characteristics of individuals: (i) by determining the hand preferred in carrying out a range of manual activities, and (ii) by recording the different achievements of the two hands on each task. Both methods have their advantages and disadvantages, the preference approach usually employs a 10 or 12 item questionnaire which enables a good deal of information to be obtained on large numbers of subjects in a short period of time. Measuring proficiency allows for a much more precise description of differences between the hands but generally requires individual testing and is time consuming [150].

The Preference measure (questionnaire approach – "Which hand do you use to do a range of activities"), places demand on the cognitive process such as memory, while the performance-based approach is much more immediate and task oriented with less focus on memory and more focus on achieving a specific goal.

Preference can be assessed adequately by means of a self-assessment questionnaire in which a subject is asked to indicate which hand is used to throw a ball, to hold a hammer, to draw and so on. The three best known handedness inventories are those of Crovitz and Zener, Annett and Oldfield [151-153]. The details of how these questionnaires were scored is given in the literature section 1.3.5. A Dutch language questionnaire (Strien) was the Preference test used in my study and the details are discussed in the Methods section 2.2a.

Performance measures refer to the greater muscle strength and skillfulness of one of the two hands. Muscle strength in each hand can be assessed by having subjects perform motor tasks, such as peg moving ,finger tapping, or dot filling task [129, 154, 155]. These are explained in the section 1.3.5.

The Card reaching test (Quantification of Hand Preference -QHP) is a Performance test where hand preference is quantified by a participant's readiness to reach across the body's midline into contralateral space [2]. This helps to determine the consistency in the hand preference and it places a demand on the motor component. This was the Performance test used in my study the details are discussed in the Methods section 2.2b.

The terms 'direction', 'degree' and 'strength' of hand preference needs to be explained here. Most of the questionnaires measure handedness as a binary variable (right and left). This dichotomy hypothesis implies that there are no theoretically important differences between subgroups of right-handers, and that the overall 'direction' is the only aspect of hand preference that has neuropsychological significance. The 'degree' refers to the proportion of different tasks for which the preferred hand is preferred (i.e. between-item consistency of hand preference). The 'strength' of hand preference refers to within-task consistency, i.e. the extent to which a person will always prefer a given hand for a specific task [(156)].

The two methods of measuring handedness may be tapping into different aspects of brain function [(157)]. Handedness may therefore not be a unidimensional trait. It would be interesting to determine handedness using multiple measures of both preference and performance.

1.2: East Flanders Prospective Twin Survey (EFPTS)

The perinatal factors and the umbilical cord characteristics used in the present study come from EFPTS.

This section will explain in detail about the history, aim, methods, subjects and results of EFPTS.

The information about EFPTS presented here was taken from the published article which can be accessed at: DEROM, C., VLIETINCK, R., THIERY, E., LEROY, F., FRYNS, J. P. & DEROM, R. (2006) The East Flanders Prospective Twin Survey (EFPTS). *Twin Res Hum Genet,* 9, 733-8.

<u>1.2.1: What is EFPTS?</u>

The East Flanders Prospective Twin Survey (EFPTS) is a prospective, population-based registry of multiple births in the province of East Flanders, Belgium. [(158)]. (See Fig1-1: Map of East Flanders region).

EFPTS has several unique features: it is population-based and prospective, with the possibility of long term follow-up; the twins (and higher order multiple births) are ascertained at birth; basic perinatal data recorded; chorion type and zygosity established; and since 1969 placental biopsies have been taken and frozen at –20 °C for later determination of genetic markers. The EFPTS is the only large register that includes placental data and allows differentiation of 3subtypes of monozygotic (MZ) twins based on the time of the initial zygotic division: the dichorionic– diamnionic pairs (early, before the 4th day after fertilization), the monochorionic–diamnionic pairs (intermediate, between the 4th and the 7th day post fertilization), and the monochorionic–monoamnionic pairs (late, after the 8-day post fertilization).

<u>1.2.1(a): History of EFPTS</u>

The East Flanders Prospective Twin Survey (EFPTS) was started in July 1964 at Ghent University, Department of Obstetrics, by Robert Derom (RD) and Michel Thiery, a twin himself. The founders became interested in twin surveys when one of them (RD), studying foetal oxygenation during labour, unexpectedly discovered that, as a rule, the second-born twin suffered from a low degree of intrauterine hypoxia [(159)]. The EFPTS adapted the design used in Birmingham Twin Survey [(160)]. This helped to establish a long term cohort for the multiple births in the region.

The Birmingham twin survey was initiated by obstetricians and midwives in the Children's hospital in Birmingham to record the birth characteristics of the twins. Zygosity was determined by the examination of blood groups, placental membranes and also by the identification of the placental enzyme, mainly alkaline phosphatase. Postnatal growth and development of the twins were also assessed by the medical team [160]. Both the antenatal characteristics and postnatal physical and mental developmental milestones of the twins were recorded in the survey.

In 1989, EFPTS moved to the Department of Human Genetics of the University of Leuven (Belgium). At present it is hosted by Twins, a non-profit Association for Scientific Research in Multiple Births. It is currently partly funded by the Department of Human Genetics of the University of Leuven, Twins, the University of Maastricht (Netherlands) and, since 2005, the Province of East Flanders.

1.2.1(b): Aims, Subjects and Methods of EFPTS

This section offers explanation about the aims and the way subjects are recruited in the EFPTS study. In addition, this also mentions the way of contact established with the parents of the multiples.

The prevalence of multiple births in a well-defined geographic area, and the number of mono, dizygotic multiple births are determined. The obstetrical (duration of pregnancy, pregnancy and birth complications, birthweight, induction of ovulation) and/or in vitro fertilization (IVF) and related techniques are also explored. Paediatric outcomes (intrauterine growth, congenital malformations, perinatal and infant morbidity and mortality) and other phenotypes such as behaviour, learning and school problems and intelligence at a later age are also evaluated. In addition to this, investigation of the causes of the multiple pregnancy and the influence of zygosity and moment of zygotic division on the investigated traits is carried out. The use of improved methods of multiple birth studies is not only used to determine the genetic predisposition of normal traits, diseases and malformations but also the role of the environment (with special emphasis on the prenatal environment) which includes both individual-specific as well as common environmental influences.

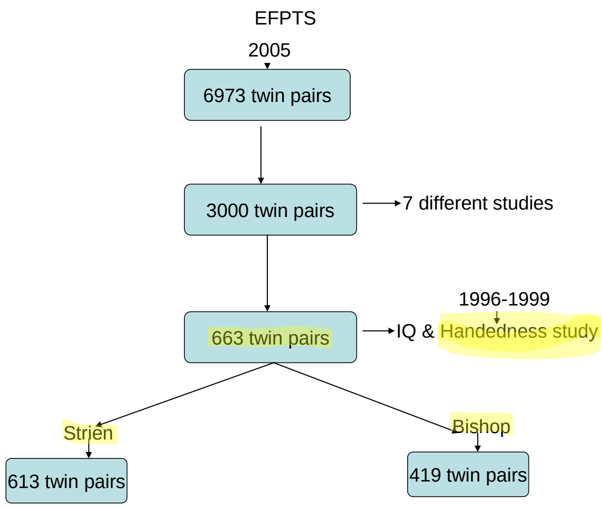
All the multiple maternities, where one of the children weigh at least 500 g or, if birthweight is unknown, where the gestational age is atleast 22 weeks and with birth in the Province of East Flanders, are eligible for inclusion in the survey.

The specific methodology of this survey is to determine the zygosity of each multiple birth with near certainty to certainty through examination of the placental membranes and vascular anastomoses, blood groups and DNA-fingerprints (if necessary). This is done by the collection of medical data by the gynaecologists and the neonatologist, and enhanced by the follow-up of the multiple through one of the studies and/or questionnaires. The establishment of a three-generation pedigree through civil birth registries is also achieved.

The families of the multiples are contacted by a biannual newsletter. In addition, a TWIN hotline is managed by a psychologist, who is backed by a team of physicians and specialists. This is to help the public and the parents of twins with psychological, educational, medical or practical problems. Three general meetings per year and evening meetings where the parents of multiples meet each other are also organised. In addition, topics which most concern the parents such as language development, behaviour and the development of individuality, schooling and education are discussed in several meetings throughout the year. EFPTS and the Association for Scientific Research in Multiple Births (TWINS) jointly provide these services to families with multiple births. Most of these services are carried out by volunteer workers.

Some of the twins and their family members are invited periodically to participate in specific follow-up studies.

1.2.2(a): EFPTS COHORT:



Flow chart 1-2: The EFPTS COHORT

From the above flow chart, we can see that between 1964 and 2005, 6973 twin pairs were registered and investigated. In addition, 214 triplets, 15 quadruplets, 5 quintuplets and one octuplet were registered. More than 3000 twin pairs have been enrolled in up to seven different studies. The parents and siblings of the twins were also examined in some studies.

Of particular relevance to the current thesis is a study that was carried out in 1996-99. In this, 663 twin pairs (1326 twins) were invited to participate in an IQ study and handedness was also measured after getting consent in a sample of 1257 twins. These are the subjects of the current thesis [161].

1.2.2(b): Study of handedness from EFPTS

It is generally claimed that the prevalence of left-handedness is higher in twins than in singletons, and for a long time discordant handedness was used as a criterion of monozygosity, in the belief that it represented mirror-imaging [162]. It has been shown since that discordance of handedness is as frequent in monozygotic as in dizygotic twins [126].

In the course of the EFPTS, handedness was assessed as part of a genealogical study [163]. Handedness was examined in 1616 twins (808 twin pairs) aged 6 to 28. Handedness was assessed by asking the following question to the parents of the twins: "Do you consider your children as right-handed or left-handed?" In 80% of the cases (N=1292) a second question was asked: "Has this always been the case?" These were asked during a telephone call of approximately 15 minutes, which was carried out by scientific staff as part of a three-generation genealogical study. Twins with unknown zygosity or chorionicity were excluded. In this sample of 1616 twins, DZ was 54%, MCMZ was 31% and DCMZ was 15%. The frequency of left-handedness was 157 (15%) in DZ, 79 (16%) in MCMZ and 43 (18%) in DCMZ twins. They reported findings as: handedness of the twins themselves ($p>0.3$) and within-pair differences as ($p>0.5$). The authors concluded that a higher frequency of left-handedness was seen in twins (17%) and it seemed to be independent of zygosity and chorionicity and the belief that discordant handedness in MZ twins represents mirror-imaging is mythical also.

1.2.3: Summary of EFPTS

In conclusion, EFPTS is the ideal resource for the long-term follow-up study of multiples in a population-based manner. Previous twin studies have failed to take chorion type into account, because retrospective determination of the placental anatomy is not possible. No large twin studies on handedness have taken chorion type into account.

The above information about EFPTS clearly justifies the use of the data from it to explore the possible relationship between the perinatal factors and handedness.

1.3: Literature search

1.3.1: Outline

In PUBMED, the search term handedness yielded about 40,000, the term Twins about 33,000, Perinatal factors about 14,500 and Umbilical cord factors about 31,000 results. Owing to the enormous results obtained, the MeSH database was used for the subsequent searches. The terms Handedness, twins and one of the perinatal and Umbilical cord factor were restricted to MeSH (Medical Subject Headings). The terms Handedness and twins got about 55 articles. In this, when birth weight was added to the search, it gave about 2 articles and the term birth order came with about 9 articles. I explored the articles with factors of interest to me, but have looked into all the articles which have explained handedness and have used and referenced them accordingly. The search was carried out in 2009. The years of publication ranged from 1921 to 2009. Since then, I have registered with the NCBI (National Center for Biotechnology Information) at the U.S. National Library of Medicine. This facility has enabled me to get updates on my topic of research.

Below is a compilation of work done in handedness based on birth order, birth weight, umbilical cord twists, and chorion differences. I have included a critical review of handedness in twins. I have also looked into articles which have explored the best ways of assessing handedness.

1.3.2: Handedness, perinatal (birth) factors and umbilical cord characteristics

1.3.2(a): Handedness and birth factors

One of the possible potential pathological factors in determining handedness would be birth factors, which I have used as a collective term for factors such as: birth order, birth stress factors (which would include difficult labour, mode of delivery, birth position), birth weight, and chorion type.

1.3.2(b): Handedness and birth order:

The birth order of a child (1st or later born) could be implicated in determining the hand preference later. Bakan proposed that left-handedness might be a result of 'neurological insult associated with pre-natal or delivery factors'. Bakan showed that birth ranks (1, 4+: first born and a child born as the 4th in the family), had a higher frequency of left-handedness than the lower birth ranks (2, 3: born as the 2nd and the 3rd child in the family). He showed that 59% of 95 were left-handed and 45% of 553 were right-handed in his sample of singleton students. The incidence of left-handedness was also raised in the children of older mothers (greater than 30 years). He showed that birth stress and left-handedness may be related to a variety of genetic or environmental factors, such as maternal physique, parity, nutrition, drug ingestion, smoking or even pain sensitivity which may influence the amount of anaesthetic used [104].

Dusek and Hicks failed to find a birth order effect. The relationship between a set of birth risk factors (parity and maternal age) and handedness was examined in a set of 600 elementary school singleton children and none of these birth risk factors were significantly related to handedness [164].

Christian et al found a significant relationship between birth order and handedness in monozygotic twins and also found an excess of left-handedness among first-born twins (n=104 twin pairs, $p<0.01$). The birth order in twins is not the same thing as birth order in singletons. The twins used in their sample was six years of age or older and they differed in their handedness when compared to their co-twin. No such relationship was found in dizygotic twins. The authors suggested that this could be due to genetic factor causing handedness as the significant result was found in monozygotic twins and they share more between them than the dizygotic twins [165].

Handedness was examined in relation to birth order and maternal age for 1,097 subjects and in relation to the season of birth in 1,186 kindergarten singleton children. Tasks on which hand preference was observed included three pencil and paper tests (name writing, human figure drawing, geometric form copying), cutting with scissors and throwing a ball. Handedness was defined as right or left, if only one hand was used and as mixed, if they switched hands between tasks. Questionnaires filled out by most parents gave the birth order, maternal age for 1,097 children and the date of birth was available for all 1,186 subjects. More left-handedness was seen in boys of birth orders 1 and 4 (n=282) than two and three (n=266), ($p<.01$), but maternal age did not have an effect in causing left-handedness. For each of the fall and winter months (September to February), the proportion of left-handed male births was higher (21%) than that for the spring and summer months (11.9%). Seasonal environment variables (e.g. climate, nutrition, disease, hormone levels), operating at specific times in the gestation period or early postnatal life, may be factors in the elevated rate of left-handedness observed among children born in fall or winter. For girls, no significant effects for birth order, maternal age or season of birth of handedness was observed [101].

1.3.2(c): Handedness and birth stress:

Bakan also proposed that left-handedness could be attributed to hypoxia-induced brain changes in the foetus which could be due to pregnancy and birth complications. He suggested that maternal smoking during pregnancy would produce prenatal hypoxia and which may result in low birth weight and birth complications. He also hypothesized that maternal smoking during pregnancy results in a leftward shift of handedness in the offspring. He compared the distribution of handedness in the offspring of mothers who did and did not smoke cigarettes during pregnancy. Information on maternal smoking, handedness and birth complications were analyzed for 803 university singleton students. There was a significant shift to the left in the distribution of handedness scores for the offspring of smoking mothers (n=216), as compared to those of nonsmoking mothers (n=587). Offspring of smoking mothers also reported significantly more birth complications. He concluded that left-handedness could be associated with pathological neurodevelopment (166) .

Coren employing a sample of 133 left-handed and 1165 right-handed singleton subjects and using the subjects' reports as to whether or not various birth complications from their own births, reported that the incidence of breech birth, instrument delivery, Rh-incompatibility, Caesarean birth and multiple births were all significantly higher for left than right-handed subjects (167).

The handedness of 942 singleton subjects was ascertained by a 14-item questionnaire by Tan and Nettleton. The mothers of the subjects supplied information about maternal age at birth, birth weight, and birth stress (as indicated by birth history, birth complications and assisted delivery methods). No relationship between maternal age, birth weight or reported birth stress factors or the birth order and left-handedness was found. The results have to be read with caution as the birth factors were subjectively filled in by the mothers and the reliability becomes questionable [108].

McKeever et al studied the relationship between handedness and pregnancy and birth risk factors (such as maternal age, parity, birth weight, preterm births, vaginal bleeding during pregnancy, maternal diabetes, maternal heart, kidney, thyroid problems, forceps use, caesarean birth, breech presentation, baby breathing difficulties at birth and prescribed medications during pregnancy). Participants were undergraduate students (singletons) from two universities in Ohio and were recruited, over a period of approximately 15 years into a number of studies concerned with language lateralisation, spatial and verbal abilities. All the participants were asked for permission to contact their mothers regarding questions about pregnancy and birth risk factors. The mothers were provided with a questionnaire that inquired about these factors. A total of 942 mothers were contacted and 805 returned reasonably completed questionnaires, for a return rate of 85.5%. Since the students were at university, the hand used for writing which was used as a criterion of handedness was meaningful.

The mean age of mothers of left-handed daughters was 26.72 years and that of right-handed daughters was 26.00 years. The analysis of variance showed that the maternal age of mothers of left-handed daughters was significantly greater than that of mothers of right-handed daughters ($p<.02$). No such effect was seen between left and right-handed sons. Chi-square analyses of the data of all daughters and sons showed that the incidence of left-handedness did not differ between those born in different birth orders. Despite the finding that maternal age was related to left-handedness of offspring, the difference in the mean maternal ages of mothers of left- and right-handed offspring was weak (only 0.72 years) [168].

Prospective studies of handedness of children for whom perinatal events were recorded at the time of birth are rare. The evidence for an association of birth stress with left-handedness has the disadvantage that it has depended on the questionnaire responses of students describing their own birth histories. Annett and Ockwell used oral reports of parents, usually mother, during visits to over 100 families to measure hand preference and skill. Family visits were made over a period of 6 years and all the families volunteered after appeals were made for the study. The sample had 175 right-handed and 84 left-handed singleton children. Observations of hand preference and motor skills were made for each member of the family and followed by routine questions about birth order and perinatal history. The criterion for left-handedness in this study was the use of that hand for writing and also history of forced dextral writing was included. They found out that Reported Birth Stress (RBS) was as frequent for right-handers as for left-handers. The incidence of RBS was greater for first born than children of later birth ranks (100).

McManus I.C conducted two surveys to study the relationship between left-handedness and birth factors (which included maternal age, paternal age, gestational age, birth weight and birth stress factors, i.e. mode of delivery and the presenting position of the foetus). No evidence of association between left-handedness and any of the above factors was seen.

Survey I (n=936) was a questionnaire, which was completed by undergraduate singleton students who reported any known history of complications during birth. Survey II (n=1245) was by a questionnaire completed by singleton students and their parents who reported birth complications. Both the surveys were completed based on the memory of the students and their parents and will have an element of subjectivity.

No evidence for a relationship between handedness and birth complications were shown in both the surveys [106] .

1.3.2(d): Handedness and birth position of foetus:

Roos studied 486 cases in an endeavour to ascertain the relationship between the presenting position of the foetus in singletons and their dextrality. The age of the children ranged from six months to two years. Two government penny postal cards on the same sheet of cardboard were folded together. One card contained instructions to offer the child ten times each day some object he desires, to hold the desired object directly in front of the child and to record on the accompanying record card the number of times the child reaches with his right hand and the number he reaches with his left hand. The other card contained the author's address on one side and on the opposite, a record for 1 and 2 above and had the questions as: if any of the child's relatives are left-handed and approximately at what age did your child have the first tooth?

Of the 486 subjects, 81.7% were right-handed and 18.3% left-handed. Further, 81% of the LOA (left occipito anterior) position group and 79% of the ROA (right occipito anterior) position group are right-handed. The percentage of right handedness was nearly the same for both the major birth positions and the results of this study clearly seem to indicate that the dominant position of the child in the uterus of the mother is not causally related to its handedness [103].

Churchill et al reported an association between the presenting position of the foetus and left-handedness. The sample consisted of 1,102 singleton cases, all born in a single obstetric unit and recorded as having been born in the left occipito-anterior (LOA) or right occipito-anterior (ROA) position. Their handedness was observed at two years of age by examiners ignorant of the birth position. Of the 93 left-handed children, 62.4% were born by ROA position when compared to 42.7% of 836 right-handed children [68] .

1.3.2(e): Handedness and birth weight

Pregnancy and some birth related variables have been reported to be associated with left-handedness. For example, very low birthweight (VLBW), greater maternal age and infant resuscitation have been found to be associated with left-handedness.

137 VLBW children (singletons) and 162 controls (singletons) were tested by a handedness questionnaire at 12 years of age. A significant proportion of VLBW children were left-handed [112].

Reduced foetal growth is strongly associated with a number of chronic conditions later in life. This increased susceptibility results from adaptations made by the foetus in an environment limited in its supply of nutrients. These chronic conditions include coronary heart disease, stroke, diabetes, and hypertension [169]. The factors which would contribute to reduced foetal growth will often be seen more in twins as two of them have to share the same nutrients within their intra-uterine environment. Large birth weight differences can often be one indication of the transfusion syndrome and hence may provide a more convenient indication of late-splitting than detailed observations of asymmetries.

Hay and Howie examined birth weight and handedness in 16 sets of MZ (monozygotic) and 13 sets of DZ (dizygotic) twins aged between 5 and 25 years. They found out that left- or mixed-handedness was far more common in one individual of MZ pairs with birth weight differences above 450 grams than in other MZ pairs or in DZ pairs with a difference above 450 grams. This is the first report of a connection between birth weight differences in MZ and handedness [170].

1.3.2(f): Handedness and chorion type

Carlier and Spitz tested manual performance, direction and degree of laterality in MZ (monozygotic) twins of known chorion type and DZ (dizygotic) twins (8 to 12 year old). All birth records from three hospitals from 1980 to 1985 were analyzed. Ninety-six families living in France were traced. The final sample included 79 twin pairs (38 boys and 41 girls). The zygosity was ascertained using molecular genetic analyses and parents filled out a questionnaire based on physical similarities. Further DNA analyses classified 55 sets of monozygotic and 24 sets of dizygotic twins. Chorion type was established by a pathologist specialized in the study of the placenta, without knowing the zygosity diagnosis. This gave 20 monochorionic-monozygotic, 24 dichorionic-monozygotic and 24 dizygotic twin pairs. Parents signed informed consent before coming to the laboratory with the twins.

Three manual tasks were used: dot-filling, tapping and peg moving tasks. The degree of laterality was defined as the relative difference between the mean of the preferred hand and the other hand. Chi-squared test was used to compare hand preference. The raw data were adjusted for age and gender by regression analyses. In the mixed model, zygosity was considered a fixed effect and twin pairs nested in zygosity was the random effect. No chorion effect was seen. The monochorionic and dichorionic monozygotic twins differed neither for frequency of discordant pairs nor for handedness, laterality measurements, and manual performance. The sample size was small and a simple interpretation would be that the resemblance in twins and siblings in manual performance is due to common environment and the effect being higher in twins. As performances in the three manual tasks were not correlated (correlations between laterality scores were close to zero), the common environmental factor may be thought to be task specific [171].

1.3.2(g): Handedness and umbilical cord factors

Prenatal survival does depend on the function of the umbilical cord [172]. The umbilical cord plays a paramount role in intrauterine existence because it is the only connection between the foetus and mother [117]. The twist (winding in my study) is thought to arise as the result of embryonic/foetal movement [119]. Twisting of the cord seems to facilitate the turgidity, strength, and flexibility of the umbilical cord while reducing the risk of torsion and entanglement [173, 174]. The direction of the umbilical cord twist can be determined on gross examination of the placenta and, in most cases, may also be identified antepartum by sonography [175]. The coiling index, or number of twists per centimetre of umbilical cord, is relatively constant, as is the direction of the spiral [119]. Many studies have documented a strong predominance of leftward twists [119, 122, 173, 176, 177].

Lacro et al. evaluated 2801 singleton placentas and noted a 7:1 dominance of left to right twists. They postulated that the predominance of left twisting might be related to handedness, but this was not borne out by the data.The purpose of this study was to evaluate the origin, direction and relevance of the umbilical cord twist. They initially hypothesised that the direction of the helix or twist of the human umbilical cord at birth correlated with the eventual handedness of the child.

Families were initially interviewed by telephone. A handedness questionnaire by Oldfield was completed for both the child and both the parents. A medical and developmental history and complete family history for left-handedness were obtained. Each child was then examined doing coloring, figure drawing, eating, clapping, placing marbles into a small hole and placing key-shaped pegs into a grooved pegboard. The families and examiner were blinded with respect to the umbilical cord twist of the child. The results of the handedness evaluation were confirmed during a follow-up interview 6 to 12 months after the actual examination. 45 children (3 and 4 year old) with previously documented umbilical cord twists were evaluated with respect to hand preference and performance. Of the 45 children evaluated for handedness, 23 were from left twist group (17 were right-handed and 6 were left-handed) and 22 were from the right twist group (19 were right-handed and three were left-handed). There were no medical or developmental problems with either group. There were only four mothers who were left-handed, indicating that the twist is independent of the handedness of the mother. Further, the prevalence of right versus left handed mothers was not significantly different between the two groups. So, the authors concluded that the direction of the cord twist was independent of the handedness of the child as well as the mother [122].

1.3.3: What are Twin Studies?

The twin study is a potent tool for advancing the understanding of neurodevelopmental and ontogenetic aspects of behaviour. The classic twins-reared-together design includes both identical, monozygotic twins (MZ) who share 100% of their genes and non-identical, dizygotic twins (DZ), who share on average 50% of their genes. MZ twins are more similar to each other than DZ twins for a heritable trait. The heritability coefficient is the proportion of the differences among individuals within a population for a particular trait that are due to genetic differences. Twin studies are equally informative for studying environmental effects. Environmental influences are partitioned into shared environmental influences that create similarities among individuals, and non-shared environmental influences that create differences among individuals [178].

1.3.3 (a): Twin studies estimate the relative contributions of genetic and environmental factors:

The phenotypic variance of a trait at a particular age is partitioned into its genetic and environmental underpinnings. A heritable trait is not necessarily present at birth or unmodifiable. Results from twin studies suggest that the influence of genes changes over the lifetime. For example, variation in neonatal temperament (irritability, activity) is due to environment, whereas temperamental variation later in infancy is heritable [179, 180].

1.3.3 (b): Twin studies increase the power of molecular genetic designs:

Molecular genetic designs such as linkage and association studies require well-defined phenotypes that are highly heritable for good statistical power. The twin design is useful in identifying highly heritable traits that can then be subjected to molecular methods. Even more striking, the power of molecular genetic techniques is increased by using them within the context of a twin design using both MZ and DZ twins. The

DZ twins are used to determine whether or not genetic similarity at a particular chromosomal region is related to phenotypic similarity [(178)].

1.3.3 (c): Twin studies provide a unique method of studying sex differences:

Opposite-sex twins provide a unique opportunity to study the development of sex differences. Twin studies offer advantages over the traditional comparison of single-born girls and boys, because opposite-sex twins are precisely the same age, they simultaneously experience a range of family variables (such as socio-economic status, family constellation and parental attitudes) and they share on average half of their genes. Longitudinal twin studies can determine whether estimates of heritability become significantly different for boys and girls across age, suggesting that genes may also contribute to later-appearing sex differences [(178)].

1.3.3 (d): Twin studies elucidate the etiology of the co-occurrence of two or more phenotypes:

Rates of the co-occurrence or co-morbidity of childhood psychiatric disorders exceed chance. These disorders may really be components of the same overarching disorder, or they share a genetic liability. The co-occurrence of anxiety and depression in both childhood and adulthood is high. The twin design can elucidate co-morbidity by indicating whether the basis of the observed association is common genetic effects, common environmental effects or both. For example, a twin study illustrated that Tourette syndrome, ADHD (attention deficit hyperactive disorder) and conduct disorder share a common genetic influence in childhood [(181)].

1.3.3 (e): Twin studies evaluate environmental risk factors free from genetic contamination:

Twin designs offer a research context for studying unambiguous environmental influences by controlling for genetic effects on environmental measures. For example, the relationship between parenting style and child behaviour has been found to be partially due to shared genes. By controlling for the genetic component of the covariance within a twin design, true environmental influences can be uncovered.
Of course, identifying these environmental influences is important for designing effective interventions. Evidence stemming from twin studies underscores the important role of the non-shared environment on behaviours in childhood. One way to identify non-shared environmental influences is to study monozygotic co-twin differences. Because monozygotic twins are genetically identical, the environment is at the root of their differences [182].
To conclude, elucidating the genetic and environmental under-pinnings of the associations among alleles, physiological pathways and complex behaviours using the twin method has tremendous power for creating links between divergent fields (genetics, biology, psychology).

1.3.4: Handedness in twins: summary of findings

In this, I have compiled work done not only on handedness in twins, but also on other measures of lateralisation. This includes footedness, eyedness and earedness. This reference for sidedness (hand or foot) is an expression of cerebral hemisphere lateralisation. The distribution of handedness in twins is of critical importance for theories of a genetic influence on handedness. Twins have been shown to have a greater frequency of left-handedness than singletons [145]. Collins claimed that the distribution of concordant and discordant monozygotic handedness pairs approximates to that of a binomial distribution [183].

Davis, A and Annett, M (1994):

Davis and Annett showed that there were 11.7% left-handers among twin births and only 7.1% among single births in their study. In 1982, they carried out the study from individuals who were drawn from a representative national sample of households in Great Britain (n=10,777) who responded to a questionnaire. The questionnaire asked about the year of birth, sex, were they born a twin and with which hand do they normally write. They also found that the largest factor affecting handedness was age-group, with left-handers accounting for 11% of single births at age 18-30 years, but only about 2% for the 71 and older age group [184].

Sicotte, N.L (1999):

In a meta-analysis of twins and singletons, a higher incidence of left-handedness was seen in twins when compared to singletons. Sicotte et al reviewed the literature using the keywords as twins and handedness for studies from 1966 to 1999. All studies with at least 10 twin pairs were considered. All the studies had to have both the monozygotic and dizygotic twins. A total of 33 studies were included for analysis. 5 studies did not have information on zygosity and were excluded. A total of 28 studies were analysed that included handedness and zygosity information for a total of 9969 twin pairs. Most studies assigned individuals as either right or left-handed. Zygosity was most frequently determined by questionnaire and/or physical similarities. These data were analysed two ways: as individuals (n=19,938) to compare overall handedness in monozygotic and dizygotic twins and as pairs (n=9969) to compare concordant/discordant handedness in monozygotic and dizygotic twins. Odds ratio and corresponding two-tailed 95% confidence intervals were calculated for each study independently. Two-tailed p-values were also computed individually for each study again across all studies for the null hypothesis that the odds ratio was equal to 1. The logarithms of the upper and lower confidence intervals for the odds ratio were calculated and graphed for each individual study. In this eight studies individually support the hypothesis that left-handedness is more common among twins ($p<0.02$, $p<0.05$, $p<0.001$, $p<0.01$, $p<0.001$, $p<0.03$, $p<0.05$ and $p<0.05$). The results did not support the hypothesis that monozygotic and dizygotic twins have different frequencies of left-handedness. The estimated common odds ratio across all studies was 0.99 and did not differ significantly from an odds ratio of 1 ($p=0.75$), indicating that the overall incidence of left-handedness was the same in both the twin groups. This suggests that there is nothing specific about the monozygotic twinning process per se that contributes to an excess of left-handedness in twins. In twin pair analyses, left-left pairs were significantly higher among monozygotic twin pairs than dizygotic twin pairs. This finding is difficult to explain by a cultural model of handedness, as monozygotic and dizygotic twins are presumably subjected to very similar parental and societal influences affecting handedness. These results provide strong support for the hypothesis that genetic factors play a significant role in the determination of hand preference in humans [(185)].

McManus, I.C and Bryden, P (1992):

In a meta-analysis carried out by McManus and Bryden on 72,600 offspring, the left-hand proportion of both sons and daughters increased from 10% in children from right-right parents to about 20% in children from right (father) - left (mother) parents. Left-handedness among daughters did not increase when father was left-handed, but sons on the other hand were more susceptible to become left-handed if their father was left-handed. One possible explanation for this would be exposure to prenatal environmental condition [186].

Orlebeke, J, F et al (1996):

Twin family data can cast light on the longstanding problem about the influences of genes and environment on the etiology of left-handedness. So, hand preference was assessed in 1700 adolescent twin pairs and their parents. In 1989, families with a twin pair were recruited by asking all 720 city counsels in the Netherlands for addresses of twins aged 12-22 years. After contacting these families by letter, 2375 families replied that they were willing to participate and finally 1700 families returned a mailed questionnaire. The mean age of the twins was 17.8 years. Questionnaires asking about health, alcohol and tobacco use, zygosity, personality and hand preference were mailed to all the families. Hand preference was assessed in both parents and twins with one question with two choices as: "Do you consider yourself predominantly right-handed or predominantly left-handed? Parental hand preference data were complete (for both the parents) for 1388 couples. For 3326 twins, hand preference data were available for both the first and second born twins. Of the 3326 twin subjects, 471 were left-handed. In the parents, 11.3% of the fathers and 9.6% of the mothers were left-handed. There was no relationship between the left-hand prevalence of parents and the zygosity of their twin offspring (n=1430, p=.99). But when at least one parent is left-handed, then the probability that one or both of their twin children is left-handed too, was slightly enhanced (n=2751, p=.02). This appeared for sons only: for sons (n=1266 and p=.03) and for daughters (n=1485 and p=0.17). The authors concluded that both boys and girls were more likely to be left-handed when their mother was left and the left-handedness of fathers did not affect the probability of left-handedness in their daughters but did so in their sons. This could be possible if left-handedness was caused by exposure to some prenatal environmental condition (which itself can be genetically based) in the mother. The genes concerned were sometimes transferred to the offspring and sometimes not and could form the underlying basis for left-handedness "running in families". In addition, the data also suggested a y-chromosomal contribution because of the stronger association between left-handedness in father and their sons. They also speculated that, under suboptimal intra-uterine conditions, leading to growth retardation, consequent low birth weight and conditions of enhanced birth stress, the foetus was supposedly more vulnerable for such hormonal influences (187). This idea was further supported by the fact that parental handedness is associated with handedness of the twins and it suggests a chromosomal based environmental cause (126).

Rife, D.C (1950):

Rife has shown from his study on 554 twin pairs who were asked a question if they used their left hand to do any task found that there were a far higher proportion of left-handed pairs amongst those MZ twins with a family history of left-handedness than in those without a history of left-handedness (188).

Medland, S.E et al (2003):

Medland, S.E et al studied the effects of birth order, intra-uterine crowding and mirror imaging in handedness in 3657 MZ (monozygotic) and 3762 DZ (dizygotic) twins. Handedness was asked by a questionnaire (filled by both the parents and self) with two questions as: which hand would you use to write a letter and which hand would you use to throw a ball to hit a target? The participants were offered three responses as left, right or either. Parental and self-reported questionnaires showed high correlations (r=.97) suggesting that parental reports can be considered as accurate as well. Placentation was used as an indicator of chorionicity and was assessed by asking: how many placentas were there at birth? With responses as: single, joined, separate or don't know. Age (defined as year of birth) was used as a moderator variable. There were no differences between left-handedness in twins and their siblings. No influence of placentation on the prevalence of left-handedness was observed. While writing hand was highly correlated with throwing hand (r=.94), the influence of social/cultural pressures on the hand used for writing was stronger than those on the hand used for throwing. It could be possible that a range of heterogeneous environmental confounds might be acting to increase the amount of variance due to common environmental factors on the writing hand measure thereby obscuring a possible genetic effect. The authors concluded that their study found no evidence to suggest that handedness was influenced by either the experience of twinning per se or the timing of the twinning event in monozygotic twins [(189)]. The study found no evidence to suggest mirror imaging to have a major effect on the handedness of monozygotic twins as seen in other studies [(163, 171)]. The only evidence in favour of monozygotic twins having a higher incidence of left-handedness than dizygotic twins was obtained prior to 1930, when we assume that classification of laterality was not entirely independent of zygosity determination.

<u>James, W.H and Orlebeke, J.F (2002):</u>

James and Orlobeke have speculated that the hazards associated with being first-born in twin pairs (trauma) are more closely associated with left-handedness than are the hazards associated with being second-born (hypoxia). In their study, they had 6 categories of twin pairs, (monozygotic males, monozygotic females, dizygotic males, dizygotic females, dizygotic male-female and dizygotic female-male), and has shown that the first born twin was more likely to be left-handed. In the sample (n=303 twin pairs), 183 pairs of the first born twin was left-handed, $p<.0005$. They have suggested that some environmental factor plays a part and is possibly responsible for the excess of left-handedness in twins [97].

<u>Reiss, M et al (1999):</u>

Reiss et al used information on handedness, footedness, eyedness and earedness from 33 monozygotic and 67 dizygotic pairs. The age of the sample was from 10 to 25 years and all twins were born in Dresden, Germany. Zygosity was determined by serological analysis, incorporating 16 immuno-genetic tests (Department of Forensic science).

Handedness (hand preference) was assessed with a test battery including 12 tasks such as using a hammer, playing a dice, drawing and so on. Hand preference was also assessed using a modified paper and pencil performance test which as a dexterity test to be performed with maximal speed and precision to dot the circles. They got a continuous score for hand dominance ranging from -100(consistent left) to +100, (consistent right).

Footedness (foot preference) was assessed with five tasks: hopping, kicking a ball (standing), kicking a ball (sitting), drawing a figure with the foot (standing) and drawing a figure with the foot (sitting).

Eyedness (eye preference) was asked with one question: “Which eye do you use when peeping through a key hole?”

Earedness (ear preference): The twin was required to listen to a clock in a box.

Left-preference in a task was scored as one point, right as zero and no preference as a half point. This procedure established a continuous score for hand preference ranging from 12, consistent left to 0, consistent right. For foot preference, the scores ranged from 5, consistent left to 0, consistent right. In case of eye and ear preference, scores ranged from 1, left-sided to 0, right-sided. Their findings indicated that the incidence of left-handedness in twins was not found to be higher than singletons. Similar results were found for other lateralities. No differences in incidence of non-right sidedness between monozygotic and dizygotic twins were found. Their results do not confirm a genetic hypothesis of determination of sidedness in humans [(190)].

Ooki, S (2006):

Ooki looked into the factors related to handedness and footedness of twins using two of the largest databases on Japanese twins. Handedness was measured by the mother's report of which hand a twin used to write a letter and footedness by which foot a twin used to kick a ball. Placentation which could be used as an indicator of chorionicity was assessed by asking, "How many placentas were there at birth?" and mothers identified the number of placentas as one, two or don't know. The first group consisted of 1,131 twin pairs, all school children who were 11 or 12 years of age and the second group consisted of 951 twin pairs of 1-15 years of age group. The data on handedness was gathered by a questionnaire. The prevalence of left-handedness was 14% in males and 13% in females. There were no significant differences in the prevalence between identical and non-identical twins and irrespective of sex of the twin. It was concluded that factors that affect handedness or footedness such as sex, birth year, parity, neonatal asphyxia, gestational age, birth complications and family history seem to have stronger effects on handedness and footedness than being a twin, although retrospective maternal repots were used and a certain unexpected bias might have been introduced [191].

Gurd, J et al (2006):

The differences between right and left handers reported in the literature on fine motor tasks, has traditionally been interpreted relative to purported functioning of the cerebral hemispheres. However, conclusive evidence for performance differences which are intrinsic to handedness per se is difficult to obtain unless left and right handers are compared who are similar in their genetic and environmental background. To explore this further, Gurd et al employed a monozygotic twin design which minimizes differences in genetic variation between the two groups. Forty female monozygotic twin pairs were selected on the basis of discordance of writing hand. Their laterality preferences were assessed and they were tested for differences on hand performance tasks (dot filling, finger tapping and peg moving). The results revealed that, the right-handers were more strongly lateralized than their left-handed sisters and that the left-handers had greater variation in their laterality scores. However, the results have to be read with caution as the sample size was small (n=40) [192].

1.3.5 : Measurement of handedness: Preference and Performance

There are two ways in which individuals can be classified into handedness groups. The first one is done directly by measuring hand preference for unimanual activities. This employs the use of questionnaires which are subjective and memory-dependant on the participants. The second method is done indirectly through measures of hand performance, which are objective and can be used to predict levels of hand preference [15].

The questionnaire data may reflect a more cognitive aspect of hand preference and the performance-based data may reflect a motor component. Corey et al have also shown that measures of hand preference and performance may not correlate significantly with one another. But, preference scores will act as accurate predictors of self-reported handedness. According to them, preference measures yield a bimodal distribution (two distinct groups) and performance would yield a unimodal distribution, with a slight shift to the right (no clear division between the two groups).

Research utilising observational methods of measuring hand preference has found that while the direction of handedness may be established early, the strength and consistency of handedness may take longer to develop [193].

McManus and colleagues have embraced the 'dichotomy hypothesis', which maintains that handedness is a binary variable and it is a mistake to measure it on a continuous scale. They argued that because it is possible to quantify handedness, it does not mean that it makes sense to do so. They also stressed that this would be the most meaningful way of assessing handedness as long as there were no strong cultural pressures against left-handedness. The dichotomy hypothesis implies that there are no theoretically important behavioural differences between subgroups of right-handers, and that overall direction is the only aspect of hand preference that has neuropsychological significance [194, 195].

The most popular research tool in measuring handedness is the handedness questionnaire, which provides a quantitative index, rather than a binary classification, and is quick and convenient to administer, without requiring equipment or even the presence of an examiner.

Several studies have shown that, with appropriate instructions, the preferences people report on questionnaires are closely similar to the preferences observed when they carry out the same activities [196, 197]. The test-retest reliability has been shown to be reasonable [197, 198]. Three common questionnaires are commonly used in handedness research. These are: Annett questionnaire [151], Crovitz-Zener inventory [152] and the immensely popular Edinburgh Handedness Inventory [153].

The Crovitz-Zener inventory [152] is a 14-item scale. By summing across all the 14 questions, a quantitative scale is derived with a range from 14 (always right-hand) to 70 (always left-hand). The Annett questionnaire simply codes for a number of items whether preference is left or right. It does not simply sum the number of items preferred for each side, but usually groups individuals into classes depending on the specific items endorsed, with the classification being derived from association analysis [151] . In the Edinburgh Handedness Inventory, people are asked to rate their preference on a five-point scale. Rather than summing the scores, a laterality quotient is computed, as 100* (R-L)/ (R+L), where points for the right (R) and left (L) hand are given according to the strength and direction of preference [153].

The different performance tasks for measuring handedness would be: peg-moving test by Annett, tapping test by Peters & Durding and dotting within a boundary by Tapley & Bryden [129, 154, 155].

Peg-moving test: This task uses 10 dowel pegs and a pegboard and the participant who stands in front of the pegboard must move all the pegs from the back to the front as fast as possible with one hand. Three trials will be given for each hand starting with the right hand and then alternating between the hands [129].

The tapping test: For this task, the participant uses a tally counter and the thumb is used to depress a key which advanced the counter. After a practice period during which participants familiarise themselves with he counter, there are three trials with each hand, starting with the right hand and then alternating between the hands. On each trial, participants are instructed to tap as fast as possible for 20 seconds. The score was the mean number of taps registered on each trial with each hand [154].

Dot-filling test: For each trial, the participant will be given a sheet of A4 paper ruled with 140 squares, each 1cm wide and 1 cm apart. The task is to place a discrete pen mark in as many as boxes as possible in a 30 seconds time period. Trials with the right hand began at the top right hand corner of the page, went right to left along the top row, and then left to right for the next row, continuing this snaking movement down the page. A sheet of paper containing three rows of squares will be provided for participants to practice the task and to ensure that they adopted the correct direction of movement. The mean number of those boxes marked inside the box boundary across three trials will be scored for each hand [155].

A behavioural measure for quantifying consistency of hand preference was devised by Bishop et.al [2]. The task was to pick up playing cards, an activity with a substantial loading on primary hand preference. Use of the non-preferred hand was encouraged by varying the spatial position of the cards in relation to the body's midline. The prediction was that all right-handed writers would show predominant use of the right hand in reaching for the cards, but those reporting a weak preference would be more likely to switch to the left hand when cards were placed on the left of the body's midline.

Hand preference and performance are simply different forms of expression of the factor which determines the laterality of an individual [61]. Some authors have reported a close agreement between the two tests. A linear relationship between results obtained from the two measures has been reported [129, 199]. In a study of 144 university students, Lake and Bryden, found a significant positive correlation of 0.78 between the two measures of handedness [200]. Similar results were obtained by Tapley and Bryden [155]. The authors reported a high correlation of 0.75 between the two measures on a sample of 1556 university students. But when tested separately, the correlation dropped to 0.17 for the right-handers only and 0.20 for the left-handers only. They suggested that the right and left-handers might represent two distinct subsamples of the general population.

Provins et al. had explained that the preference score comes from a variety of manual activities and the performance score relates to performance on a single task [201]. Hence, the ideal practice would be to do these two different measures of handedness on the same sample.

1.4: Validation of Strien Questionnaire and Card reaching test by Bishop:

For the validation of the Dutch questionnaire, a selection of questions were made from the most reliable and valid preference items from Crovitz and Zener, Annett and Oldfield questionnaires. Two other, not previously used items (turning a key, unscrewing top) were added. Items that were ambiguous (such as top hand on broom) or that referred to gross movements (such as using glass and carrying books) were not selected. Completed questionnaires were obtained from 456 (213- male, 243- female) university students and staff members who were recruited through university newspaper advertisements. Ages ranged from 16 to 45 years. Each item was coded from 0 to 2 (0=left, 1=both and 2=right). The total score would range from 0 (extremely left-handed) to 32 (extremely right-handed). Writing hand was not included, because writing is thought to be more influenced by cultural factors [(1)].

Bishop et al, proposed a novel approach to quantify hand preference using a single behavioural measure. In this test, the participant is observed picking up cards which are positioned in different spatial locations relative to body midline. Details about the way the test is conducted is given in 'Methods' section. A simple measure of laterality can be obtained by counting the proportion of cards that are picked up with the right hand. The rationale of the test was that the person's preference for using one side is pitted against the case of making a motor movement to a particular spatial position. If a person had no preference, each card would be picked up with the nearest hand, so that cards on the left side of the body would be picked up wth the left hand, and those on the right side of the body with the right hand. The stronger the preference, the more likely is that the person will persist in using the preferred hand even when this means reaching across the midline. Hence this test gives a quantitative behavioural index of handedness as a continuum. The first step was to explore how this test compares with more traditional approaches that measure handedness (questionnaire). Undergraduate volunteers were recruited from the Subject Panel at the Department of Experimental Psychology, University of Oxford and from posters distributed around the university. Participants were selected from an initial group of 60 right-handed and 60 left-handed writers. All were paid £3.50 for participating. Bishop et al argued that it was trivial to demonstrate that a laterality index could differentiate left from right-handers and a much more stringent test was neded to see how far an index could differentiate between degrees of handedness within the right-handed population. They compared exclusive strong right-handers (n=18, those who stated they always used the right hand for more eight or more of the 10 activities, and usually uesd the right hand for the remainder) and exclusive weak right-handers (n=15, who usually used the right hand for three or more activities, and always used the right hand for the remainder), Predominant right-handers (n=18, those who preferred the right hand for the most of the activities, but who used the left hand for at least one activity) classified by the Edinburgh handedness Inventory [153]. Each participant was tested individally by the same experimenter. These groups did not differ on the relative skill of the two hands as measured by peg moving, tapping and dotting tests. The exclusive strong right-handers and the exclusive weak right-handers were indistinguishable in terms of the hand used for reaching the cards placed in different spatial locations. However, the test did discriminate group Predominant right-handers, which included some individuals who reached predominantly with the left-hand. It was concluded that performance batteries should quantify degree of hand preference and the Card reaching test shows promise as a method for providing a unitary scale of hand preference. The authors also concluded that while some questionnaire responses may be contaminated by cultural and training effects, the behavioural measures used in this test largely overcame these objections. Furthermore, this will be suitable for young children, patient groups who might show poor compliance on more demanding tasks and for cross-cultural comparisons [2].

1.5: Aim of the study

The aim of the current study was to investigate the influence of perinatal factors and umbilical cord characteristics on handedness in twins. The underlying hypothesis is that study factors indicative of increased stress during the prenatal and perinatal period will be associated with a higher prevalence of left-handedness in twin subjects.

Though the genetic aspects were not be explored in this study, the effects of zygosity was adjusted for the analysis and chorionicity was treated as an effect-modifier.

1.5 (a): What is known so far?

Different studies of handedness have yielded contradictory findings. It is hard to reconcile the different results from the different studies in the literature search section. This could be because of different subjects (singletons in some and twins in some studies) and different definitions of handedness being used. Also accounting for this would be the variable quality of data measurement and small sample sizes in some studies.

1.5 (b): What will this study achieve?

It is a comparatively large study using prospectively collected perinatal data, and so should produce reliable results. Both the preference and performance scores of handedness will be taken into account. To our knowledge no comprehensive investigation into all the perinatal factors and handedness has been conducted so far. Moreover, no study used both preference and performance scores to evaluate the relationship between handedness and perinatal factors. By using twins in the study, it will be possible to establish the relative importance of environmental and genetic influences on handedness.

1.5 (c): Objectives

Research Objectives of the study:

- To characterise and describe the EFPTS subjects who participated in this study
- To examine their handedness, using two separate testing methods
- To compare handedness results within subjects from the two methods
- Briefly to review the concordance of handedness within twin pairs
- To compare the distribution of demographic and perinatal characteristics in left and right handed subjects
- To determine how various demographic and perinatal factors affect the risk of being left handed, using univariate and multivariate statistical models (with adjustments for the twinned nature of the data)
- To determine whether differences within twin pairs may influence the risk of being left handed (for continuous factors)

CHAPTER 2: Methods

This project involved the analysis of perinatal (predictor) and handedness (outcome) data from a pre-existing cohort study of twins (the EFPTS). The contents of this database as used in this study are now described.

2.1 The data source – predictor variables

In the East Flanders Prospective Twin Survey (EFPTS), for each twin, perinatal and placental data was recorded by gynaecologists and neonatologists. Umbilical cord length, windings and knots were recorded at delivery. Gestational age was taken as the number of complete weeks of pregnancy. Birth weight was recorded from the obstetric notes within 24 hours of delivery. Other perinatal factors recorded included maternal age, paternal age, parity, delivery mode, and presentation of the twins (i.e., position in which foetus lies within the uterus). The method of conception of the twins (natural or by assisted methods) was also used as a predictor variable. Chorion type was recorded based on a standardized protocol [(202)].

The EFPTS is, to our knowledge, the only large-scale register that includes placental data and that allows differentiation of three subtypes of monozygotic (MZ) twins based on the time of initial zygotic division [(203)]. The zygosity of each twin was determined by sex, placentation, blood groups, and, since 1982, by examination of five highly polymorphic DNA markers. Different-sex twins and same-sex twins with at least one different genetic marker were classified as dizygotic and monochorionic twins were classified as monozygotic. For the same-sex dichorionic twins with the same genetic markers a probability of monozygosity was calculated using a LOD-score method. After DNA fingerprinting, a probability of monozygosity of 0.999 was reached. [(204)]. The LOD score (logarithm (base 10)) of odds is a statistical test often used for linkage analysis in humans. The LOD score compares the likelihood of obtaining the test data if the two loci are indeed linked to the likelihood of observing the same data purely by chance. Positive LOD scores favour the presence of linkage, whereas negative scores indicate that linkage is less likely [205].

2.1.1: Data preparation

A few obvious data entry errors, (mainly in dates of birth- where one twin had an improbable date which was clearly a typographical error) were corrected after careful scrutiny of the data. For some analyses, the continuous predictor variables were grouped into convenient categories based on the existing knowledge on these factors. Some categorical data were also re-classified. This is given in Table 2-1.

Table 2-1: Categorisation of the Perinatal and Umbilical cord data.

Factors	**Original data format**	**Categories used for this study**
Birth year	continuous in years	1977-1979 1980-1982 1983-1985 1986-1988 1989-1991
Mother's age	continuous in years	<25 years 25-35 years 35+ years
Father's age	continuous in years	<25 years 25-35 years 35+ years
Gestational age	continuous in weeks	32 weeks 32-36 weeks 36+ weeks
Birth weight	continuous in grams. Also examined in 100grams units in models.	<1500grams 1500-1999grams 2000-2499grams (baseline category) 2500-2999grams >=3000grams
Parity	6 categories (1-6) Ordinal (6=6 or more)	1st parity Other parity
Delivery mode	10 categories	Spontaneous & breech Instrumental & extraction Section

Table 2-1: continued.
Categorisation of the Perinatal and Umbilical cord data.

Factors	**Original data format**	**Categories used for this study**
Presenting position of the foetus	4 categories	Cephalic Breech Other
Mode of conception	4 categories	Assisted Spontaneous Unknown
Umbilical cord length	continuous in cms	<40 cms 40-60 cms >60 cms
Umbilical cord windings	4 categories	Clockwise Counter- clockwise Undefined & mixed
Umbilical cord knots	2 categories	False knots No & other knots

When the categorical variables were used in the regression models, the lowest level was taken as the baseline, unless otherwise indicated. Smaller numbers was the usual reason for choice of another category.

Umbilical Cord knots was categorised as 'false', 'no' knots and 'other'. Due to the low numbers in 'true' and 'Other' knots, this category was incorporated as "No & other" category.

2.2: Main outcome measures (Preference and Performance tests)

As part of the EFPTS investigation, 1257 twins born between 1977 and 1991 and registered on EFPTS were invited to participate in a study on handedness. In this, the total number of monochorionic-monozygotic twins was 460(37%), dichorionic-monozygotic was 14(1%) and the dizygotic twins was 783(62%). All twins were invited to the Study Centre between 1996 and 1999, where Strien hand preference questionnaire and Bishop card reaching test were administered by the research team of EFPTS [1, 2]. The measures on handedness were done as a sub-study on the twins on whom some cognition tests were administered. The inclusion criteria were that the twins must be born in the East Flanders region and both the twins had to be alive and not suffering from severe mental retardation. The participants were recruited on a voluntary basis and the purpose of the study was explained to the parents.

2.2a: Preference handedness: Strien's Questionnaire

The Strien's questionnaire referred as the 'Preference test' (Figure 2-1), ascertains hand preference for 17 common manual activities by subject's self report. The questionnaire was filled in by the twins (by the mothers for the younger twins). The questionnaire includes questions on the preferred hand used in performing specific activities such as cutting with scissors, drawing (full list given in Figure 2-1). Although the questionnaire asked about writing, the writing hand was not included as a preference item, because writing is thought to be more influenced by cultural factors than any of the other manual activities. If not sure, twins were asked to visualize the relevant activity, and if still not sure, it was assumed that both hands were used. Scores indicating handedness were assigned to each activity (left=0, both hands=1, right=2) and aggregated into a sum score. The sum score ranged between 0 and 32 for the 16 questions used. This constituted extreme left-handers (score of 0) and extreme right-handers (score of 32).

For analysis purposes, I derived a laterality quotient, by dividing the sum score of each individual by the maximum score achievable in the test.

Having defined a continuous score of 0-1, I then dichotomized the score in to left or right. This gave a handedness proportion which indicates the proportion of tasks performed by the right and the left hand (0-0.50: left-handed; 0.51-1: right-handed preference.)

2.2b: Performance handedness: Bishop's card reaching test

The Bishop's card reaching test, also referred to as the 'Performance test' (Figure 2-2), gives a quantitative behavioural index of handedness as a continuum. For this test, the subject is asked to pick up picture cards placed in front of him. Seven positions each placed 30 degrees apart from one another and within the child's reach (this varied according to the length of the arms of each child) were marked on a cardboard template (see Fig. 2-2). Position 4 is the midline. Positions 1, 2 and 3 are situated on the left-hand side of the semicircle, positions 5, 6 and 7 on the right-hand side of the semicircle. The template was placed on a table and a stack of picture cards showing easily nameable items was placed at each position. Children stood in front of the template in the centre of the baseline. The distance of cards from the central box was adjusted to be within comfortable reach of the contra lateral arm.

The subjects were asked by the experimenter to pick up a specific, named card and to place it in a box located directly in front of them. The experimenter recorded the hand used to pick up each card. No time constraints were imposed. The card order was random but the sequence of positions was the same for all participants. The child was not informed of the experimental interest in hand preference [2]. Three playing cards were placed at each position. The aim was to count the total number of cards reached by the right and the left hand. Scores were assigned as (right=1, left=0) and individual scores were aggregated into a sum score.

As in the Strien's test, a Bishop's laterality quotient (0-1) was compiled (test score/maximum test score) and this was dichotomised to determine handedness. The range from 0-0.5 was classified as left and from 0.51-1.00 as right-handers. When compared to the Strien's questionnaire, fewer twins completed the test owing to the time taken to perform this test.

A complication now arose in that the Bishop's test format varied in the EFPTS database. The number of cards used at each position varies in the literature; the earliest reports used 3 cards, but later studies increased the number of cards up to 7. In the EFPTS study, some subjects had 3 cards placed in the 7 positions giving a score ranging from 0 to 21 (0=left and 21=right handed). However, the majority of subjects had scores ranging from 0 to 49 indicating use of a larger card set, with 7 cards at each position. The data set reveals that a decision to increase the card set was apparently made two years after the start of the study.

In previous research with adults, the number of cards was doubled from 3 to 6 and an additional card was placed at each spatial position to avoid any orientation strategy for the last reach. This gave a score ranging from 0 to 42 (0=left and 42=right)[206].

For the purpose of the present study, a decision was required on how to deal with this heterogeneity. There were two factors to consider here- Firstly.: numerical considerations – excluding those with only 21 cards would reduce sample size considerably.

Secondly: are the 21 card test and 49 card test results equivalent?

To decide the best strategy, I compared the handedness results after 49 cards to the results in the same subjects after the first 21 cards had been drawn. After 21 cards, 28% of subjects were left-handed and after 49 cards, only 25% were left-handed. This raised the possibility that the two tests were different. I then investigated the effects of the number of cards at specified positions.

The Bishop test is based on the theory that in the absence of any hand preference, the right hand is more likely to be used to pick cards on the right hand side of the midline and vice-versa. This means that the proportion of reaches made with the right hand should increase as the participant moved from card positions 1 (left) to 7 (right most). A preliminary plot of handedness according to card position in the EFPTS database suggested a trend towards increased right-handedness was present for the 21 card experiment but not for the 49 card experiment. (See Fig 2-3). So, the decision that the 21 cards was the best option for analyses was made. Using only the first 21 cards from those with 49 selections would maximise the available subjects, if this was added to those who only did 21 cards.

These results can also be compared to those obtained when handedness is defined for each subject using all the available cards (sensitivity analysis: see 2.3.3b).

2.3: Statistical methods

The statistical analyses were run using the software package- STATA (version 10).

The aim of the study was to determine if there is a statistical association between handedness and perinatal factors - if perinatal factors can "predict" handedness. However, the characteristics of the dataset (paired data, not normally distributed) provided a number of methodological challenges to the analysis, which will be discussed later (in sections 2.3a).

2.3.1: Initial Exploratory analysis

Firstly, the perinatal characteristics of target and study cohort were compared (using percentages) to show the extent to which the study cohort (those completing the handedness test) represents the target cohort (all EFPTS recruits). The completeness of the data was also assessed.

Next the outcome variable was examined. Histograms were used to examine the frequency distributions of the laterality quotients derived from Strien (Preference) and Bishop (Performance) tests.

The outcome variables were also examined as dichotomised handedness scores. The proportions that are left-handed were examined according to the year of birth and age at research to determine whether the prevalence of left-handers varied over time or with the age at testing. Chi-square tests (standard and test for trend) were used to determine whether any differences were significant.

807 subjects completed both Strien and Bishop's tests. This helped to see how many were left-handed in both the tests. Also, this enabled to compare the percentage of left-handers in Strien and Bishop's test independently.

As my data set is on twins, the handedness was compared within twin pairs using Pearson's correlation-coefficient (r), with its associated hypothesis test (i.e. r=0). This would indicate if there is an association in handedness within the twin pairs. Also, the distribution of handedness within twin pairs according to zygo-chorionicity was examined.

2.3.2: Association between handedness and perinatal and umbilical cord factors

The paired structure of data arising from twins has been a challenge in the construction, estimation and interpretation of regression models and also to the more basic statistical analysis. This is because standard statistical tests assume independence of data and these data were derived from twins.

Moreover, the outcome data (handedness) expressed as a quotient from 0 to 1, did not have a normal distribution. As consistent with the existing literature, my data had a 'j' shaped distribution. I plotted histograms to confirm this; I also used the STATA command "gladder" to explore a variety of transformations. The transformation of the data using the formula (-ln[sqrt (%L + 1)] as suggested by Bishop D.V.M (2001) was unsuccessful [(207).] Non-parametric tests could not be used as they assume independence and the clustered nature of the twin data means this assumption would be violated. I therefore decided that for most of the analyses and for modelling, I would dichotomise the laterality quotient in to right and left handed though this would result in the loss of information.

Due to the intrinsic clustering of the twin data, standard statistical tests such as t-test and chi-squared tests were inappropriate. So, I used methods in STATA for comparing means and proportions between groups which adjust for clustering.

For the comparison of continuous variables in left and right handers, I used the 'Means' command in STATA which is able to take clustering into account. I then used the 'test' command to determine if the means in L + R (left and right) handers were equal.

For example: to test if father's age differs between left and right handers:

mean <father's age>, over (handedness score) vce (cluster <twin identifier>)

matrix list e (b)

test [father's age] Left = [father's age] Right

For the categorical variables, I used the 'Proportions' command to determine the percentage of each category within the left and right handed twins after taking the clustering into account. I have also tested if the proportions in each category were equal in left and right handers.

For example: in zygochorionicity:

proportion zygochorionicity, over (handedness score) vce (cluster <twin identifier>)

matrix list e (b)

test [MCMZ] Left = [MCMZ] Right

test [DZ] Left = [DZ] Right

Since it would be redundant to test all categories, the category with smallest numbers was excluded in each case.

<u>2.3.3(a): Modelling risk (odds) of left-handedness</u>

The paired nature of both outcome and predictor data from twin studies requires special consideration when selecting appropriate modelling techniques. The model for my data has been derived from the article named 'Regression models for twin studies: a critical review' [208] .

Some data are the same in both the twins (e.g. mother's age, father's age, gestational age), while others differ (e.g. birth weight, umbilical cord length). Hence, I decided to use random-effects logistic regression to determine if handedness (Strien or Bishop) is associated with any of the perinatal factors or the umbilical cord characteristics. For predictor variables that do not vary within twin pairs (e.g., mother's age) population average mixed-models were used and these may also be used where predictors do vary within twin pairs (personal communication with Dr.K.Hemming, Senior Lecturer, Public Health, Epidemiology and Biostatistics, University of Birmingham). The STATA command xtlogit was used. Results for univariate analysis were presented in the form of Odds ratios (odds of being left-handed) with 95% CIs. The factors which yielded significant results in univariate models were tested in bivariate models, after controlling for the type of twin i.e., zygo-chorionicity to see if the significance persists. This factor was included since genetics of handedness is uncertain.

For the continuous factors which differ between twins, I used the same model to explore within-twin pair and between- twin pair differences. For instance, is a low birthweight twin with a high birthweight co-twin more or less likely to be left-handed than a low birthweight twin with a low birthweight co-twin? Here, I entered both the mean birthweight for each pair and the difference between the pairs in to the model.

2.3.3 (b): Sensitivity analysis:

Finally the modelling results for Bishop's test were repeated defining handedness from all the available cards (rather than the first 21) to see if the results were sensitive to changes in the definition of handedness.

Chapter 3: Results

3.1 The study subjects

3.1.1: Subject Characteristics

Table 3-1 shows the characteristics of all eligible subjects (the target cohort) and those who took part in the handedness study (the study cohort). It reveals the completeness of the data and indicates how far the study cohort represents the target cohort.

Table 3-1: Comparison of characteristics of Target (n=4502) and Study cohort (n=1257)

Baseline characteristic	**Target cohort**		**Study cohort**	
	Frequency	**Percentage**	**Frequency**	**Percentage**
Birthyear	**(n=4502)**		**(n=1257)**	
1977-1979	772	17.1	92	7.3
1980-1982	764	17.0	51	4.1
1983-1985	736	16.4	265	21.1
1986-1988	1094	24.3	458	36.4
1989-1991	1136	25.2	391	31.1
Missing	0	0.0	0	0.0
Zygochorionicity	**(n=4502)**		**(n=1257)**	
MCMZ	1752	38.9	460	36.6
DCMZ	106	2.4	14	1.1
DZ	2644	58.7	783	62.3
Missing	0	0.0	0	0.0
Birth order	**(n=4502)**		**(n=1257)**	
1	2251	50.0	626	49.8
2	2251	50.0	631	50.2
Missing	0	0.0	0	0.0
Gender	**(n=4500)**		**(n=1257)**	
Male	2211	49.1	626	49.8
Female	2289	50.9	631	50.2
Missing	2	0.0	0	0.0

Table 3-1: continued.

Comparison of characteristics of Target (n=4502) and study cohort (n=1257)

Baseline characteristic	Target cohort		Study cohort	
	Frequency	Percentage	Frequency	Percentage
Mother's age	**(n=4396)**		**(n=1233)**	
<25 yrs	1140	25.3	257	20.5
25-35 yrs	2960	65.5	881	70.0
35+ yrs	296	6.6	95	7.6
Missing	106	2.4	24	1.9
Father's age	**(n=3430)**		**(n=905)**	
<25 yrs	448	10.0	97	7.7
25-35 yrs	2450	54.4	657	52.3
35+ yrs	532	11.8	151	12.0
Missing	1072	23.8	352	28.0
Gestational age	**(n=3864)**		**(n=1125)**	
<32 weeks	286	6.4	57	4.5
32-36 weeks	460	10.2	121	9.6
36+ weeks	3118	69.3	947	75.4
Missing	638	14.1	132	10.5
Birthweight	**(n=4463)**		**(n=1249)**	
<1500 grams	338	7.5	52	4.1
1500-1999 grams	543	12.1	128	10.2
2000-2499 grams	1358	30.2	394	31.3
2500-2999 grams	1545	34.3	469	37.3
>=3000 grams	679	15.1	206	16.5
Missing	39	0.9	8	0.6
Parity	**(n=4424)**		**(n=1233)**	
1st parity	2190	48.7	634	50.4
Other parity	2234	49.6	599	47.7
Missing	78	1.7	24	1.9
Delivery mode	**(n=4468)**		**(n=1250)**	
Spontaneous & Breech	2693	59.8	715	56.9
Instrumental & extraction	780	17.3	194	15.4
Section	995	22.1	341	27.1
Missing	34	0.8	7	0.6
Presentation of foetus	**(n=4404)**		**(n=1238)**	
Cephalic	3037	67.5	847	67.4
Breech	1117	24.8	321	25.5
Other	250	5.6	70	5.6
Missing	98	2.2	19	1.5

Table 3-1: continued.
Comparison of characteristics of Target (n=4502) and study cohort (n=1257)

Baseline characteristic	Target cohort		Study cohort	
	Frequency	Percentage	Frequency	Percentage
Mode of conception	**(n=4502)**		**(n=1257)**	
*Assisted	796	17.7	261	20.8
Spontaneous	3404	75.6	967	77.0
Unknown	302	6.7	29	2.3
Missing	0	0.0	0	0.0
Cord length	**(n=4413)**		**(n=1246)**	
<40cms	3051	67.8	859	68.3
40-60cms	1224	27.2	355	28.3
>60cms	138	3.1	32	2.5
Missing	89	2.0	14	1.1
Cord windings	**(n=4414)**		**(n=1238)**	
Clockwise	2863	63.6	807	64.2
Counter-clockwise	629	14.0	175	13.9
Undefined & mixed	922	20.5	256	20.4
Missing	88	2.0	19	1.5
Cord knots	**(n=4382)**		**(n=1237)**	
False	1080	24.0	371	29.5
+No & Other	3302	76.0	866	70.5
Missing	0	0.0	0	0.0

* Assisted (ovulation-induction + in-vitro-fertilisation and other related techniques like ICSI (intra-cytoplasmic sperm injection), Gift (Gamete Intrafallopian Transfer), Zift (Zygote Intrafallopian Transfer)

+ No & other: added together due to low numbers in 'other' category.

3.1.2: Completeness of data

Table 3.1 shows that for the variables mode of conception, twin birth year and zygochorionicity, entry was complete with no missing data in the cohorts.

For father's age, data entry was not complete and had the most missing data in both the cohorts. The missing data was just under a quarter of the sample size in target and over a quarter in the study population.

This was followed by gestational age in which missing data contributed to about 14% in target and about 10% in the study group.

For the variables, mother's age, delivery mode, parity, birthweight, presentation, umbilical cord characteristics, the percentage of missing data was low.

3.1.3: Representativeness of study sample

All twins born from 1977 to 1991 were invited to participate in the study, but Table 3.1 shows that dizygotic twins (62%) were over-represented in the study sample. In addition, twins born later (1986-1991), who were younger than the rest, are also over-represented in the sample. The sample comprised of about 7% from the years 1977 to 1979 and about 88% from the years 1983-1991. This could be due to the fact that younger twins were taken to the test centre by the parents. As this test was conducted as a sub-study of the main IQ tests, parents with young twins may have been more motivated to have their children's IQ measured, than teenagers or adults would be to take part themselves.

In both the maternal and paternal age, more participation came from the parents who were aged between 25-35 years of age. About 50% of the mothers were primiparous (1^{st} parity) in both the cohorts.

In almost all the variables, the study cohort is found to be similar to the target, eligible cohort. No major, obvious differences are seen between the two cohorts. There is therefore no evidence of major response bias in the study cohort, though there is perhaps some indication of attrition bias as older subjects are under-represented. In conclusion, the study cohort seems to be reasonably representative of the target cohort based on the observations in Table 3-1.

3.2: Outcome measures

Results are available for handedness from Strien's questionnaire (Preference test) for 1226 twins (response rate was 97.5%) and Bishop's card reaching test (Performance test) for 838 twins (response rate was 66.6%). At the time of taking the tests, the age of the participants ranged from 7 to 20 years (median age was 11 and inter-quartile range was from 9-13).

3.2.1: Distribution of the outcome measures- (Laterality quotients (LQ))

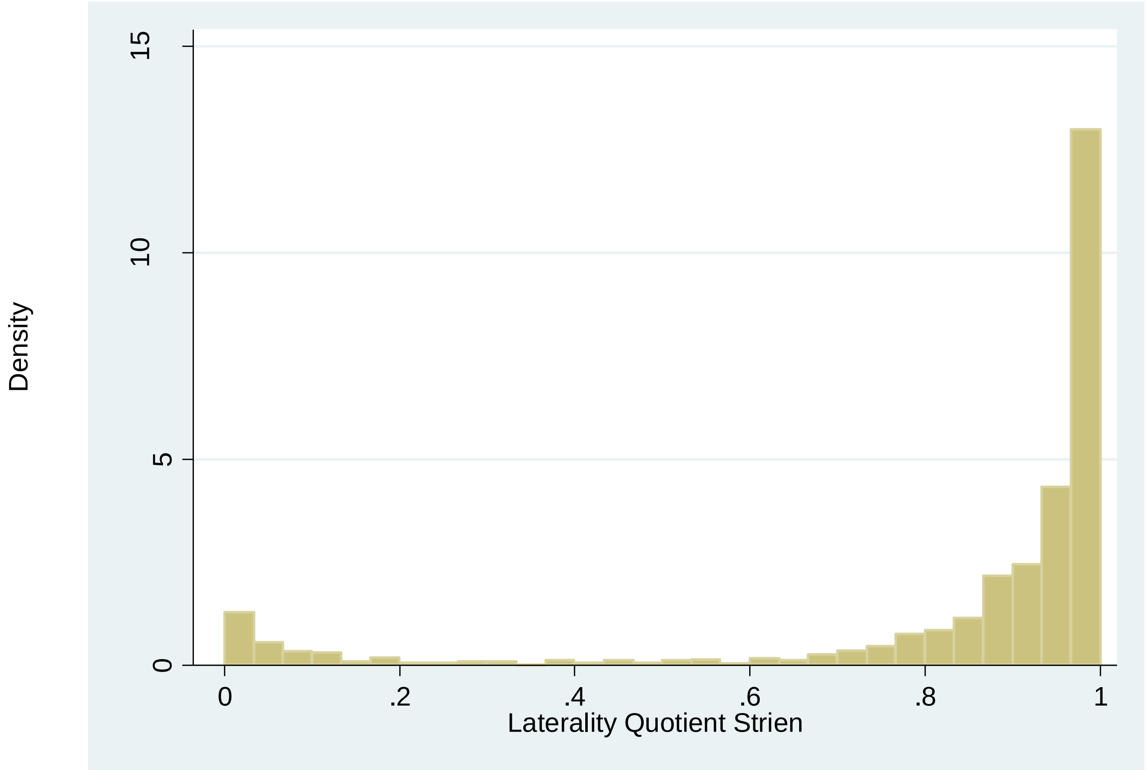
The frequency distributions of the laterality quotient for both tests are shown in the histograms in Figure 3.1. Table 3-2 shows the distribution on subjects after splitting the LQ into 5 equally-spaced categories.

The Strien test showed less polarisation than the Bishops test which also classifies more subjects within the ambi-dextrous categories. In table 3-2, the proportions within each category of LQ (chi-square test on the numbers) differed significantly between the two tests ($p<0.001$), though this test may be unreliable if the data are not independent. For the Strien test, only 11% of subjects had LQs from 0.2 to 0.8, as compared to the 28% in Bishop's test (Table 3.2).

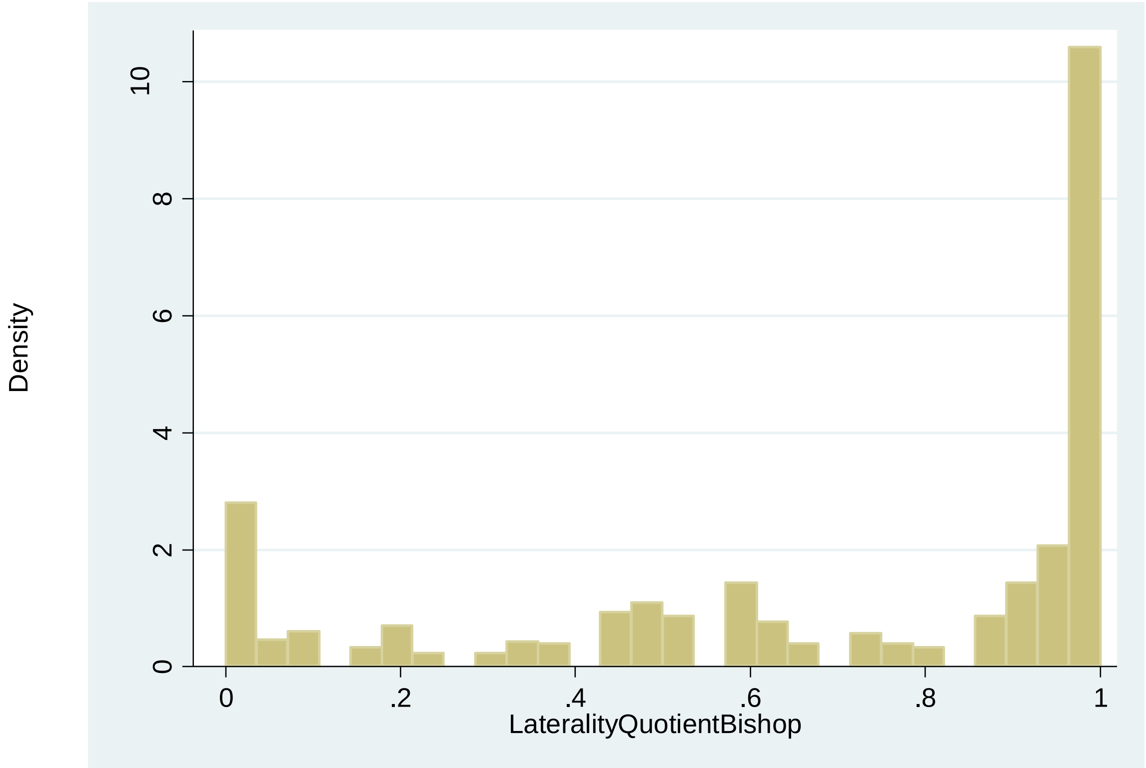
Table 3-2: Comparison of distribution of laterality quotient scores in Strien and Bishop's tests.

Laterality quotient	Strien (n=1226)	Bishop test (n=838)
0-0.20	9.4% (115)	17.5% (147)
0.21-0.40	1.6% (20)	4.7% (39)
0.41-0.60	2.0% (25)	15.5% (130)
0.61-0.80	7.3% (89)	7.6% (64)
0.81-1.00	79.7% (977)	54.7% (458)

Fig 3-1 :(A) Histogram of Strien laterality quotient for 1226 subjects:



(B): Histogram of Bishop laterality quotient for 838 subjects:



3.2.2: Handedness scores (dichotomisation of the LQ)

Table 3-3 shows the proportion of subjects who were classified as left-handed (LQ between 0 and 0.5; values of 0.51 and above were classified as right-handed), along with a breakdown by birth year and age at research. Since social attitudes towards handedness and preference vary over time it would be appropriate to look the percentages of left-handers in the different years. For both the tests the age at test administration ranged from 7 to 20 years.

Table 3-3: Proportion of subjects classed as left handed (LQ 0.5 or lower) for Strien (preference test) and Bishop (performance) tests according to birth year and age at handedness testing.

Percentage (number/total) classified as left-handed		
	Strien test	***Bishop test***
Overall	12.2 (149/1226)	29.5 (247/838)
By Birth year		
1977-1979	10.9 (10/92)	35.4 (29/82)
1980-1982	13.8 (7/51)	30.8 (8/26)
1983-1985	14.3 (38/265)	36.8 (21/57)
1986-1988	11.5 (51/442)	27.9 (83/298)
1989-1991	11.4 (43/376)	28.7 (106/369)
Chi-square p-value	0.78	0.65
p-value for trend	0.62	0.31
By age at testing		
<10 years	11.3 (36/319)	28.6 (89/311)
10+ years	12.5 (113/905)	30.0 (158/527)
Chi-square p- value	0.57	0.64

There is no evidence in Table 3-3 of variation in left-handedness by age or birth cohort for either test. The p-values for difference and trend were not significant. However, these results may be invalid since these tests assume independence of the data, which may not be true for twin data.

Nevertheless, it is striking that 30% were found to be left-handed by Bishop and only 12% by Strien test. Since the tests were carried out on different cohorts (1226 did Strien and 838 did Bishop's test), might the two cohorts have differed in their prevalence of left- handedness? To test this, I examined test results in the sub-cohort who completed both the tests.

3.2.3: Comparison of the two tests

807 subjects completed both the tests of handedness. In this cohort, 615 (76%) showed the same result in both the tests. 66 (8.2%) were left-handed and 549 (68.0%) right-handed in both the tests. Overall, 10.7% were left-handed in Strien test and 29.5% were left-handed in bishop's test. This is similar to the results in table 3.3 and confirms that the Bishop's test is more sensitive to left-handedness.

The correlation-coefficient between the laterality quotient from the Strien and Bishop's test was 0.36- indicating only moderate agreement. For the dichotomised scores, I used Kappa test for agreement and found the tests to have only a fair level of agreement (kappa = 0.30). Therefore, findings from both the tests will be presented separately in this report.

3.2.4: Handedness within twin pairs

For both the tests, handedness was examined within twin pairs, comparing the first born and the second born twins. Findings for the Strien test are given in Table 3-4a and Table 3-5a for Bishop's test below. Also shown are the within-pair correlation-coefficient and its p-value.

Strien Preference test

Table 3-4a: Distribution of handedness within twin pairs: Strien (Preference test).

All twins: correlation coefficient = -0.0008 p-value= 0.98				
		1st born twin		
		Left	Right	Total
2nd born twin	Left	9	62	71
	Right	67	458	525
	Total	76	520	596

In the Strien test, 78.4% (9+458/596) of twin pairs were concordant for handedness and 1.9% (9/467) of the concordant pairs were both left-handed. Left-handedness was slightly more common in the first born twins (12.8% (76/596) vs. 11.9% (71/596)). The correlation co-efficient was -0.0008. It may be concluded that, although these data are paired, the Strien test outcome data shows no evidence of any appreciable correlation within twin pairs.

The above tests were repeated for the different types of twins based on the zygo-chorionicity. There were 147 MCMZ twins. In this, 76.9% (4+109/147) of twins were concordant for handedness, out of which 4 (3.5%) of the concordant pairs were both left-handed. The first born twins were more likely to be left-handed (17%) when compared to the second born twins (11.6%). There were 75 DCMZ twins. In this, 80% were concordant for handedness and 3.3% of the concordant pairs were both left-handed. In the first born twins left-handedness was seen in 9.3% when compared to the second born twins (16%). There were 374 DZ twins. In this, 78.6% of twins were concordant for handedness, out of which 3 (1.02%) of the concordant pairs were both left-handed. The first born twins were more likely to be left-handed (11.8%) when compared to the second born twins (11.2%).Visual inspection of these tables suggests little evidence that zygochorionicity affects the degree of concordance in the Strien test.

Table 3-4b: Distribution of handedness within twin pairs according to zygo-chorionicity in Strien (Preference) test.

MCMZ: Correlation coefficient = 0.0628 p-value= 0.45				
		1st born twin		
2nd born twin		Left	Right	Total
	Left	4	13	17
	Right	21	109	130
	Total	25	122	147

DCMZ: Correlation coefficient = 0.1100 p-value= 0.35				
		1st born twin		
2nd born twin		Left	Right	Total
	Left	2	10	12
	Right	5	58	63
	Total	7	68	75

Dizygotic (DZ): Correlation coefficient = -0.0510 p-value= 0.33				
		1st born twin		
2nd born twin		Left	Right	Total
	Left	3	39	42
	Right	41	291	332
	Total	44	330	374

Bishop's (Performance) test

Table 3-5a: Distribution of handedness within twin pairs: Bishop (Performance test).

All twins: correlation coefficient = 0.06				p-value= 0.22
		1st born twin		
		Left	Right	Total
2nd born twin	Left	41	77	118
	Right	83	207	290
	Total	124	284	408

In the Bishop test, 60.8% of twin pairs were concordant for handedness and 16.5% of the concordant pairs were both left-handed. Left-handedness was slightly more common in the first born twins (30.4 vs. 28.9%). The correlation co-efficient was 0.06. It may be concluded that, although these data are paired, the Bishop test outcome data shows no evidence of any appreciable correlation within twin pairs.

The above tests were repeated for the different types of twins based on the zygo-chorionicity. There were 97 MCMZ twins. In this, 53.6% of twins were concordant for handedness, out of which 9 (17.3%) of the concordant pairs were both left-handed. The first born twins were more likely to be left-handed (36.1%) when compared to the second born twins (28.9%). There were 49 DCMZ twins. In this, 69.4 % were concordant for handedness and 20.6% of the concordant pairs were both left-handed. In the first born twins left-handedness was seen in 26.5% when compared to the second born twins (32.7%). There were 262 DZ twins. In this, 61.8% of twins was concordant for handedness, out of which 25 (15.4%) of the concordant pairs were both left-handed. The first born twins were more likely to be left-handed (29.0%) when compared to the second born twins (28.2%).Visual inspection of these tables suggests little evidence that zygochorionicity affects the degree of concordance in the Bishop test.

Table 3-5b: Distribution of handedness within twin pairs according to zygo-chorionicity in Bishop (Performance) test.

<table>
<tr><td colspan="5">MCMZ: Correlation coefficient = -0.0523 p-value= 0.61</td></tr>
<tr><td colspan="5">1st born twin</td></tr>
<tr><td rowspan="4">2nd born twin</td><td></td><td>Left</td><td>Right</td><td>Total</td></tr>
<tr><td>Left</td><td>9</td><td>19</td><td>28</td></tr>
<tr><td>Right</td><td>26</td><td>43</td><td>69</td></tr>
<tr><td>Total</td><td>35</td><td>62</td><td>97</td></tr>
<tr><td colspan="5">DCMZ: Correlation coefficient = 0.2716 p-value= 0.06</td></tr>
<tr><td colspan="5">1st born twin</td></tr>
<tr><td rowspan="4">2nd born twin</td><td></td><td>Left</td><td>Right</td><td>Total</td></tr>
<tr><td>Left</td><td>7</td><td>9</td><td>16</td></tr>
<tr><td>Right</td><td>6</td><td>27</td><td>33</td></tr>
<tr><td>Total</td><td>13</td><td>36</td><td>49</td></tr>
<tr><td colspan="5">Dizygotic (DZ): Correlation coefficient = 0.0660 p-value= 0.29</td></tr>
<tr><td colspan="5">1st born twin</td></tr>
<tr><td rowspan="4">2nd born twin</td><td></td><td>Left</td><td>Right</td><td>Total</td></tr>
<tr><td>Left</td><td>25</td><td>49</td><td>74</td></tr>
<tr><td>Right</td><td>51</td><td>137</td><td>188</td></tr>
<tr><td>Total</td><td>76</td><td>186</td><td>262</td></tr>
</table>

The correlation-coefficient reveals very little association between handedness within twin pairs. So, our outcome variable may be treated as an independent variable and the chi-square tests reported earlier will be valid. However, some of the predictor variables (e.g.: mother's age) are necessarily associated (equal) within twin pairs and so standard statistical tests which assume independence of subjects will still be inappropriate.

3.3: Association of handedness with perinatal and umbilical cord factors:

3.3.1: Examination of perinatal factors by handedness

Due to the intrinsic clustering of the twin data, standard statistical tests such as t-test and chi-squared tests were inappropriate. So, I used methods in STATA which adjust for clustering in parametric tests.

Continuous factors:

For these analyses, continuous factors were examined in their original format rather than categories.

For the comparison of continuous variables in left and right handers, we calculated 'Means' in STATA after taking clustering into account.

The results are given in Table 3-6 below.

Table 3-6: Means of the continuous predictor variables by handedness category.

Factor	**Preference test Strien(n=1227)**			**Performance test Bishop(n=838)**		
	Left	*Right*	*p-value*	*Left*	*Right*	*p-value*
Mother's age (years)	27.4	28.0	0.11	28.2	28.3	0.79
Father's age (years)	29.0	30.0	0.02	30.0	30.6	0.14
Gestational age (weeks)	36.5	36.6	0.71	36.5	36.7	0.36
Birthweight (grams)	2458	2499	0.38	2482	2533	0.19

Table 3-6: continued.

Means of the continuous predictor variables by handedness category:

Factor	**Preference test Strien(n=1227)**			**Performance test Bishop(n=838)**		
Cord length (cms)	35.1	35.1	0.97	34.4	35.2	0.36
*Birthweight difference**	-87.9	-51.3	0.44	-58.7	-50.8	0.85
*Cord length difference**	-4.1	-3.0	0.44	-4.5	-3.3	0.34

Birthweight and cord length difference*: within-pair difference (first born minus second born twin).

The means of the mother's age did not differ between the left and right handers and the one significant 'p' value observed in the father's age in the Strien test need to be read with caution as it could be due to chance. Also, the means of all the other continuous predictor variables described above showed no difference between the left and right handed twins in all the tests of laterality.

<u>*Categorical factors*</u>:

For the categorical variables, I calculated the 'Proportions' of each category within the left and right handed twins in STATA after taking the clustering into account.

The results are given in Table 3-7. For consistency with the layout in Table 3-6, I compared the proportion in each category for left and right handers, e.g. 58% of left-handers were dizygotic, compared to 63% right handers (p=0.29). For each categorical variable, I excluded significance tests for one category level (the smallest) because of redundancy.

Table 3-7: Proportions of the categorical predictors over dichotomised handedness scores:

	Preference test Strien (n=1226)				**Performance test Bishop (n=838)**			
Factor	Freq	Left	Right	p-value	Freq	Left	Right	p-value
Twin birthyear								
1977-1979	(n=92)	0.07	0.08	0.70	(n=88)	0.12	0.10	0.49
1980-1982	(n=51)	0.05	0.04	0.72	(n=26)	0.03	0.03	0.88
1983-1985	(n=265)	0.26	0.21	0.25	(n=57)	0.09	0.06	0.24
1986-1988	(n=442)	0.34	0.36	0.61	(n=298)	0.34	0.36	0.45
1989-1991	(n=376)	0.29	0.31	0.61	(n=369)	0.43	0.45	0.68
Zygochorionicity								
MCMZ	(n=306)	0.29	0.24	0.23	(n=202)	0.27	0.23	0.26
DCMZ	(n=155)	0.13	0.13		(n=99)	0.12	0.12	
DZ	(n=765)	0.58	0.63	0.29	(n=537)	0.62	0.65	0.34
Parity								
1st parity	(n=622)	0.60	0.51	0.03	(n=423)	0.52	0.51	0.84
Other parity	(n=580)	0.40	0.49		(n=405)	0.48	0.49	
Delivery mode								
Spontaneous & Breech	(n=700)	0.62	0.57	0.28	(n=484)	0.59	0.58	0.65
Instrumental & extraction	(n=184)	0.16	0.15		(n=120)	0.18	0.13	
Section	(n=335)	0.23	0.28	0.16	(n=229)	0.23	0.29	0.07
Presentation								
Cephalic	(n=824)	0.70	0.68	0.61	(n=568)	0.69	0.68	0.78
Breech	(n=315)	0.28	0.26	0.64	(n=211)	0.25	0.26	0.94
Other	(n=68)	0.02	0.06		(n=49)	0.05	0.06	
Mode of conception								
Assisted	(n=253)	0.21	0.21	0.96	(n=202)	0.26	0.23	0.44
Spontaneous	(n=944)	0.75	0.77	0.58	(n=608)	0.68	0.75	0.06
Unknown	(n=29)	0.04	0.02		(n=28)	0.06	0.02	
Cord windings								
Clockwise	(n=788)	0.70	0.65	0.17	(n=532)	0.64	0.65	0.89
Counter-clockwise	(n=170)	0.06	0.14		(n=118)	0.14	0.14	
Undefined & Mixed	(n=249)	0.14	0.22	0.02	(n=172)	0.21	0.21	0.87

Table 3-7: continued.

Proportions of the categorical predictors over dichotomised handedness scores:

	Preference test Strien (n=1226)				Performance test Bishop (n=838)			
Factor	Freq	Left	Right	p-value	Freq	Left	Right	p-value
Cord knots								
False	(n=362)	0.30	0.30		(n=261)	0.31	0.32	
No & Other	(n=844)	0.70	0.70	0.91	(n=559)	0.69	0.68	0.74
Birth order								
1	(n=609)	0.52	0.49		(n=418)	0.51	0.49	
2	(n=)617	0.48	0.51	0.49	(n=420)	0.49	0.51	0.55
Gender								
Male	(n=619)	0.53	0.50		(n=420)	0.47	0.51	
Female	(n=607)	0.47	0.50	0.49	(n=418)	0.53	0.49	0.45

Table 3-7 reveals that Strien left-handers were more likely to be born to Primiparous mothers (60% vs. 51%, p=0.03) but this was not confirmed in Bishop's test. Also of significance, is the undefined and mixed category of umbilical cord windings which seems to have less left-handers in the Strien test only (p=0.02).

Twins born by 'caesarean section' were less likely to be left-handed in Bishop test with a marginal level of significance (p=0.07). Twins conceived spontaneously were more right-handed in the bishop test and with a marginal level of significance (p=0.06). All the values with a marginal level of significance need to be addressed with caution as it could be due to chance or could be due to some confounding effect. These values should be assessed to see if the significance still persists in the multivariate analysis. The 'proportions' of all the other categorical variables did not seem to differ between the left and right-handed twins.

3.3.2: Modelling the risk of left-handedness

Mixed models in STATA

For the predictor variables that do not vary within twin pairs, population average mixed models were used.

When testing if birthweight had an influence on handedness, I also adjusted for the length of gestation, since it has been previously shown that birthweight rises on average with length of gestation[209].

For the continuous factors which differ between twins, (i.e. birthweight and cord length), I used the random-effects model to explore within-twin pair and between-twin pair differences. For instance, is a low birthweight twin with a high birthweight co-twin more or less likely to be left-handed than a low birthweight twin with a low birthweight co-twin? Here, I entered both the mean birthweight for each pair and the difference between the pairs (first-born minus second-born) in to the random effects model.

The results from the models are given in Table 3-8a for continuous and Table 3-8b for categorical variables in Strien. Table 3-9a gives the results from continuous variables and Table3-9b for the categorical variables in Bishop's test.

The use of continuous variables assumes a linear relationship with handedness. So, I explored modelling these variables in categories too. Results are shown in the Appendix (Table A1 for strien and Table A2 for Bishop's test). However, no significant non-linear effects were observed.

3.3.2a: Population averaged model: Strien (Preference test)

The odds ratio shows the odds of being left-handed for every one unit increase in the predictor variable. For example, the odds of being left-handed decreases by 3% for one year increase in mother's age. In the categorical variables, the odds ratio shows odds of being left-handed in different categories compared to baseline category. For example, the odds of being left-handed increases by 4% for those twins who were breech when compared to those who were cephalic in their presenting position.

Table 3-8a: Population averaged model: Strien (Preference test) giving the odds ratio-odds of being left-handed for every one unit increase in the predictor variable

Factor	**Odds Ratio**	**95% C.I**	**P value**
Birth year	0.99	0.94-1.04	0.68
Mother's age (years)	0.97	0.93-1.01	0.14
Father's age (years)	0.95	0.91-1.00	0.04
Gestational age (weeks)	0.99	0.92-1.06	0.70
Birthweight (100grams)	0.99	0.95-1.02	0.36
Umbilical cord length (cms)	1.00	0.98-1.02	0.98

Table 3-8b: Univariate models showing odds of being left-handed in different categories compared to baseline category in Strien (Preference) test

Factor	**Odds ratio**	**95% C.I**	**P value**
Zygochorionicity			*+0.478*
MCMZ	1.00		
DCMZ	0.86	0.48-1.52	0.59
DZ	0.79	0.53-1.16	0.22
Parity			*+0.030*
1st parity	1.00		
Other parity	0.68	0.48-0.97	0.03
Deliverymode			*+0.417*
Spontaneous & breech	1.00		
Instrumental & extraction	0.96	0.59-1.56	0.86
Section	0.76	0.50-1.15	0.19
Presentation of twin			*+0.169*
Cephalic	1.00		
Breech	1.04	0.70-1.54	0.84
Other	0.33	0.10-1.07	0.07

Table 3-8b: continued.

Univariate models showing odds of being left-handed in different categories compared to baseline category in Strien (Preference) test

Factor	Odds ratio	95% C.I	P value
Mode of conception			*+0.370*
Assisted	1.00		
spontaneous	0.96	0.63-1.47	0.87
unknown	1.87	0.71-4.95	0.21
Cord windings			*+0.130*
Clockwise	1.00		
Counter-clockwise	1.04	0.64-1.69	0.87
Undefined & Mixed	0.61	0.37-1.00	0.05
Cord knots			*+0.914*
False knots	1.00		
No knots + Other	0.98	0.67-1.42	0.91
Birth Order			*+0.486*
1	1.00		
2	0.89	0.63-1.26	0.49
Gender			*+0.511*
Male	1.00		
Female	0.89	0.63-1.26	0.51

p-value+: refers to global significance of co-efficients.

Table's 3-8a and b revealed significant associations for father's age, parity and umbilical cord windings. Specifically, the risk of being left-handed decreases as father's age increases and primiparous births were more likely to be left-handed and the undefined and mixed category of cord windings was associated with a lower risk of left-handedness, though the overall effect was not significant.

These significant factors were tested again in a bivariate model after controlling for the type of twin, i.e., zygo-chorionicity. The odds ratios did not change appreciably. Hence, it can be said that the influence of the perinatal factors on the handedness score from

Strien's test did not depend on the type of the twin. Results are given in the Appendix. (Table A3).

The observed effect of parity was then further investigated. Since parity is closely related to birthweight, mother's age and gestation, bivariate models were run for parity after adjusting for each of these factors in turn. After adjusting for gestational age and birthweight, the odds ratio and significance for parity had a p-value of 0.04 and after adjusting for mother's age, the significance remained with a p-value of 0.05.

The significant results from the univariate models above (as expected) confirm the results of the "means" and "proportions" results shown in tables 3-6 and 3-7. Here however, it is the risk that an individual twin will be left-handed that is being modelled, allowing quantification of the effect of that factor on the outcome (handedness) variable.

When the predictor variables were controlled for the gender and the age of the twins which are common confounders in many epidemiological studies, it made no difference to the overall findings. Age was used both as a continuous and as a categorical variable in these models. (Data is not shown).

3.3.2b: Population averaged model: Bishop's (Performance test)

The predictor variables were entered as continuous (Table 3-9a) and also as categorical variables (Table 3-9b) in the model and the results are given below.

Table 3-9a: Population averaged model: Bishop (Performance test) giving the odds ratio- odds of being left-handed for every one unit increase in the predictor variable.

Factor	Odds Ratio	95% C.I	P value
Birth year	0.98	0.95-1.03	0.44
Mother's age (years)	1.00	0.96-1.03	0.80
Father's age (years)	0.97	0.93-1.01	0.17
Gestational age (weeks)	0.97	0.92-1.03	0.36
Birthweight (100grams)	0.98	0.95-1.01	0.22
Umbilical cord length (cms)	0.99	0.98-1.01	0.37

Table 3-9b: Univariate models showing odds of being left-handed in different categories compared to baseline category in Bishop (Performance) test.

Factor	Odds ratio	95% C.I	P value
Zygochorionicity			*+0.525*
MCMZ	1.00		
DCMZ	0.85	0.50-1.46	0.56
DZ	0.81	0.57-1.16	0.26
Parity			*+0.838*
1st parity	1.00		
Other parity	0.97	0.71-1.32	0.84
Deliverymode			*+0.094*
Spontaneous & breech	1.00		
Instrumental & extraction	1.31	0.85-2.00	0.22
Section	0.77	0.53-1.11	0.15
Presentation of twin			*+0.913*
Cephalic	1.00		
Breech	0.98	0.69-1.38	0.89
Other	0.87	0.45-1.68	0.67
Mode of conception			*+0.017*
Assisted	1.00		
spontaneous	0.82	0.58-1.17	0.28
unknown	2.49	1.10-5.66	0.03

Table 3-9b: continued.

Univariate models showing odds of being left-handed in different categories compared to baseline category in Bishop (Performance) test.

Factor	Odds ratio	95% C.I	P value
Cord windings			+*0.977*
Clockwise	1.00		
Counter-clockwise	0.99	0.64-1.53	0.96
Undefined & Mixed	1.04	0.71-1.51	0.85
Cord knots			*+0.723*
False knots	1.00		
No knots + Other	1.06	0.77-1.47	0.72
Birth Order			*+0.558*
1	1.00		
2	0.92	0.69-1.22	0.56
Gender			*+0.332*
Male	1.00		
Female	1.16	0.86-1.57	0.33

p-value+: refers to global significance of co-efficients.

From the above table, mode of conception was highly significant but it seemed to be the unknown category (n=29) that caused it. When the unknown category was excluded, the effect was not significant (p-value for global significance of co-efficients was 0.29). This effect is probably a statistical artefact as it is based on smaller numbers (n=29), there is no biological explanation for the finding and it is not found in the Strien test. It is not possible to explain this category further as this category was entered as unknown in the data set. My measures to find out from the current EFPTS research team failed as they did not have any further information on this already collected data.

However, the significance persisted even after controlling for the type of twin (p-value of 0.05), mother's age (p-value of 0.04), gestational age (p-value of 0.02) and the parity (p-value of 0.03).

When the predictor variables were controlled for the gender and the age of the twins (both as continuous and categorical), it made no difference to the overall findings. (Data is not shown).

3.3.2c: Sensitivity analysis:

Analyses in Tables 3-9a and 3-9b were repeated with handedness defined from all the available cards. The results are shown in Table A5 and Table A6 in the appendix. Results were very similar, indicating that the findings are not sensitive to changes in the definition of handedness based on the number of cards used.

3.3.3: Random effects model: Modelling between and within twin-pair effect

Finally, the effect of the differences within twin pairs were examined for the continuous variables, birthweight (adjusted and unadjusted for gestational age) and cord length.

Table 3-10: Testing for evidence of between pair and within pair effects for continuous variables using random effects model in Strien (Preference) test.

	Odds Ratio	95% C.I	P value	+ICC
Birth weight*				
Pair diff	0.99	0.92-1.06	0.73	0.035
Pair average	0.96	0.86-1.08	0.53	
Birth weight* after adjusting for gestational age				
Pair diff	0.99	0.91-1.08	0.89	0.017
Pair average	0.94	0.79-1.12	0.51	
Umbilical cord length				
Pair diff	0.99	0.96-1.02	0.47	0.001
Pair average	1.00	0.99-1.02	0.69	

* Birthweight entered in 100grams units than a one gram unit for the more practical interpretation of results.
+ICC: Intraclass correlation coefficient.

Table 3-11: Testing for evidence of between pair and within pair effects for continuous variables using random effects model in Bishop (Performance) test.

	Odds Ratio	95% C.I	P value	+ICC
Birth weight*				
Pair diff	1.01	0.95-1.07	0.75	0.012
Pair average	0.95	0.87-1.04	0.27	
Birth weight* after adjusting for gestational age				
Pair diff	1.00	0.92-1.07	0.89	0.013
Pair average	0.98	0.85-1.13	0.81	
Umbilical cord length				
Pair diff	1.00	0.97-1.02	0.69	0.086
Pair average	0.99	0.98-1.01	0.40	

* Birthweight entered in 100grams units for the more practical interpretation of results.
+ICC: Intraclass correlation coefficient.

In the above model, the effects of inter and intra pair variation on appropriate continuous variables were checked. Also the effect of gestational age was controlled when the birth weight was entered in the model.

No significant results were found from both the tests. There is no evidence that disparity in birthweight and cord length between the twins influences the risk of left-handedness.

3.4: Summary of findings

In summary:

In Strien (Preference test) handedness, left handedness is significantly more common in twins of younger fathers, and in primiparous pregnancies. Also, undefined and mixed category of umbilical cord windings was significantly associated with lower risk of left-handedness but factor as a whole did not reach significance. Therefore, it is likely that this is a chance finding. None of these factors was affected by adjustment for the type of twin. Also, no effect of inter and intra twin variation on birthweight (adjusted and unadjusted for gestational age) and umbilical cord length were observed.

The results of Bishop (Performance test) handedness showed no evidence of association between any perinatal factors and handedness. Only one co-efficient (unknown category of mode of conception) attained statistical significance and this is difficult to interpret and may well be a chance finding especially since it is based on only 29 subjects.

Chapter 4: Discussion

4.1: Main findings

This study attempted to examine if perinatal factors and umbilical cord characteristics showed any association with handedness as measured by subjective (Strien questionnaire) and objective (Bishop's test) methods.

In Strien (Preference) test, more left-handedness was seen in twins born to younger fathers, primiparous mothers and less left-handedness was seen in those with undefined and mixed categories of umbilical cord windings.

In Bishop (Performance) test, neither the perinatal factors nor the umbilical cord characteristics (cord length, cord knots, cord windings) had any influence on handedness except for unknown category of the mode of conception. It is not possible to explain this finding further as this was the category entered as unknown in the data set.

Comparison of the two methods of measurement has shown that the Bishop's test is more likely to classify subjects as left-handed. This means that the Bishop's test can be expected to be more statistically powerful. But it did not find any association between left-handedness and any perinatal or umbilical cord factors. Although the data was paired, neither test showed any evidence of appreciable correlation of handedness within twin pairs.

4.2: Comparison with previous research

Perinatal factors which have been explored previously include maternal age, paternal age, gestational age, birth weight, parity , birth stress (mode of delivery, presenting position of the foetus at birth), and birth order and gender.

Maternal age

With respect to mother's age, we did not find a significant relationship with handedness. Our findings agree with McManus I.C who also failed to find a significant association between maternal age and handedness [(106).]

McKeever et al found that the age of mothers of left-handed daughters (but not sons) was significantly greater than that of mothers of right-handed daughters ($p<.02$). The size of the difference was small (only 0.72 years). Thus, even if there is a real relationship of maternal age to left-handedness and given the failure to find such a relationship in other studies, its impact becomes quite small [(168)] .

Paternal age

When examining father's age, we did find a significant relationship with left-handedness being more common in children of younger fathers when tested by Strien questionnaire. In the regression model, the p-value was marginal (0.04) and the

95% C.I approached unity and this could be a chance finding. Moreover, a large proportion of data on paternal age was missing. It is difficult to explain biologically; perhaps this might be a cultural effect and maybe older fathers were more likely to impose right-handedness on their left-handed offspring, but these are only speculations. This was however not supported by McManus I.C, who reported that paternal age at the time of birth does not appear to be related to patterns of handedness [(106).]

Gestational age

Gestational age of the foetus did not seem to have an influence on handedness in both of my tests. This was in agreement with the results from the surveys by McManus I.C [106].

Birthweight

Birthweight of the foetus did not seem to have an influence on handedness in both my tests. This was similar to the results from the surveys by McManus I.C [106]. Also, in my study, there is no evidence that disparity in birthweight between the twins influences the risk of left-handedness in both the tests.

Hay D.A et al, found that left-handedness was more common in monozygotic (MZ) pairs, with birthweight difference above 450grams than the other monozygotic or dizygotic (DZ) twin pairs (n=16 MZ pairs, 13 DZ pairs) [170]. These results have to be read with caution as the sample size is small and the significance seen could have been due to chance.

Parity

In my study, Strien (Preference) test (n=1226), which is administered as a questionnaire shows that more left-handedness is seen in twins born to mothers who were primiparous since birth related stresses might be expected to be greater in the first pregnancy.

In another study on singletons by Annett and Ockwell using oral reports of parents, (usually the mother), from visits to over 100 families, found out that Reported Birth Stress (RBS) was as frequent for right-handers as for left-handers. The incidence of

RBS was greater for first born than children of later birth ranks. But again, though birth stress factors were based on the report by the mothers, it would be difficult to substantiate through personal reports of this kind as positive findings could be due to some mothers having lower criteria than others of what constituted stressful birth for both themselves and their children (100) .

But Bishop's (Performance) test did not find any association between parity and left-handedness. This finding is not confirmed by other studies.

Previously, more left-handedness has been observed with increasing parity for female offspring (105). In that study, the mothers supplied information on the handedness of their children (n=1079) and also reported information on parity and birth complications. Although that study was of a similar size to my study, handedness was assessed based on the information given by the mothers and this means some degree of subjectivity would have played a role in the finding.

In another study, the relationships between a set of birth-risk factors, i.e., parity, and maternal age, and handedness were computed for 600 elementary school children in a questionnaire. But parity was not significantly related to handedness. (164)

Both the above two studies used questionnaire to examine the association between parity and left-handedness and so should be comparable with the results from the Strien test. To the best of my knowledge, no study has used Bishop's test to investigate handedness and the perinatal factors that I have used in my study.

Birth stress

Birth stress has been shown to be caused by difficult pregnancies, and is contributed by delivery mode, presenting position of the foetus and the mother's physique.

Investigations of the association between sinistrality and stressful birth have reached contradictory conclusions, with many having negative as much as positive findings [105, 106]. In the studies where there was an association of birth stress with left-handedness, it was found to be weak and conflicting. This could be due to the fact that the questionnaires were completed by the students describing their own birth histories. Knowledge of own birth history must be of variable reliability. In my study, the birth factors were entered by the medical team at the time of birth of the twins and therefore, will have a high degree of accuracy.

Delivery mode

I did not find any relationship between left-handedness and the delivery mode of the twins. This was similar to the results from the surveys by McManus I.C [106].

Coren employing a sample of 133 left-handed and 1165 right-handed singleton subjects and using the subjects' reports as to whether or not various birth complications from their own births, reported that the incidence of breech birth, instrument delivery and caesarean births were all significantly higher for left-handed subjects. Obviously, the accuracy of the reported birth complications is questionable [167].

Presenting position of the foetus

I did not find any relationship between left-handedness and the presenting position of foetus (breech and other versus cephalic). This was similar to the results from the surveys by McManus I.C [106].

Churchill J.A et al reported an association between the presenting position of the foetus and left-handedness in normally presenting fetuses. The sample consisted of 1,102 singleton cases, all born in a single obstetric unit and recorded as having been born in the left occipito-anterior (LOA) or right occipito-anterior (ROA) position. Their handedness was observed at two years of age by examiners ignorant of the birth position. Of the 93 left-handed children, 62.4% were born by ROA position when compared to 42.7% of 836 right-handed children. However, rather than position at birth being a determinant of handedness, it seems more probable that congenital asymmetries of the neuromuscular organisation of the foetus influence its position in the antepartum period. It is possible that the typical human bias to the left hemisphere contributes to the tendency of the foetus to adopt the LOA position. If fetuses lacking the typical bias are more likely to adopt other, less advantageous positions, some increased experience of birth stress could be a result (and not a cause) of their potential left-handedness [(68)].

Umbilical cord characteristics

This will include umbilical cord length, cord windings and cord knots. To my knowledge, no one else has looked into umbilical cord characteristics other than cord windings.

In my study, umbilical cord length and knots did not have any relationship with handedness. Fewer left-handers were seen in the undefined and mixed category of umbilical cord windings when tested by Strien questionnaire. This contradicted the only other study done on umbilical cord twists where no relationship between the direction of the twist of the umbilical cord and the handedness of the child was found [(122)]. But, it is difficult to account for the finding from my study as it is not possible to biologically explain the undefined and mixed category of umbilical cord. But it is unlikely to be a statistical artifact since the number was large. As cord windings are thought to be caused by foetal movements, one could speculate that definite left or right-sided windings indicate strong preference for movements in one direction, while cords without obvious windings might indicate frequent changes in direction or fewer movements in total.

Also, in my study, there is no evidence that disparity in umbilical cord length between the twins is associated with the risk of left-handedness in both the tests.

Birth Order

First born twins might be expected to experience more birth trauma and second born twins are expected to suffer more hypoxia.

Christian J.C et al found an excess of left-handedness among the first born twins (n=104 twin pairs) suggesting that delivery stresses may play a role. Handedness and birth order was assessed by a questionnaire which was completed by the twins and their parents. The authors speculated that the relationship between first-born twins and left-handedness could be due to extreme sampling deviation and the small sample size [(165)].

Even though this study used a questionnaire to determine birth details, birth order is likely to be well-reported.

I did not find a significant relationship between left-handedness and the birth order of the twins in both my tests, though the direction of the effect found was in agreement.

Gender

More left-handedness was observed in boys (16.4% of n=592) when compared to the (14.1% of n=594) girls in the study of singletons by Badian N.A. This was an incidental finding from a study of season of birth on handedness. [(101)].

I did not find any relationship between left-handedness and the gender of the twins in both my tests. Similar to this, Briggs and Nebes did not find any relationship between gender of the participants (1599 college singleton students) and handedness. When tested by Annett's questionnaire, they found out that a family history of left-handedness was significantly related to the handedness of the subjects, but not the gender [210].

4.3: Study strengths and limitations

Strengths

One reason I used twins in my study was to increase study power, since left-handedness is more common in twins. The use of twins has also allowed me to study the between and within twin pair differences in causing left-handedness. In this study of twins, determining handedness by administering both the Preference (Strien questionnaire) and Performance (Bishop's test) was novel.

In comparison to many published studies, the study sample size was large for Strien (n=1226) and Bishop tests (n=838). The perinatal data is of good quality as it comes from a prospective twin register. Though EFPTS is a voluntary register it is population based and more twin research is taking place from the data collected.

Limitations

We have to bear in mind that this sample was a sub-cohort of the study in which IQ tests were conducted. This would have contributed to the increase in the younger cohort in the study and also some degree of attrition bias. The sample comes from Belgium and though this is a growing database, my study sample comes from 1977-1991 and the tests of handedness were conducted from 1996-1999. This meant the data was readily available but the major task was to understand and analyse it. It was difficult to understand the coding and categorisation of the raw data collected by the research team. But the major disadvantage arose from the paired nature of the data and the difficulty of finding the appropriate statistical tests to account for the lack of independence within the same. Also, despite the construction of an index of laterality, the data had to be dichotomised for modeling, with consequent loss of information. Furthermore, the large number of statistical tests carried out makes the interpretation of significant results problematic- a common problem in sub-group analysis.

While 97.5% of subjects participating in the EFPTS follow-up study completed the Strien questionnaire, the response rate for the Bishop's test was only moderate (66.6%). The administration of Bishop's test is likely to be time consuming, especially for 49 cards and hence the recruitment rate was lower than with Strien's questionnaire. The two different types of Bishop tests (3 cards versus 7 cards) led to difficulties in making decisions to select one over the other in the analysis.

Pooling the results from 21 and 49 tests would have led to heterogeneity in the outcome measure. So, a decision was made to only include the first 21 cards from those who did the extended test. This maximised the sample size, but I cannot exclude the possibility that heterogeneity remained, since those given the longer version would not have realised that they have completed the test after 21 cards.

However, the results from the sensitivity analysis done on all the available cards were similar, indicating that the number of cards used to define handedness may not be a crucial factor in defining handedness.

Also, though twins were used in my study, the genetic component was not analysed.

The Apgar scoring system directly evaluates a newborn's condition at birth and low Apgar scores have been shown to be associated with decreased oxygen supply and increased incidence of neurological abnormality. It was disappointing to be unable to include the Apgar score in determining handedness in my sample since EFPTS did not have Apgar entered for my study sample.

4.4:Implications of the study

The results from this study indicate that out of the different perinatal factors and umbilical cord characteristics, only three significant factors were identified.

Left-handedness is significantly more common in twins born to younger fathers and primiparous pregnancies and less common in the undefined and mixed categories of umbilical cord windings when the subjects were administered the Strien questionnaire. However, the Bishop's test was unable to confirm these results, with the possible exception of 'mode of conception' where the results may be spurious.

The initial hypothesis that birth stress would lead to an increase in left-handedness was not proved in my study. Though, it is worth mentioning here that, the stigma associated with left-handedness as a detrimental trait has decreased in recent years. Even if strong association with left-handedness had been found, it is unlikely that clinical practice in the perinatal period would change based on this.

Nevertheless, it is still important to understand more regarding the neurodevelopmental aspect of handedness. In order to make scientifically sound conclusions (after taking the limitations discussed above into consideration), I have proposed suggestions for future research below.

4.5: Suggestions for further research

A definitive answer to the question of determinants of handedness may require a large prospective study with accurately recorded predictor variables to be able to make any scientifically sound conclusions. Also, it would be recommended to use computerised birth records and this means Apgar score will be available for us to determine the neurological aspect of handedness. It may not be possible to justify the costs involved in this study and therefore might be helpful to use existing birth cohort studies (e.g. ALSPAC: the Avon longitudinal study of parents and children) [(211).]

Many Scandinavian/European countries maintain extensive databases of the health records of their population and some may include such factors as APGAR scores. Record linkage studies may offer a cost-effective approach, but we would still need data on handedness.

Also, from this study, it has become obvious that the Bishop's test is time consuming and current research has failed to show any relationship with left-handedness. We need to bear in mind that using the Bishop's test is not cost effective for the purpose of determining handedness in the general population and it would be advisable to administer the Strien questionnaire only. This is easy to administer, not time consuming and we have shown that it has found significant relationship with left-handedness with some of the perinatal factors.

Finally, although a remarkable proportion of the variance in handedness could be explained by the environmental factors, it would be fulfilling to analyse the genetic variance which contributes to it as well. The best study design would be to analyse the genetic variance in the different types of twins. Understanding the genetic basis of handedness can help define the relationships between handedness, language, cerebral asymmetry and neurodevelopmental disorders.

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Appendix:

Table A1: Strien Preference test: Univariate models showing odds of being left-handed in different categories (continuous data presented as categorical variables).

Factor	**Odds ratio**	**95% C.I**	**P value**
Twin birthyear			*+0.776*
1977-1979	1.00		
1980-1982	1.31	0.47-3.66	0.61
1983-1985	1.37	0.66-2.88	0.40
1986-1988	1.07	0.52-2.19	0.85
1989-1991	1.06	0.51-2.19	0.88
Mother's age			$^{+}$*0.789*
<25years	1.00		
25-35years	0.89	0.59-1.35	0.58
35+years	0.79	0.38-1.68	0.54
Father's age			$^{+}$*0.263*
<25years	1.00		
25-35years	1.07	0.56-2.06	0.84
35+years	0.63	0.27-1.48	0.29
Gestational age			$^{+}$*0.609*
<32weeks	1.00		
32-35weeks	1.13	0.44-2.92	0.80
36+weeks	0.86	0.38-1.96	0.72
Birthweight			$^{+}$*0.333*
<1500gr	1.31	0.56-3.08	0.54
1500-1999gr	1.77	1.01-3.09	0.05
2000-2499gr	1.00		
2500-2999gr	1.07	0.70-1.63	0.76
>=3000gr	1.05	0.61-1.79	0.87
Cord length			+0.615
<40 cms	1.00		
40-60 cms	0.97	0.66-1.42	0.86
>60 cms	0.49	0.11-2.06	0.33

p-value^{+}: refers to global significance of co-efficients.

Table A2: Bishop Performance test: Univariate models showing odds of being left-handed in different categories (continuous data presented as categorical variables).

Factor	**Odds ratio**	**95% C.I**	**P value**
Twin birthyear			*+0.674*
1977-1979	1.00		0.84
1980-1982	0.90	0.34-2.39	0.64
1983-1985	1.19	0.58-2.43	0.37
1986-1988	0.79	0.47-1.33	0.45
1989-1991	0.82	0.49-1.37	
Mother's age			$^{+}$*0.917*
<25years	1.00		
25-35years	0.93	0.62-1.38	0.71
35+years	0.89	0.47-1.71	0.73
Father's age			
<25years	1.00		$^{+}$*0.190*
25-35years	0.56	0.30-1.05	0.07
35+years	0.59	0.29-1.21	0.15
Gestational age			$^{+}$*0.276*
<32weeks	1.00		
32-35weeks	1.07	0.45-2.51	0.88
36+weeks	0.74	0.35-1.54	0.42
Birthweight			$^{+}$*0.416*
<1500gr	1.11	0.51-2.42	0.80
1500-1999gr	1.40	0.82-2.42	0.22
2000-2499gr	1.00		
2500-2999gr	0.90	0.63-1.30	0.58
>=3000gr	0.79	0.50-1.25	0.32
Cord length			+0.962
<40 cms	1.00		
40-60 cms	0.96	0.69-1.35	0.82
>60 cms	1.07	0.40-2.89	0.90

p-value^{+}: refers to global significance of co-efficients.

Table A3: Strien test: Bivariate model showing odds of being left-handed after controlling for the zygo-chorionicity in variables which yielded significant results in the univariate analysis.

Factor	Odds ratio	95% C.I	p- value
Birthweight			*+0.434*
<1500gr	1.26	0.53-2.98	0.60
1500-1999gr	1.76	1.00-3.07	0.05
2000-2499gr	1.00		
2500-2999gr	1.08	0.71-1.65	0.72
>=3000gr	1.06	0.62-1.81	0.85
Parity			*+0.109*
1st parity	1.00		
Other parity	0.67	0.47-0.95	0.03
Cord windings			*+0.195*
Clockwise	1.00		
Counter-clockwise	1.03	0.63-1.68	0.91
Undefined & Mixed	0.60	0.37-0.99	0.05

p-value+: refers to global significance of co-efficients.

Table A4: Bishop test: Bivariate model showing odds of being left-handed after controlling for the zygo-chorionicity in the variable which yielded significant results in the univariate analysis.

Factor	Odds ratio	95% C.I	p- value
Mode of conception			*+0.037*
Assisted	1.00		
Spontaneous	0.76	0.52-1.10	0.15
Unknown	2.30	1.00-5.29	0.05

p-value+: refers to global significance of co-efficients.

Table A5: Population averaged model: Bishop (Performance test- all the 49 cards) giving the odds ratio- odds of being left-handed for every one unit increase in the predictor variable.

Factor	**Odds Ratio**	**95% C.I**	**P value**
Birth year	0.96	0.92-1.02	0.46
Mother's age (years)	1.00	0.95-1.03	0.68
Father's age (years)	0.97	0.93-1.01	0.18
Gestational age (weeks)	0.97	0.91-1.03	0.35
Birthweight (100grams)	0.99	0.96-1.02	0.38
Umbilical cord length (cms)	0.99	0.98-1.01	0.52

Table A6: Univariate models showing odds of being left-handed in different categories compared to baseline category in Bishop (Performance-all the 49 cards) test.

Factor	Odds ratio	95% C.I	P value
Zygochorionicity			*+0.330*
MCMZ	1.00		
DCMZ	0.75	0.43-1.31	0.31
DZ	0.67	0.46-1.12	0.24
Parity			*+0.807*
1st parity	1.00		
Other parity	0.96	0.69-1.33	0.81
Deliverymode			*+0.537*
Spontaneous & breech	1.00		
Instrumental & extraction	1.14	0.73-1.79	0.56
Section	0.86	0.59-1.26	0.44
Presentation of twin			*+0.930*
Cephalic	1.00		
Breech	1.07	0.75-1.53	0.70
Other	1.03	0.53-1.99	0.93
Mode of conception			*+0.003*
Assisted	1.00		
spontaneous	0.97	0.66-1.42	0.88
unknown	3.85	1.65-8.96	0.002
Cord windings			*+0.961*
Clockwise	1.00		
Counter-clockwise	1.06	0.68-1.67	0.78
Undefined & Mixed	1.02	0.69-1.50	0.94
Cord knots			*+0.583*
False knots	1.00		
No knots + Other	1.09	0.78-1.54	0.58
Birth Order			*+0.847*
1	1.00		
2	1.03	0.77-1.38	0.84
Gender			*+0.959*
Male	1.00		
Female	1.01	0.74-1.38	0.95

p-value^{+}: refers to global significance of co-efficients.

Table A7: Bishop test (all the 49 cards): Bivariate model showing odds of being left-handed after controlling for the zygo-chorionicity in the variable which yielded significant results in the univariate analysis.

Factor	**Odds ratio**	**95% C.I**	**p- value**
Mode of conception			*+0.0031*
Assisted	1.00		
Spontaneous	0.85	0.57-1.28	0.44
Unknown	3.44	1.46-8.12	0.005

p-value+: refers to global significance of co-efficients.

Fig 1-1: Map of East Flanders Prospective Twin Survey – (EFPTS)

Fig 1-1: Map of East Flanders region

Fig 2-1: Strien Questionnaire:

R = right L= left B = both

QUESTIONNAIRE: With which hand do you ...

- Vrschr = 'write'
- Vrkni = 'cut with scissors'
- Vrtek = ‘draw’
- Vrdop = ‘screw the top off a bottle ’
- Vrkaa = ‘distribute playing card’
- Vrtan = ‘brush your teeth'
- Vrfle = 'hold a bottle-opener'
- Vrbal = ‘throw away a bal'
- Vrham = 'hold a hammer'
- Vrnaa = ‘put a thread in a needle’
- Vrrac = 'hold a tennis racket'
- Vrkle = ‘open the lid of a cardboard box’
- Vrsle = ‘turn a key’
- Vrmes = ‘hold a knife to cut a rope'
- Vrroe = ‘stir with a spoon'
- Vrgom = ‘rub out something'
- Vrluc = ‘strike a match'

Figure 2-1: Adopted from the Strien questionnaire in Dutch and translated in to English.

Fig 2-2: Bishop's test:

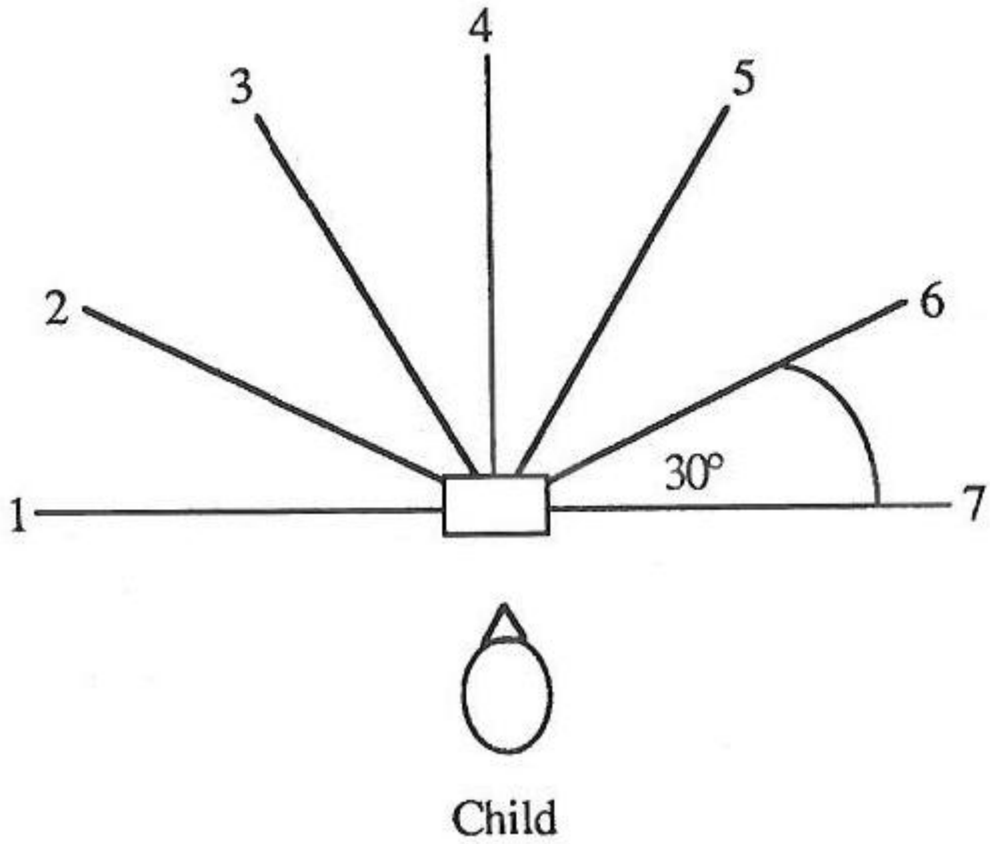
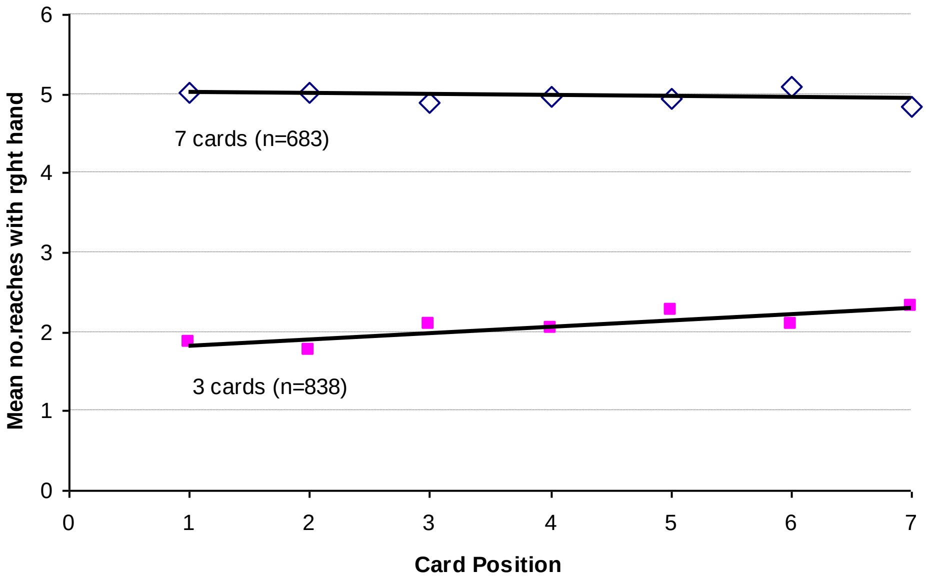


Figure 2-2: Set-up for the task of hand preference (taken from Bishop et al., 1996).

The participant reaches for three cards at each of the numbered locations and places them in the central box. The distance of cards from the central box was adjusted to be within comfortable reach of the contra lateral arm.

Fig 2-3: Handedness according to card position (21 vs. 49) in Bishop's test:



From the above figure, it is obvious that Bishop 21(3 cards) test shows a positive gradient but the gradient for 49 (7 cards) is not statistically different. The co-efficient for 3 cards was 0.079 and p-value was 0.014. The co-efficient for 7 cards was -0.012 and p-value was 0.473.

Fig 2-4: Proportional representations of the body parts in the motor cortex: