Pathological left-handedness revisited:

origins and later life health outcomes

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Pathological left-handedness revisited:

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(met een samenvatting in het Nederlands)

Thesis

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Dipersembahkan untuk orang tuaku dan kakak-kakakku tersayang,

untuk Aleš tersayang, hati dan senyumku.

Contents

Chapter 1.

General introduction

Pathological left-handedness revisited

Asymmetry in nature

One meaning of the term handedness is a tendency to use one hand rather than the other. Another meaning is the property of an object (as a molecule) of not being identical with its mirror image, or, either of the two configurations of an object that may exist in forms which are non-identical mirror images.[1] This diversity in meanings may be a reflection of the fact that asymmetry is common in nature. Natural asymmetry is not merely a characteristic of the human species, but also of animals, plants and even molecules and atoms.[2]

In his book on handedness, 'Right hand, left hand: the origins of asymmetry in brains, bodies, atoms, and cultures',[3] McManus describes some of the milestone discoveries on handedness in its wider sense. The first to show that molecules are asymmetrical in their actions was Louis Pasteur. He found that, although chemically identical to tartaric acid, racemic acid differs in its effect on polarized light. Micro-organisms, Pasteur discovered, could survive and breed on the racemic acid which turned light clockwise, but could not metabolize the racemic acid that turned the light anticlockwise.[2] This total dominance of one type over the other applies to almost every living organism found on earth.[3]

At the cellular level, the asymmetry remains both in structural and functional property: single cell organisms may look virtually symmetrical on the outside; however, they are mostly asymmetrical in their inner structure.[4] In higher organisms, such as mammals, left-right asymmetry arises early in embryogenesis, reproducibly, and consistent across species.[2]

Structural and functional asymmetry in mammals

Virtually all mammals, including the human species, are symmetrical at first glance with the saggital plane as the midline reference. However, the visceral organization and the organs themselves are highly asymmetrical, with for instance in human, the heart, stomach, and spleen being normally on the left side, whereas the liver is located at the right side. Furthermore, the right lung contains more lobes (consequently the bronchus branches are following the number of lobes) than the left lung, the left kidney is located higher than the right kidney.[3] Asymmetry is also present in the structure of the organ which most differentiates *Homo sapiens sapiens* from its ancestors and other mammals, the

brain. The left brain is generally bigger than the right, the planum temporale is bigger on the left side, and the Sylvian fissure is longer in the left side.[2;5]

Human beings, as the most complex of mammals, are probably also the most asymmetrical in terms of hand preference, with the ratio between the use of dominant (right) and non-dominant hand (left) in the population being 9:1.[3] In performing high level tasks, other primates such as gorillas and orangutans show motor asymmetry at the population level.[6] However, the direction of the preference is inconsistent across species and type of activity, with orangutans showing a significant left-hand bias, gorillas showing a trend toward right-handedness, and chimpanzees reportedly exhibiting a population-level right-hand bias for nut cracking but left-hand bias for termite fishing.[7;8] However, in such primates this one side bias is not as strong as in humans, with a 2:1 ratio between dominant compared to non-dominant hand use in the population.[7]

The first visible asymmetry in human body structure is shown as early as 15 days of gestation, when the cardiac tube bends towards the right and forms the D-shaped cardiac loop, which later develops into the heart.[2;3] As for handedness, the earliest sign was recently reported to be detectable at the age of 10 weeks of gestation.[9]

The origin of human handedness

From recent observations of lateralization in tool use by wild chimpanzees it is inferred that throughout time, since about 5 millions years ago before the Pan-Homo split, our ancestors have used mainly the right hand.[7] Researchers have analyzed the arts of people living as early as 15,000 years ago, and they found a similar distribution of around 10% left-handedness as what we find today, which was also shown to be stable across different geographic areas.[10;11] Recent studies in the distribution of hand preference across different geographic areas and cultures revealed some differences in frequency, however strong right hand dominance in those populations remained the rule.[12]

There are several theories which try to explain the origin of handedness. Generally, there are both genetic and environmental theories. Human handedness is already long believed to be genetical in origin, supported by a vast number of studies in this field. However, a simple Mendellian genetic model fails to cover all aspects of handedness or laterality[13] and twins studies failed to show that handedness is purely genetical in origin.[14;15] Currently, the most plausible genetical theories of handedness or laterality which can answer many questions related to handedness come from

Annett[13] and from McManus.[3] Annett introduced the Right Shift (*RS+*) gene, while Mc Manus named the gene Dextral (*D*). The presence of these genes would induce right side dominance, while the absence of them would introduce the role of chance in side preference (Annett called the factor *RS-* and McManus called the allele *C* for chance). The main difference between these theories lies in the definition of handedness: as a continuous phenomenon in Annett's theory (handedness should be measured quantitatively, e.g.using pegboard) and as a dichotomous phenomenon (handedness as simply left and right) in McManus' theory. It has been hypothesized that the gene that programmes the left-right asymmetry of handedness is a mutation of a gene that is responsible for the asymmetry of other body structures in humans.[3]

There are also recent observations linking the hair-whorl trait to hand preference and suggesting a single gene two-allele random recessive model.[16] Recently, a study concluded that, although handedness variation may be etiologically complex, there is at least one polymorphic genetic influence that is located on 2p12-q11.[17] This same chromosome was found to be also linked with schizophrenia/schizoaffective disorder,[18] as non right-handedness has been concluded to be moderately related to these disorders.[19]

As the handedness trait is largely heritable it is puzzling why left-handedness should persist in the evolution. For left-handedness to persist it should have specific benefits. There are indeed observations that such advantages exist. Left-handers are on average better in dual confrontation sports, but also in fights.[20;21] Such observations are frequency dependent, the advantage of being left-handed being higher when the left-handedness frequency is lower. Both left and right handed persons get less chance to practice against left-handed opponents when such opponents are rarer. This phenomenon probably reflects a negative frequency dependent selection mechanism.[22]

An important question is whether handedness is solely influenced by genes or whether there may also be environmental influences. Indeed, there have been a number of theories postulated about a variety of environmental influences. Environmental influences, in terms of timing, may act prenatally, peri-natally or post-natally. As for prenatal environmental influences, the most intriguing theory comes from Geschwind (Behan) and Galaburda (GBG theory),[5] who stated that asymmetry of the brain which influences hand preference is caused by high intrauterine hormonal exposure (testosterone or other sex hormones). However, the GBG theory acknowledged the importance of genetic influence. High exposure to sex hormones was thought to delay the early life growth of the left hemisphere, induce inter hemispheric compensation and finally result in left-

handedness. This exposure was also proposed to alter other structures such as the thymus and therefore induce atypical changes in the immune system.

In the perinatal period, the human brain is susceptible to adverse environmental influences such as hypoxia. Birth trauma or other conditions, such as maternal anxiety,[23] ultrasound during pregnancy,[24] and prematurity[25] which may result in brain hypoxia[26] or other types of brain injury,[25;27] are proposed to cause atypical handedness (left or mixed handedness), although mechanisms are not fully understood. This particular mechanism has been designated as pathological left-handedness.[27]

After birth, subsequent social exposures (cultural, educational, physical environment) may alter the existing innate handedness towards what is locally believed to be socially acceptable. Often still and particularly in specific cultures and religions, left-handed children learn to be right-handed in order to get more accepted in the right-handed world. Although societal pressure against left-handedness has become less of an issue in Western societies, the results of previous strong discouragement of left hand use are still commonly encountered among older individuals.[28-31]

Recently, Rogers and Vallortigara have described and discussed their interesting theory that, given genetic influences on handedness, alignment of the direction of behavioral asymmetries at the population level arises as an evolutionary stable strategy under 'social' pressures. Such social pressure would occur if individually asymmetrical organisms have to coordinate their behavior with that of other asymmetrical organisms of the same or different species.[32]

By nature, many of the hypotheses described above act in very early life or even in gestation when there is at most restricted access to children for direct measurements. For this reason many of these hypotheses are not easily studied in humans. However, it is commonly accepted by now that handedness is indeed predominantly determined by gene(s), nevertheless, environmental influences also clearly play a role in the handedness phenotype.[3;5;13;27.]

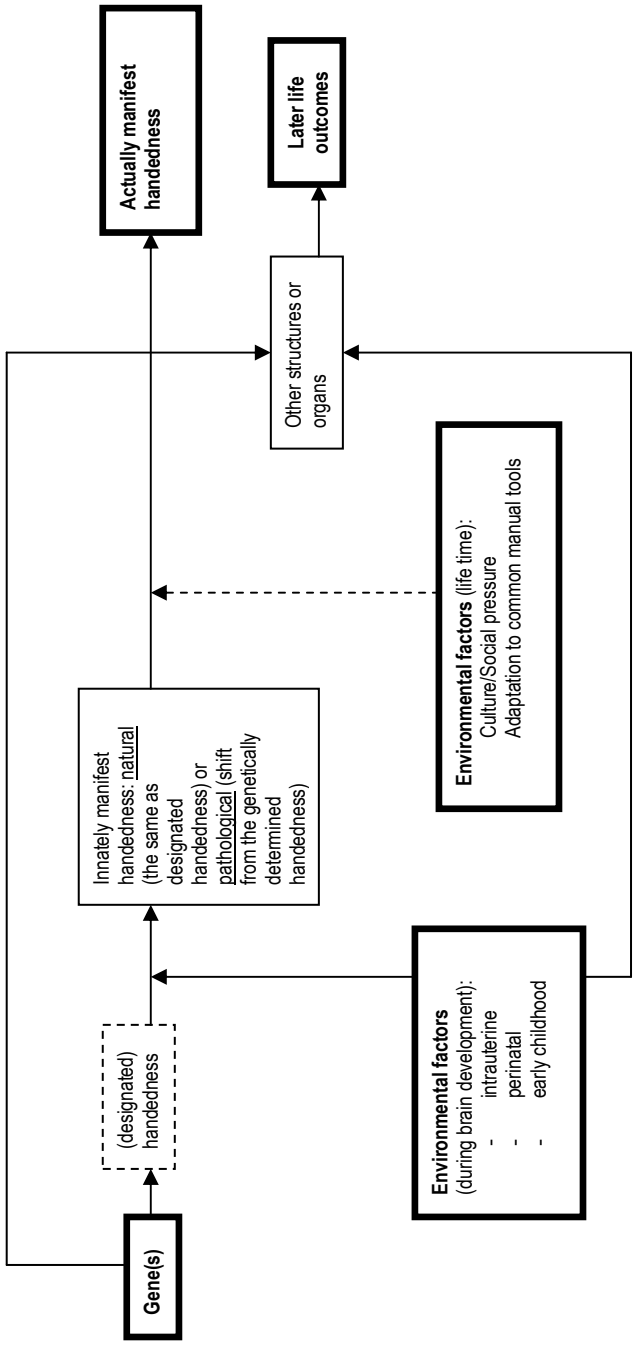

Fig1. Scheme of proposed origins of human handedness

Measuring human handedness

Measuring handedness or rather laterality in humans is complex.[33] If we just ask for people's handedness, the answer will mostly be simply based on the hand they use for writing. However, handedness in humans is actually not so simple and is not restricted to a few manual tasks, moreover, preference and performance (hand skill and strength) should be differentiated. Human handedness is believed to be a continuous variable, a spectrum, which may vary from extremely right-handed, mixed of left and right to extremely left-handed.[13;34] Measuring handedness by using only one question about for instance the writing hand is believed to simplify the matter, thus inducing misclassification.[13;34] Particularly, the writing hand is the item for which most left-handed children were forced to change.[35]

There are several methods to measure handedness (sidedness) in the most valid manner, for instance the use of batteries such as the Oldfield (Edinburgh) inventory.[34] This inventory is composed of several questions in which the subject is asked about which hand (also foot and eye) they prefer to use to perform several tasks such as writing, drawing/painting, throwing, using scissors, using a tooth brush, using a knife without fork, using a spoon, using a broom, striking a match, opening a box, kicking, and looking with one eye. The results are then transferred into a laterality quotient which ranges from -100 (extremely left-handed) to +100 (extremely right-handed).

Another method, which is mostly used in children is observed performance, where children are asked to do several tasks, such as writing, throwing, moving a peg on a board, and finger tapping task.[33;35]

Despite of all the complexity of hand preference measurement, self reported hand preference was shown to be consistent with the actually performed common tasks, and therefore can be used as the crude approximation of the actual handedness.[36;37]

Why epidemiology of handedness?

Epidemiology has a general interest in disease occurrence as a function of determinants, and as a discipline it is best described as occurrence research. Hand preference, as a measure for cerebral lateralization, is in that sense of interest if it is possibly associated with disease or other health states. Such associations, particularly concerning handedness and mortality, have been suggested in the

past but also refuted.[38-50] However, there are also observations linking handedness to disease/disorders that are still subject to research.[5;19;35;51-55]

Overall, there is common scientific consensus that hand preference should generally be considered a result of normal genetic variation. However, part of the handedness distribution may have a pathological origin and be a proxy for early life developmental problems that may themselves underlie later life diseases. The reasoning along such lines is not much different from for instance the 'developmental origins of adult diseases' or Barker hypothesis.[56-58] In epidemiologic studies, hand preference can then be used as an approximation, albeit crude, for an aggregate of early life causes for later disease.

The studies presented in this thesis tried to find new causes for pathological left-handedness and advancing knowledge of associations between handedness and diseases in later life.

Handedness research in this thesis: some premises

For research on handedness, particularly as a possible disease risk indicator (see later in this thesis), it was important to take account of the varying suggested origins. The point of departure for this thesis was that in essence variation in handedness or laterality is natural (genetic) variation with no obvious pathophysiological relation to disease or other health states. However, we also included the concept of pathological left-handedness, covering all putative adverse early life environmental effects that may cause excess left-handedness irrespective of genetic preference. Left-handedness thus defined, may have later life consequences. Since we were unable to distinguish the physiological from the pathological, we were bound to have determinant (handedness) misclassification. Such misclassification has consequences for the interpretation of any effects that we were to encounter in the studies of this thesis.

A second premise, although seemingly obvious, is that handedness itself is no cause of disease but a possible marker of processes that may lead to disease. This was important as handedness may itself be directly related to specific health states such as accident proneness[36;59;60] and such associations were not the object of study in this thesis.

A third premise for the set-up of this thesis was based on a very wide variety of published observations concerning handedness or laterality. The fact that so many associations with handedness were found could mean that the processes involved in laterality are so central that any

disturbances in these processes may indeed have consequences in a variety of health areas. Alternatively, we should acknowledge that this variety might also be considered an indication that associations are biased, confounded. In this thesis we started from the view that laterality is a central essential phenomenon.

A fourth and final premise is that handedness in its meaning of manual preference, is a measure that we have to rely on in much of the research in this thesis mainly for logistic reasons. However, as pointed out above, it is a measure for a wide variety of human functions that are lateralized. As the actual interest is in cerebral lateralization, the use of hand preference as just one indicator for lateralization would undoubtedly further contribute to determinant misclassification. Again, this would have to be taken account of in interpretations of findings in this thesis.

Outline of the thesis

In this thesis, the presented studies were based on several cohorts and cross-sections. The first study (chapter 2.1) was based on a study in prematurely born children in an academic hospital in Utrecht, the Netherlands, who had been followed up for 8 years. As neonates, these children underwent a serial cranial ultrasound (US) to determine brain lesions. At school age (median age 8 years), these children were asked for their hand preference, as well as being observed while doing certain manual tasks. Magnetic Resonance Imaging (MRI) was performed to make sure that all brain lesions were observed. We studied the relationship between brain lesions and later handedness.

In chapter 2.2, we investigated whether brain insults, such as bacterial meningitis, occurring in early childhood, may increase the chance of children to become left-handed. We studied the association between a meningitis severity score, derived from clinical and laboratory signs and symptoms which were previously reported to be predictive of more severe bacterial meningitis, and the chance of becoming left-handed. Furthermore, we also studied whether the left-handed children had a different neuropsychological, hearing, and motor skill performance at school age compared to the right handed children. This study was performed in a cohort of Dutch children followed up for 7.4 years from the age when bacterial meningitis was first diagnosed until reaching school age (mean age 9.7).

In chapter 2.3 we studied whether a functional polymorphism in the promoter area of IGF-1 gene and left-handedness are related, as a possible explanation for associations found between handedness and breast cancer. This cross-sectional study was performed in a birth cohort of young

adult women who were included from the Utrecht area. The polymorphism was determined at first inclusion in young adulthood and handedness was measured using the Edinburgh inventory 4 years after inclusion.

In chapter 3.1, we studied the association between left-handedness and breast cancer incidence in middle age women. We used a cohort of a breast cancer screening program in Utrecht, the Netherlands. At inclusion, these women were asked for their innate hand preference and from then onwards, they were followed for the occurrence of breast cancer for 16 years. Data on demography and reproductive history were obtained using a questionnaire at inclusion.

In chapter 3.2 we investigated the association between left-handedness, depression, and diseases proneness. We used a cohort of adult men and women of whom at inclusion data on demography and handedness were assessed by questionnaires. Approximately 4 years afterwards, these participants were asked to fill out standardized questionnaires in the psychiatric domain and also data on CIDI (Composite International Diagnostic Interview) based depression were obtained.

In order to investigate a question about left-handedness and mortality risk that has been much debated in the past, we used the same cohort that we used in chapter 3.1. These women were followed up for the outcome for almost 13 years from inclusion (chapter 3.3).

The main results of the above studies are reviewed and discussed in chapter 4. Further discussion about the clinical relevance, public debates and suggestions for future research is also presented in this chapter.

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Chapter 2.

The origin of handedness

Chapter 2.1.

Minor intraventricular haemorrhage in the left side of the neonatal brain induces left-handedness.

Manuscript based on this chapter:

Ramadhani MK, Rademaker KJ, de Vries LS, Grobbee DE, Beek F, Uiterwaal CSPM. Minor intraventricular haemorrhage in the left side of the neonatal brain induces left-handedness. Submitted.

Summary

Background:
Prematurely born children are more often left-handed than their term born peers. It is unknown if this excess left-handedness, a marker for the extent of cerebral lateralisation, is caused by specific cerebral lesions related to prematurity.

Subjects and Methods:
In a cohort of 221 preterm born infants (gestational age < 32 weeks and/or birth weight < 1,500 grams), brains were serially examined in the neonatal period using cranial ultrasound (US) to detect intraventricular haemorrhage (IVH), periventricular leukomalacia (PVL) and other abnormalities. At age 7 to 10 years, magnetic resonance imaging (MRI) was done, and hand preference and current IQ were measured. MRI and US were classified as normal, mildly abnormal, or severely abnormal, blinded to handedness data.

Results:
Children with severely abnormal findings had an increased chance to become left-handed compared to those with normal US findings: odds ratio (OR) 4.1, 95% CI 1.6 to 10.0, p=0.003. This was confirmed by MRI scans: 4.0, 1.5 to 10.7, p=0.005. Findings were mainly attributable to IVH. Children with left-sided IVH showed a higher chance for left-handedness compared to those without IVH (OR 4.4, 1.7 to 11.3, p=0.002), whereas right-sided IVH did not. Furthermore, neonates with left-sided mild IVH (grade I and II) still showed an increased chance for left-handedness: OR 4.0, 95% CI 1.5 to 10.9, p=0.007. PVL was not related to left-handedness.

Conclusion:
Our findings strongly indicate that even a small intraventricular haemorrhage affecting the left side of the brain may induce left-handedness. This is likely related to the role of the subependymal germinal matrix in the developing brain.

Introduction

There is strong evidence in support of a genetic basis of hand preference,[1;2] but early life environmental circumstances may have an impact as well.[2-4]

The prevalence of left-handedness varies between populations but is typically around 12% for males and 9% for females.[1] There is a higher prevalence of left-handedness among children with extremely low birth weight[5;6] and among prematurely born children.[7] This suggests that early life stressors may induce a shift towards left-handedness. Prematurely born neonates are also at an increased risk for brain lesions.[8] If prematurity is related to more brain damage and to more left-handedness, it may be speculated that brain damage can induce left-handedness. That would fit the theory of pathological left-handedness, proposing an increased incidence of left-handedness in children with early life brain damage to the left hemisphere.[4] Although empirical evidence is scarce, there are observations in young children that congenital hemiplegia may cause a right-handed predisposition to shift towards left-handedness.[9]

We set out to examine whether left-handedness at school age is related to brain damage in a birth cohort of prematurely born children.

Subjects and Methods

Study population

The study pertains to children admitted soon after birth to the Neonatal Intensive Care Unit of the Wilhelmina Children's Hospital, a tertiary referral hospital. All children, born between March 1, 1991 and March 1, 1993 with a gestational age $\leq$ 32 weeks and/or a birth weight $\leq$ 1500 grams were enrolled in a cohort with follow-up through school age. The rationale and study design were extensively described elsewhere.[10] Briefly, the original cohort consisted of 375 children of whom 64 (17%) died and 28 (7.5%) were excluded because of congenital abnormalities or chromosomal disorders. At a median age of 8 years, the children were invited to visit the hospital for one day and have several tests including cranial MRI. Of the remaining 283 children, 22 (7.8%) could not be traced due to moving and the parents of 25 children (8.8%) refused to participate. Finally, 236 children (83.4%) participated.

Neonatal cranial US was available in 234 (99.2%) and MRI in 226 (95.8%), of the 236 children. MRI failed due to anxiety in 10 children. Two children with a congenital abnormality on

neonatal cranial US and one child who developed a not yet diagnosed neuromuscular disorder were excluded. This left 221 children who had both US and MRI (78% of all included). The study was approved by the Medical Ethics Committee of the University Medical Center Utrecht. Parental informed consent was obtained.

Neonatal cranial US

Cranial US was performed within six hours after admission, at least three times during the first week of life and subsequently once a week until discharge. At term age the infants were scanned in the follow-up clinic during their regular visits as long as the anterior fontanel allowed for examination. A standardized protocol was used to acquire images in a systematic way (coronal, midsagittal, parasagittal planes). An ATL UM-4 mechanical sector scanner (Philips Medical Systems, Best, The Netherlands) was used with a 7.5 MHz transducer to ensure the best possible resolution.

All US scans were analyzed by one neonatologist (LSdV) who was unaware of later handedness, MRI findings or neurodevelopmental outcome. The US scans were classified according to the most severe lesions seen at any moment (table 1). Haemorrhages were classified according to Papile.[11] Periventricular areas of increased echogenicity (PVL) were classified according to de Vries.[12] The combined US findings were classified into three groups: normal, mildly abnormal, severely abnormal.

Focal infarction was diagnosed when an area of increased echogenicity with cystic evolution was seen in a region supplied by one of the main cerebral arteries; a convexity haemorrhage was diagnosed when a unilateral area of increased echogenicity at the convexity of the brain was seen, not limited to a specific arterial territory. US scans were also analyzed for the presence of ventricular dilatation (post haemorrhagic or ex-vacuo), calcifications, germinal layer necrosis, germinal layer or choroid plexus cysts or subependymal pseudo cysts. Ventricular dilatation as a single feature was classified in the mildly abnormal group. If the ventricular dilatation existed in combination with a small haemorrhage (post haemorrhagic ventricular dilatation) and therapeutic intervention for the dilatation was required, it was listed in the severely abnormal group.

MRI

We used neonatal US findings as the primary predictor of subsequent handedness. Neonatal US is a dynamic examination that may detect transient phenomena in the brain and also aspects that would

be different from findings on MRI. Therefore, school age MRI findings were used to confirm the US results. Details on the MRI measurements have been extensively described elsewhere.[10]

Table 1. Classification of US and MRI findings

US	
Classification of haemorrhages according to Papile[11]	
Grade I	small germinal layer haemorrhage (GLH)
Grade II	GLH plus intraventricular haemorrhage (IVH), filling the ventricle <50% with blood
Grade III	Large IVH distending the ventricle in the acute phase due to blood filling the ventricle >50%
Grade IV	IVH associated with unilateral parenchymal involvement due to venous infarction
Classification of periventricular leukomalacia (PVL) according to de Vries[12]	
Grade I	periventricular areas of increased echogenicity present for 7 days or more
Grade II	periventricular areas evolving into small localized fronto-parietal cysts
Grade III	periventricular areas of increased echogenicity evolving into extensive periventricular cystic lesions involving the occipital and frontal-parietal white matter
Clinical classification	
Normal	no or minor abnormalities like germinal layer or plexus cysts, subependymal pseudo cysts or calcifications (lenticulostriate vasculopathy) as exclusive findings
Mildly abnormal	IVH grade I or II, PVL grade I or germinal layer necrosis or a combination of these features
Severely abnormal	IVH grade III or IV, cystic PVL grade II or III, thalamic lesion, focal infarction or haemorrhage at the level of the convexity
MRI	
Clinical classification	
Normal	no abnormalities
Mildly abnormal	mild gliosis, mild ventricular dilatation, irregular shape of the ventricles, thinning of the corpus callosum or a combination of these features
Severely abnormal	extensive gliosis or gliosis in combination with marked ventricular dilatation, thalamic lesions, an abnormal retrochiasmatic part of the visual system, cerebellar and cortical atrophy

Handedness at school age

Hand preference was measured as a part of the movement ABC test.[13] The chart contains three domains: manual dexterity (3 items), ball skills (2 items) and static and dynamic balance (3 items). Before the test, the children were asked to indicate their preferred hand for writing or drawing. Children indicated left, right or no preference. During the test, the investigator observed the preferred hand used for performing the tasks. All children used the hands they indicated previously as their preferred hand. Children who had no preference (n = 3) were included as non-left-handed children.

Intelligence

All children performed five subtests of the Wechsler Intelligence Scale for Children-Revised Edition (WISC-R; Dutch version): similarities, vocabulary, block design, picture arrangement and digit span. They were supervised by a child psychologist, who was unaware of the neonatal or handedness status of the child. Using the procedures and tables published by Kaufman[14] scaled scores were converted to an estimated IQ score.

Data analysis

Group differences in general characteristics were tested using student's t-test or Mann-Whitney U test when appropriate. Group differences in categorical variables were tested using chi-square tests for trend.

Logistic regression analysis was used to study the relation between handedness as dependent variable and variables indicating brain damage as predictors. Furthermore, adjustment for potential confounders namely gender, birth weight, gestational age, and age (only for MRI) was performed. Analyses were performed in the overall group (n = 221) and in the group without cerebral palsy (CP; n = 201). For subsequent more specific analyses, we used the group without CP only, because hand preference of children with CP is very much affected by the disabled limbs.

Similar logistic regression models were used to analyse the associations between type and severity of brain damage and handedness. For analyses on handedness and the side and grade of IVH, IVH grade I and II were pooled together for reasons of statistical precision. Grade III was not pooled together with grade IV due to the presence of parenchymal involvement in grade IV. We could not meaningfully analyse separately for grade III or IV because of very small numbers of children in each group.

Results are expressed as odds ratios with corresponding 95% confidence intervals and intervals not including 1 ($p<0.05$) were considered statistically significant. Data were analyzed using the SPSS for Windows statistical package (version 11.5, SPSS Inc., Chicago, IL, U.S.A.).

Results

At school age, 20.4% were left-handed, with 23% left-handed males and 17% left-handed females. Table 2 shows that left-handed children were only slightly lighter at birth than non left-handed children (p = 0.18), gestational age and school age IQ were similar, and similar proportions of children had CP.

Table 2. Characteristics of 221 prematurely born children by handedness.

	Left-handed	Non left-handed	P value
GENERAL			
N (%)	45 (20.4)	176 (79.6)	
Gender (%)			0.29
Female	17 (37.8)	82 (46.6)	
Male	28 (62.2)	94 (53.4)	
AT BIRTH			
Mean birth weight in grams	1141 (267)	1211(326)	0.18
Minimum, maximum birth weight	700, 1610	485, 2200	
Mean gestational age in weeks	29.6 (2.0)	29.4 (2.0)	0.50
Minimum, maximum gestational age	26.1, 36.0	25.0, 34.4	
Cranial US (%)			
Normal	14 (31.1)	82 (46.6)	
Mildly abnormal	17 (37.8)	72 (40.9)	0.01*
Severely abnormal	14 (31.1)	22 (12.5)	
CP (%)	4 (8.9)	16 (9.1)	0.97
AT SCHOOL AGE			
Median age in years (minimum, maximum)	7.9 (7.3, 10.5)	8.0 (7.2, 10.5)	0.52
MRI (%)			
Normal	12 (26.7)	75 (42.6)	
Mildly abnormal	21 (46.7)	83 (47.2)	<0.01*
Severely abnormal	12 (26.7)	18 (10.2)	
Intelligence Quotient	99.3 (2.4)	101.2 (1.1)	0.44

Values are mean (standard deviation), unless otherwise indicated. US = ultrasound, CP = cerebral palsy, MRI = magnetic resonance imaging,
* chi square tests for trend

Table 3 shows that neonates with severe brain abnormalities had a statistically significant 4.1 times higher chance for left-handedness than normal neonates, after adjustment for gender, gestational age, and birth weight. Excluding the children with CP strengthened the findings. The results were largely similar when using MRI results at school age. As CP could clearly influence handedness preference, all 20 affected children were excluded from further analyses.

Table 3. Associations between neonatal cranial US and school age MRI imaging classifications and handedness in prematurely born infants.

NEONATAL US								
	Left-handed (%)	Non left-handed (%)	Odds ratio	95% CI	p-value	Adjusted Odds ratio	95% CI	p-value
CP included (n=221)								
Normal (96)	14 (15)	82 (85)	-	-	-	-	-	-
Mildly abnormal (89)	17 (19)	72 (81)	1.4	0.6 to 3.0	0.412	1.5	0.7 to 3.5	0.292
Severely abnormal (36)	14 (39)	22 (61)	3.7	1.6 to 9.0	0.003	4.1	1.6 to 10.0	0.003
CP excluded (n=201)								
Normal (95)	14 (15)	81 (85)	-	-	-	-	-	-
Mildly abnormal (85)	17 (20)	68 (80)	1.5	0.7 to 3.2	0.352	1.7	0.7 to 3.8	0.221
Severely abnormal (21)	10 (48)	11(52)	5.3	1.9 to 14.7	0.002	5.5	1.9 to 15.7	0.002
MRI AT SCHOOL AGE								
	Left-handed (%)	Non left-handed (%)	Odds ratio	95% CI	p-value	Adjusted Odds ratio	95% CI	p-value
CP included (n=221)								
Normal (96)	12 (13)	75 (87)	-	-	-	-	-	-
Mildly abnormal (89)	21 (24)	83 (76)	1.6	0.7 to 3.4	0.246	1.3	0.6 to 3.0	0.470
Severely abnormal (36)	12 (33)	18 (67)	4.2	1.6 to 10.8	0.003	4.0	1.5 to 10.7	0.005
CP excluded (n=201)								
Normal (95)	12 (13)	75 (87)	-	-	-	-	-	-
Mildly abnormal (85)	21 (25)	80 (75)	1.6	0.8 to 3.6	0.211	1.4	0.6 to 3.1	0.402
Severely abnormal (21)	8 (38)	5 (62)	10.0	2.8 to 35.7	<0.001	9.7	2.7 to 35.7	0.001

Normal children are the reference categories; 95% CI: 95% confidence interval; Adjusted: gender, gestational age, birth weight (MRI data additionally adjusted for age)

Table 4 shows that neonates with IVH on US had a 2.6 times higher chance to become left-handed than those without IVH. PVL was not associated with handedness. Results for other types of abnormalities were not statistically significant.

Table 4. Associations between types of neonatal brain abnormality on cranial US of prematurely born infants and handedness at school age.

Type of brain abnormality		Left-handed (%)	Non left-handed (%)	Odds ratio	95% CI	p-value	Adjusted Odds ratio	95% CI	p-value
IVH									
	No (133)	21 (16)	112 (84)	-	-	-	-	-	-
	Yes (67)	19 (28)	48 (72)	2.1	1.0 to 4.3	0.038	2.6	1.2 to 5.7	0.015
PVL									
	No (167)	33 (20)	134 (80)	-	-	-	-		
	Yes (33)	7 (21)	26 (79)	1.1	0.4 to 2.7	0.849	1.1	0.4 to 2.8	0.852
Other									
	No (166)	31 (19)	134 (81)	-	-	-	-	-	-
	Yes (35)	9 (26)	26 (74)	1.5	0.6 to 3.5	0.354	1.5	0.6 to 3.5	0.392

Normal children are the reference categories; values pertain to 200 premature children without CP (additional exclusion: 1 child with IVH and another type of intracranial bleeding); 95% CI: 95% confidence interval; Adjusted: gender, gestational age, birth weight; Other: ventricular dilatation (post hemorrhagic or ex-vacuo), calcifications, germinal layer necrosis, germinal layer or choroid plexus cysts, subependymal pseudocysts.

Table 5 shows that neonates with a left sided IVH had a 4.4 times higher chance for left-handedness than neonates without IVH, while those with a right sided IVH had a similar chance. Those with bilateral IVH had a relative risk of 2.2 but this was not statistically significant. Categorizing the IVH based on both the clinical grading and side of the lesion showed that children with minor left-sided IVH (grades I-II) had a 4.0 times higher chance for left-handedness than children without IVH. Among the children with severe IVH (grades III or IV) there were only 4 right-sided, 4 left-sided, and 5 bilateral IVH, which did not allow for meaningful analysis of an association between handedness and grade III or IV IVH.

Forty-three percent of the children with recorded IVH grade I and II in the neonatal period had normal MRI findings during school age (51% mildly abnormal and 6% severely abnormal), compared to only 15% of the children with grade III and IV IVH (31% mildly abnormal and 54% severely abnormal).

Table 5. Associations between side and grading of IVH on cranial neonatal US in prematurely born infants and handedness at school age.

IVH	Left-handed (%)	Non left-handed (%)	OR	95% CI	p-value	Adjusted OR	95% CI	p-value
By side (n = 199)								
None (133)	21 (16)	112 (84)	-	-	-	-	-	-
Right side (19)	3 (16)	16 (84)	1.0	0.3 to 3.7	1.000	1.2	0.3 to 4.6	0.804
Left side (26)	11 (42)	15 (58)	3.9	1.6 to 9.7	0.003	4.4	1.7 to 11.3	0.002
Bilateral (21)	5 (24)	16 (76)	1.7	0.6 to 5.0	0.366	2.2	0.6 to 7.3	0.213
By side and grade (n = 186)								
None (133)	21 (16)	112 (84)	-	-	-	-	-	-
Grade I or II right side (15)	1 (7)	14 (93)	0.38	0.1 to 3.1	0.381	0.5	0.1 to 3.7	0.456
Grade I or II left side (21)	9 (43)	13 (57)	3.69	1.4 to 9.7	0.008	4.0	1.5 to 10.9	0.007
Grade I and/or II bilateral (16)	2 (13)	14 (87)	0.76	0.2 to 3.6	0.731	1.0	0.2 to 5.3	0.970

Children without IVH are the reference category; for analysis by side of IVH, values pertain to 199 premature children without CP (additionally excluded: 1 child with IVH and another type of intracranial bleeding, 1 child with unknown side of IVH); Analysis restricted to IVH grades I and II as grades III or IV (n = 13) were too rare; 95% CI : 95% confidence interval; Adjusted: gender, gestational age, birth weight.

Discussion

Our main finding, in a cohort of prematurely born neonates followed for 8 years, is that in preterm children minor IVH particularly affecting the left hemisphere is strongly related to subsequent left-handedness.

The original motive for the study was not related to the handedness hypothesis, thus rendering information bias unlikely. Handedness was directly observed by the investigator and at school age when it is considered to be almost stable.[7] The 20% prevalence of left-handedness in our cohort was similar to other populations of premature children,[15] but twice that in the general population.[1]

It is commonly taken that interactions between a strong genetic influence and early life environment (pre, peri and post-natal) and learning processes play a role in the actual manifestation of hand preference.[1;2] An adverse perinatal environment such as birth stress may induce pathological cerebral lateralisation including handedness, particularly in males.[16] Rh incompatibility, low birth

weight, premature birth, caesarean section, and breech delivery may be such stressors although relationships are weak.[16]

We know of one study by Marlow et al[15] using neonatal US in premature children that did not show a relation between brain damage and handedness. We attribute the disparities between their findings and ours to measurement differences. They found normal scans in 60% of children versus 42% in our study. This may be explained by our use of a mechanical sector scanner with a 7.5 MHz transducer instead of a linear array machine with a 5 MHz transducer, which is less well suited for the detection of lesions in the periventricular white matter. Moreover, Marlow et al used parental-reported handedness from observing their younger children for two days, whereas we assessed handedness by direct observation at school age.

Left-sided IVH was related to left-handedness in our study. This agrees with the concept of pathological left-handedness.[4] Our results further point at a specific brain location, the subependymal germinal matrix that might be critical to that concept, because particularly even mild grades of left-sided IVH did increase the chance to become left-handed. The germinal matrix is located over the head of the caudate nucleus and in the thick subependymal cell layer of the thalamostriate groove of the ventricle. It functions as a pool of neuroblasts as the origin of neurons and of glioblasts as progenitor cells of microglia, astrocytes and oligodendrocytes migrating to cerebral cortex, including the motor area.[17-19] It is most prominent between 24 and 34 weeks of gestation and has almost completely regressed by term age.[17] However, in preterm infants germinal matrix tissue is found to be still extensive.[17] At the last stage of gestation its function is mostly related to glia.[19] The function of glia is probably not restricted to the support of neurons, as astrocytes were recently found to have active roles in neuro-organization, synaptogenesis and synaptic maintenance, of the developing and adult brain.[20] Destruction of glia progenitors due to bleeding might cause atypical development of the ipsilateral brain, possibly also including the motor area.

The premature brain is susceptible to damage due to premature anatomy, haemodynamic instability, and a propensity for bleeding.[17] However, there have been reports of asymptomatic antenatal IVH in 3.8% of full term neonates,[21] without any reported adverse outcome.[21] Furthermore, full term children with brain damage due to birth stress also have a higher chance of becoming left-handed.[16] Thus, left-handedness in general may to some extent be attributable to antenatal IVH. The outcome of preterm born infants with IVH depends on the IVH severity.[18] The children with uncomplicated IVH are reported to have a reduced size of the cortical grey matter.[22] After an IVH,

rats show bilaterally suppressed cell proliferation in the germinal matrix, increased cell death in the ipsilateral striatum and germinal matrix, and astrocyte and microglia reaction.[23]

Our results indicate that IVH increases the chance of becoming left-handed while PVL does not. IVH mostly affects one hemisphere, while only in severe cases it may affect both sides.[24] Moreover, germinal matrix haemorrhage more likely involves the left side rather than the right or both sides, probably due to cerebrovascular anatomic differences or to haemodynamic stresses related to patency of the ductus arteriosus.[25] PVL is generally bilateral and is the most common type of injury causing CP, often affecting the lower limbs (diplegia) or all extremities (quadriplegia).[26] This may explain why PVL in our study was not related to left-handedness and why including CP in our analyses diluted our observations.

Although our findings indicate that even subtle brain damage in a specific critical area of the developing brain may induce left-handedness, the direct neurological impact of such damage may be limited. Following insults, a variety of compensatory changes in the brain occur, often sustaining functions through operation of undamaged residual tissue.[27] There are reports, including our own, about left-handedness having other adverse health consequences in later life.[28;29] However, whether pathological left-handedness has anything to do with such later life health is as yet unknown.

In conclusion, our findings strongly indicate that even a small intraventricular haemorrhage affecting the left side of the neonatal brain induces left-handedness. This is likely to be related to the role of the subependymal germinal matrix in the developing brain.

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Chapter 2.2.

More likely left-handed after more serious childhood bacterial meningitis: support for the pathological left-handedness hypothesis

Manuscript based on this chapter:

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Summary

Background:
Although handedness appears largely genetically determined, it has been suggested that early life brain damage promotes pathological left-handedness in some cases. Consequently, brain damage inflicted by bacterial meningitis may be a cause of left-handedness.

Subjects and Methods:
182 survivors of childhood non-*Haemophilus influenzae type b* (Hib) bacterial meningitis in the Netherlands (mean age of 9.7 years) were studied. These children were selected randomly after clustering them into those with or without parental report on academic and behavioural problems. Medical records were obtained from the hospitals, while handedness and neurodevelopmental outcome were assessed at school age. Logistic regression analysis was used to study the relationship between a severity score of bacterial meningitis and handedness.

Results:
Fifteen percent were left-handed. Severity of childhood bacterial meningitis was related to left-handedness (Odds Ratio (OR) 6.2, 95% CI 2.0 to 18.6 for those with a total severity score above the median compared to those below). Compared to non left-handed children, left-handed children had lower IQ (mean difference - 6.6, 95% CI -12 to -1.2), lower vocabulary score of WISC-r (-1.0, -2.1 to 0), and lower Beery score on visual-motor integration (– 4.9, -10.1 to 0.4). Left-handed children also had more combined academic and behavioural limitations (OR 2.7, 95% CI 0.9 to 8.6), lower manual speed of the dominant hand (mean difference -9 taps, $p < 0.05$) and better manual steadiness in the non-dominant hand (mean difference of contact's time -2.7 second, $p<0.05$).

Conclusion:
Our results support the role of early life brain damage in left-handedness. Left-handed post-meningitic children generally have worse neurodevelopmental outcome than non left-handed survivors.

Introduction

Despite the changes in clinical course and prognosis induced by the introduction of antibiotics,[1] bacterial meningitis continues to be a significant cause of morbidity and mortality in children.[2] Around five percent of children with bacterial meningitis die and, among survivors, about 15% develop severe sequelae such as sensorineural hearing loss, motor problems, seizures, and mental retardation.[3] More subtle adverse outcomes such as cognitive, academic, and behavioral problems are present in an additional 20%.[4]

Hand preference is believed to develop throughout life, especially during early life. Handedness is strongly genetically determined[5;6] but the eventual phenotype is also believed to be influenced by non-genetic factors.[7] The frequency of left-handedness in the general population is typically around 10%.[6] Observations on prematurely born and full term infants with possible perinatal brain injury suggest an increased frequency of left-handed individuals, which may be related to early brain injury,[8;9] named pathological left-handedness.[10]

Bacterial meningitis which occurs during early childhood may affect later hand preference through injury of the developing brain.[11] It was suggested that bacterial meningitis involves the right brain more often, leading towards dominance of right-handedness.[12] However, observations in meningitis survivors showed a higher chance of left-handedness compared to healthy siblings, implying that brain damage occurring before handedness is firmly established may induce a switch in hand preference.[11]

Childhood survivors of bacterial meningitis and children with left hand preferences also appear to share common neurodevelopmental or physical ailments, such as hearing impairment, lower academic and motor performance.[1;8;13]

We aimed to study characteristics of severity of bacterial meningitis in early childhood that may be causally related to hand preference of the survivors at school age. We also studied the relationship between hand preference and neurodevelopmental outcome at school age.

Subjects and Methods

Study population

The present study was initiated to develop a prediction rule of academic and/or behavioural limitations at school age of survivors of childhood bacterial meningitis. The rationale and design have been extensively described elsewhere.[14]

Briefly, in 1999, Koomen et al.[4;14] compiled a cohort of 674 Dutch school-age children who had recovered from non-Hib bacterial meningitis 5 to 10 years earlier without severe sequelae. The inclusion criteria were: birth date between January 1986 and December 1994, and recovery from meningitis caused by *Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus agalactiae, Escherichia coli,* or *Listeria monocytogenes* between January 1990 and December 1995. The exclusion criteria were: meningitis caused by Hib or other less common pathogens, 'complex onset' of meningitis (defined as: meningitis secondary to immunodeficiency states, central nervous system surgery, cranial trauma or cerebrospinal fluid (CSF) shunt infections or relapsing meningitis), mental retardation, bilateral deafness, cognitive or behavioural problems prior to meningitis, and diseases developed after meningitis (such as cancer) which could by themselves or their treatment have led to cognitive and/or behavioural problems. Fifty-seven percent of the children were boys, the mean age at infection was 2.4 (range 0 to 9.5) years. The diagnosis of bacterial meningitis was based on the isolation of bacteria in the CSF.

For efficiency reasons, Koomen, et al.[4;14] selected two equal groups of children from the cohort on the basis of data gathered by two questionnaires on complaints with respect to school achievement and behaviour. Children were classified as those whose parents had complaints about academic achievement and/or behaviour (134 children, 20%) and those whose parents had no complaints (the remaining: 540 children, 80%). One hundred of the 134 children whose parents had complaints and 101 of the 540 children, whose parents had no complaints, were randomly sampled (nested case-control approach). Of these children, the academic achievement and behaviour were assessed with the *Academic Achievement Test* (*AAT*) and the *Child Behaviour Checklist* (*CBCL*), and the medical records were reviewed for a final and thorough check of the exclusion criteria and to obtain data on the risk factors. Nineteen children were excluded due to various causes.[14] Hence, the final study population consisted of 182 children, of whom 84 had academic and/or behavioural limitations (based on *AAT* and *CBCL*). The children were assessed at a mean age of 9.7 (range 5.3-14.2) years, on average 7.4 (range 4.0-10.4) years after meningitis.

Measurements at meningitis diagnosis

Koomen, et al.[4;14] obtained data on signs and symptoms as well as physical and laboratory examination from the original medical records of the children. For the present analysis, we used variables which were previously shown to be predictors of severity of bacterial meningitis, such as certain general characteristics, signs and symptoms at presentation, laboratory indicators, and causative pathogen.[15]

Measurement at school age

Hand preference

Handedness was observed and recorded when the children performed tasks (finger tapping and manual steadiness) for another research question.[4] The tasks were part of a test to measure motor speed and steadiness of children. They were asked which hand was their dominant hand. The investigators were blinded for both the meningitis severity and the neuropsychological status of these children.

Neuropsychological assessment

Details on this assessment have been described extensively elsewhere.[4]

Assessment of the children took place individually at the University Medical Centre Utrecht between August 1999 and June 2000. For some analyses on attention, and manual speed and steadiness, all children under 7 years (n=33) were excluded, because academic skills had not yet developed to a degree that could be reliably assessed using standardised methods, hence these analyses pertained to 149 children.

Koomen, et al.[4;14] measured general cognition (using the computerised version of the Coloured (age < 11 years) or Standard (age ≥ 11 years) Progressive Matrices[16;17] and the Vocabulary subtest of the Wechsler Intelligence Scale for Children-Revised[18]), memory and learning (*Word Span* providing a measure of working memory[19]), attention (*Colour Trails,*[20] *Balloon Piercing,*[21] *Copying geometrical figures: The Developmental test of Visual-Motor Integration (Beery)* [22]), reaction times, manual speed and steadiness.[21]

Hearing impairment

Information on hearing loss after the episode of bacterial meningitis was obtained from medical records and the parents by questionnaire. Hearing loss was defined as a perceptive loss of > 25

dB4.[23] Also gathered from the medical records was information on timing and type of hearing evaluation. Children with bilateral deafness were excluded.

Minor neurological signs

A standardised neurological examination[24] was performed by a child neuropsychologist. Particular attention was paid to minor neurological signs in gross and fine motor functioning (*e.g.* walking on tiptoe, walking on heels, sequential finger-thumb opposition, imitation of gestures with arms, hands and fingers), balance (*e.g.* standing on one leg, walking a straight line) and co-ordination (*e.g.* finger-nose-test, diadochokinesia of arms and hands). Presence of at least two minor neurological signs was taken to indicate minor neurological impairment.

This study was approved by the Institutional Review Board (IRE) of the University Medical Centre Utrecht. Written informed consent was obtained from the parents or caretakers of all children and from the children themselves if they were 12 years or older.

Data analysis

General characteristics are summarized as mean (standard deviation) or median (minimum, maximum) for continuous data and as frequencies with percentages for categorical variables. Missing values were imputed using multiple imputation methods.

To reproduce the estimated prevalence of left-handedness in the whole population from the selected group, we used a weighting factor derived from the sampling fraction (weight: 5.4 (540/101) for those whose parents had no complaints about academic achievement and/or behaviour and 1.3 (134/100) for those whose parents had complaints).

Logistic regression analysis was used to study the relation between handedness (left or non-left) as dependent variable and variables indicating severity of meningitis as predictors (univariable, multivariable and as a severity score). First, we performed univariate analyses with all available variables to find out which were predictors of more severe meningitis (we took the variables with OR > 1). Subsequently, multivariate analysis was performed to assess whether these variables were mutually independently related to hand preference. Then, to derive the severity score, we took a summary of all positive values (presence) of variables that univariately predicted left-handedness, without taking into account the weight of the beta coefficient of the variables. Then, the total severity score was dichotomized with the median value as the cut off.

To analyze the relation between handedness and post-meningitis neurodevelopmental outcome at school age, we performed logistic regression (minor neurological impairment, hearing loss, academic and/or behavioural limitation) and linear regression analysis (IQ, vocabulary score of WISC-r, Beery score, memory, learning, attention, response speed, manual speed, and manual steadiness).

The results are expressed as odds ratios or linear regression coefficients with corresponding 95% confidence intervals and intervals not including 1 for odds ratios or 0 for linear regression coefficients ($p<0.05$) were considered statistically significant. Data were analyzed using the SPSS for Windows statistical package (version 12.0.2, SPSS Inc., Chicago, IL, U.S.A.).

Results

Of 182 childhood survivors of bacterial meningitis with and without parental report of behavioural problems, 15.9 % were left-handed. Taken into account the weighting factor to extrapolate this to the original post-meningitic population, 15% were left-handed (95% CI 10% to 21%).

The median age of presentation at the hospital with the signs and symptoms of meningitis was 1.8 years (minimum 0 and maximum 7 years). The most frequent pathogen found in the CSF culture was *N. meningitidis* (n = 146, 80%), followed by *S. pneumoniae* (n = 26, 14%), *S. agalactiae* (n = 5, 3%), *E. coli* (n = 4, 2%), and *L. monocytogenes* (n = 1, 1%). There were more boys (64.7%) than girls (35.7%). Forty eight percent of the children had at least one parent with low education and 90.7% were of Dutch ethnicity. The mean birth weight was 3443 (SD 517) grams and mean gestational age was 40 (SD 1.6) weeks.

As shown in table 1, from univariate analysis, presence of focal neurological signs, presence of few petecchiae, signs of impaired peripheral circulation, macroscopic haemorrhagic CSF, *E.coli* positive CSF culture and high CSF leucocytes counts seemed to indicate a higher risk to become left-handed (for the definitions we refer to the legend of table 1). Putting all the variables into one model did not change most of the relations substantially, except for age.

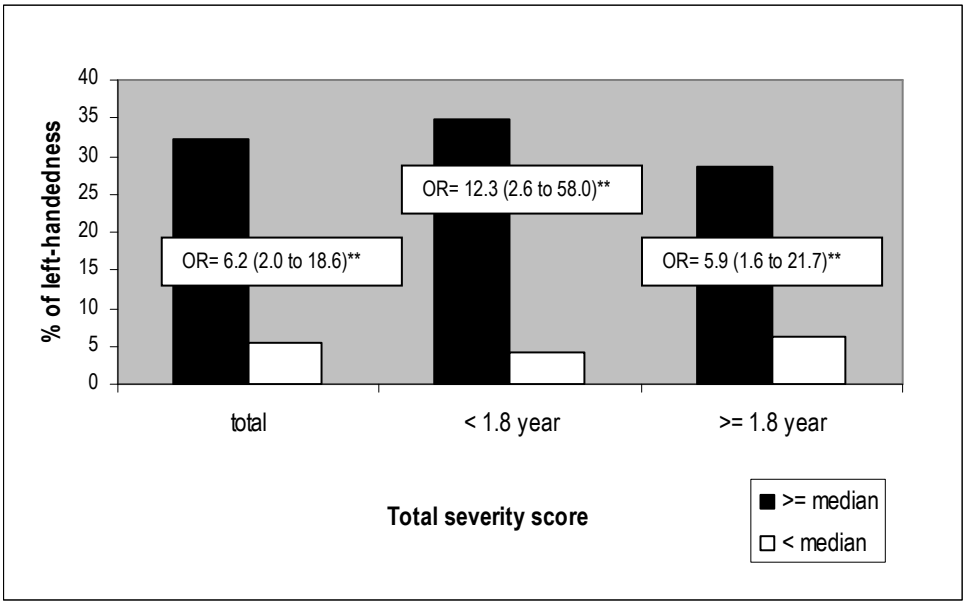
Table 1. Predictors of left-handedness among survivors of childhood bacterial meningitis without severe sequelae

Predictors	Left-handed (n = 29)	Non Left-handed (n = 153)	Crude OR (95% CI)	Adjusted OR (95% CI)
Demography				
Age at meningitis diagnosis < median (1.8 year) (%)	17 (58.6)	74 (48.4)	1.5 (0.7 to 3.4)	0.9 (0.3 to 2.6)
Low parental education (%)	16 (55.2)	72 (47.1)	1.4 (0.6 to 3.1)	1.3 (0.5 to 3.5)
Male gender (%)	22 (75.9)	95 (62.1)	1.9 (0.8 to 4.6)	1.7 (0.6 to 4.8)
Neurological related variables				
Presence of focal neurological signs (%)	7 (24.1)	9 (5.9)	5.1 (1.7 to 15.1)	5.8 (1.5 to 22.8)
Seizures before presentation (%)	4 (13.8)	14 (9.2)	1.6 (0.5 to 5.2)	1.4 (0.3 to 5.8)
Circulation related variables				
Presence of few skin petecchiae (%)	6 (20.7)	10 (6.5)	3.7 (1.2 to 11.2)	3.3 (0.9 to 11.5)
Impaired peripheral circulation (%)	19 (65.5)	73 (47.7)	2.1 (0.9 to 4.8)	2.4 (0.9 to 6.3)
Laboratory variables				
Macroscopic haemorrhage apearance in CSF (%)	3 (10.3)	3 (2.0)	5.8 (1.1 to 30.1)	4.7 (0.6 to 34.7)
Positive E. coli CSF culture (%)	3 (10.3)	1 (0.7)	17.5 (1.8 to 175.1)	16.1 (1.1 to 244.9)
High leucocyte count in CSF (3rd tertile) (%)	17 (58.6)	42 (27.5)	3.7 (1.3 to 10.2)	5.8 (1.7 to 20.0)

All at admission, except for seizures; missing data were imputed using multiple imputations. Included in focal neurological signs were ataxia/dysartria, increased or decreased strength of arms/legs, increased or decreased tonus of arms/legs, increased or decreased reflexes of arms/legs, and deficits of coordination. Many petecchiae was not related to left-handedness. Impaired peripheral circulation measured by clinical examination (cold acra, delayed capillary refill, cyanosis, blue nails). OR of becoming left-handed, presence of sign compared to absence or the highest tertile compared to the lowest tertile. Adjusted OR: from multivariable logistic regression with all variables listed in the table simultaneously included.

Figure 1 shows that children with a meningitis severity score above the median had a 6.2 times higher risk of becoming left-handed at school age compared to those below the median (95% CI 2.0 to 18.6). Furthermore, those who contracted meningitis below the median age of 1.8 years had a 12.3 times higher risk (95% CI 2.6 to 58.0) compared to a 5.9 times higher risk (95% CI 1.6 to 21.7) among children who contracted meningitis at older age.

Figure 1. The frequency of left-handed children by group of total severity score (n=182)



Legend to figure 1. OR: Odds ratio (95% confidence interval), independent variable = total severity score (below or above median); dependent variable = handedness at school age (left or non left-handedness). Total severity score was calculated by adding all the positive items in table 1 and then dichotomized with the median value as the cut off (for age specific analysis, age was not included in the total score calculation). **P < 0.01.

Table 2 shows that left-handed children had an almost 3 times higher chance of reported combined academic and behavioural limitations than non left-handed children, although this was only borderline statistically significant (p = 0.09).

Table 2. The relation between handedness and post-meningitic neurodevelopmental outcome at school age

Neurodevelopmental outcome	Left-handed (n = 29)	Non Left-handed (n = 153)	Crude OR (95% CI)	Adjusted OR (95% CI)*	P-value*
Neurological outcome					
Minor neurological impairment (%)	6 (20.7)	29 (19.0)	1.0 (0.4 to 2.8)	0.9 (0.3 to 2.6)	0.90
Hearing loss (%)	2 (6.9)	13 (8.5)	0.8 (0.2 to 3.7)	0.8 (0.2 to 3.7)	0.77
School performance					
Children with an academic and/or behavioural limitation (%)	17 (58.6)	67 (43.8)	1.8 (0.8 to 4.1)	1.7 (0.7 to 3.8)	0.21
with academic limitation (%)	8 (27.6)	36 (23.5)	1.6 (0.6 to 4.2)	1.5 (0.6 to 4.1)	0.43
with behavioural limitation (%)	3 (10.3)	17 (11.1)	1.3 (0.3 to 5.0)	1.2 (0.3 to 4.9)	0.77
with combined limitation (%)	6 (20.7)	14 (9.2)	3.1 (1.0 to 9.5)	2.7 (0.9 to 8.6)	0.09

Presence of at least two minor neurological signs (gross and fine motor functioning, balance, and coordination) was taken to indicate minor neurological impairment.
* Adjusted for gender and age at examination.

More specific analyses on cognitive function are shown in table 3, with left-handed children generally performing worse on the cognitive tests. Left-handed children had an almost 7 points lower IQ (p = 0.018), a 1 point lower vocabulary score of WISC-r (p = 0.061), and an almost 5 points lower Beery score (p = 0.069) than their non left-handed counterparts. The left-handed children also had a worse performance in memory tests, especially for total immediate recall and recognition. They performed worse in the trails test (longer time difference between trail A and B), but better in sustained attention test (balloon piercing test: less numbers of touched non-target balloons), than the non left-handed group.

Table 3. Performances in cognitive domains: comparison between the left-handed and non left-handed survivors of bacterial meningitis

Cognitive domains	Left-handed	Non Left-handed (reference)	Crude mean difference (95% CI)	Adjusted mean difference (95% CI)*	P-value*
Intelligence (n = 182)					
Mean CPM/SPM IQ (SD)	96.0 (13.3)	101.8 (14.5)	-5.8 (-11.5 to -0.1)	-6.6 (-12.0 to -1.2)	0.02
Mean vocabulary standard score of WISC-r (SD)	8.1 (3.2)	9.1 (2.6)	-1.0 (-2.1 to 0.1)	-1.0 (-2.1 to 0.0)	0.06
Visual motor integration (n = 182)					
Mean Beery score	95.2 (14.9)	100.4 (12.6)	-5.2 (-10.4 to 0.0)	-4.9 (-10.1 to 0.4)	0.07
Memory and Learning (n = 182)					
Mean word span (SD):					
Forward	4.0 (1.1)	3.9 (1.0)	0.1 (-0.3 to 0.5)	0.2 (-0.2, 0.5)	0.35
Backward	2.8 (0.7)	3.2 (0.9)	-0.3 (-0.7 to 0.0)	-0.3 (-0.6 to 0.1)	0.10
Mean score learning locations (SD):					
total immediate recall	51.7 (11.9)	56.6 (10.4)	-4.9 (-9.2 to -0.6)	-3.8 (-7.8 to 0.1)	0.06
proactive interference	5.2 (2.2)	5.9 (2.2)	-0.7 (-1.5 to 0.2)	-0.5 (-1.4 to 0.3)	0.23
retroactive interference	11.0 (3.0)	12.2 (3.0)	-1.2 (-2.4 to 0.0)	-0.9 (-2.0 to 0.2)	0.13
delayed recall	12.0 (3.0)	13.1 (2.6)	-1.1 (-2.1 to -0.0)	-0.9 (-1.9 to 0.1)	0.09
Recognition	13.8 (1.9)	14.7 (1.4)	-0.9 (-1.5 to -0.3)	-0.8 (-1.3 to -0.2)	0.01
Attention (n = 149)					
Color trails:					
Median time difference trails B-A in sec. (minimum, maximum)	78 (9, 233)	60 (-74, 230)	-	-	0.05†
Median corrected + uncorrected errors (minimum, maximum)	0 (0, 6)	1 (0, 8)	-	-	0.67†
Balloon Piercing:					
Mean wandering time targets in ms (SD)	3321 (482)	3362 (585)	-41 (-297 to 215)	-96 (-342, 150)	0.44
Errors of omissions (yes)	5 (22%)	49 (39%)	0.4 (0.2 to 1.3)‡	0.4 (0.1 to 1.2)‡	0.09
Errors of commissions (yes)	11 (48%)	89 (71%)	0.4 (0.2 to 0.9)‡	0.3 (0.1 to 0.9)‡	0.02

* adjusted for gender and age at examination, † Mann Whitney U-test, ‡ Odds ratio; Abbreviations: CPM = Colored Progresive Matrices; SPM = Standard Progressive Matrices; IQ = Intelligence Quotient; WISC-r = Wechsler Intelligence Scale for Children-revised; ms = milliseconds; sec = seconds. Ommisions = number of missed target balloons (0 and $\geq$ 1); Commissions = number of touched non target balloons (0 and $\geq$ 1).

Table 4 shows that left-handed and non left-handed children differed in their motor speed and steadiness. Compared to the non left-handed group, left-handed children performed worse on the manual speed test (the number of taps) with their dominant hand (p = 0.05), but seemed to perform better with their non-dominant hand. In the test of manual steadiness (time of contacts), left-handed children performed better with their non-dominant hand than the non left-handed children (p = 0.01).

Table 4. Performances on measures of speed and steadiness: comparison between left-handed and non left-handed survivors of bacterial meningitis

Measures	Left-handed (n=23)		Non left-handed (reference) (n=126)		Adjusted mean difference*	P value*
	Mean	SEM	Mean	SEM		
Response speed						
Total reaction time (ms)						
Simple light	520	17	518	7	-13 (-45 to 18)	0.40
Simple sound	467	19	457	8	-6 (-40 to 28)	0.73
Disjunctive two lights	658	20	629	7	12 (-26 to 50)	0.53
Disjunctive light and sound	671	23	632	10	21 (-20 to 63)	0.31
Manual speed						
Number of taps						
Dominant hand	133	5	146	2	-9 (-17 to -0.1)	0.05
Non-dominant hand	122	4	118	2	7 (-1 to 15)	0.09
Manual steadiness						
Number of contacts						
Dominant hand	99	17	97	7	-7 (-42 to 28)	0.69
Non-dominant hand	144	21	164	9	-33 (-74 to 8)	0.11
Time of contact (s)						
Dominant hand	4.6	0.8	3.5	0.3	0.7 (-1 to 2)	0.40
Non-dominant hand	4.9	1.1	7.0	0.5	-2.7 (-5 to -1)	0.01

* adjusted for gender and age at examination; SEM = Standard Error of the Mean

Discussion

Our findings in a cohort of bacterial meningitis survivors followed up for 7 years support the hypothesis that early life brain damage may induce left-handedness.

To appreciate these findings, there are several issues to be addressed. This cohort was selected on the basis of whether or not the parents had reported academic and/or behavioural problems. This limits the ability to calculate absolute risks, but not relative risks. Groups were selected without prior knowledge of handedness, thus excluding the possibility of selection bias. Handedness was assessed while performing motor speed and steadiness tests and information bias is therefore unlikely. At the conduct of the original study there was no research question involving handedness. This adds to the validity of our findings because the relevant measurements for the present study cannot have been biased by prior knowledge of such research questions. Our findings are probably an underestimation because the vast majority of left-handedness in our cohort is likely to be non-pathological, which has most likely diluted the observed effects.

Interactions between genes, early life environment (pre-, peri- and post-natal) and learning processes play a role in determining handedness.[6] Any insult occurring during a period of rapid brain growth and development may induce pathological cerebral lateralisation including handedness.[9] To our knowledge, only one previous study tried to link bacterial meningitis and left-handedness.[11] Despite the small number of participants (n=28), that study reported a higher rate of left-handedness in children surviving bacterial meningitis compared to their healthy siblings. All of the left-handed children had had meningitis during infancy, in line with our results suggesting that the severity indicators are more strongly associated with handedness in younger children than older children. In another recent study it was argued that bacterial meningitis may have a favourable effect towards the dominance of right-handedness, because severe meningitis predominantly affects the right hemisphere. The authors did not analyse handedness directly, but used the direction of forced deviation to predict localization of brain damage.[12]

In agreement with one earlier study,[11] the 15% prevalence of left-handedness in our cohort is somewhat higher than the 10% reported for the general population.[6] Higher rates of left-handedness are also found in association with other early life problems involving the developing brain, such as premature birth.[25] In line with our prior hypothesis, this may indicate that indeed some of the excess left-handedness may have to do with aspects of certain diseases. As indicated previously, severe (lateralized) brain damage will affect lateralization of cerebral functions, which may

include handedness.[26] We assumed that in the developing brain particularly also more subtle damaging effects may play a role in the occurrence of (excess) left-handedness. A further premise was that the effect of meningitis on outcome, including left-handedness, would depend on the age at diagnosis as an, albeit rough, indicator of brain development.[11;27] Outcomes of damage in such early stages of brain development depend on interaction between brain plasticity and vulnerability, as functions of lesion severity and nature, age at onset, gender, and psychosocial context.[27] Indeed, specific signs and symptoms at the time of the meningitis were related to school-age left-handedness. These were general clinical signs and symptoms of bacterial meningitis of which most (younger age, low parental education, male gender, focal neurological signs, convulsion, petecchiae, impaired circulation, *E. coli* CSF culture) were previously shown to reflect severity.[15] The fact that having few petecchiae (and not many) was related to left-handedness indicates that it is the meningitis rather than sepsis that is related to left-handedness. Possibly, clinical conditions which predispose to a more localized instead of generalized brain lesion are better predictors of left-handedness. Macroscopic haemorrhagic appearance of the CSF was associated to left-handedness. Even though some of it may be due to 'contamination' with blood as a result from the puncture procedure, there is no reasonable explanation as to why this would preferably happen in left-handed children. The fact that *E. coli* stood out as associated with left-handedness stresses the importance of the timing of damage (infancy), since in the CSF this organism is almost exclusively found in infants with meningitis.[28]

Our study further shows that the reasons for excess left-handedness may also relate to a less favourable developmental outcome at school age. Obviously, the lower levels of various IQ scores in left-handed children are clinically subtle. However, left-handed children had a three times higher risk of combined academic and behavioural limitations at school age, further explained by a generally lower performance in various tests measuring memory, learning, and attention. The neurodevelopmental gap between post-meningitic survivors and their peers increase with age,[27] therefore subtle deterioration at school age should not be ignored.

Differences in motoric abilities between left and non left-handed children may be of interest. In our study, left-handed children showed a worse performance with the dominant hand (left) but better performance with the non-dominant hand (right), than the non left-handed group. This contradicts with findings of Bishop et al[29] who reported that left-handed children were clumsier with their non-dominant hand. We speculate that where left-handedness is a pathological shift in children who were innately right-handed, the non-dominant right-hand performance may be better than the

left-hand performance of naturally manifest right-handers. This is supported by previous studies showing that the self-reported right-handed group in the population consists of mostly strongly right-handed individuals (performing very well with their right hand but poorly with the left hand in various tasks) while the left-handed group comprises mostly more ambidextrous individuals.[5]

In summary, our findings show that clinical signs and symptoms indicating severity of disease in children suffering from bacterial meningitis in early life predict left-handedness at school age. These results are compatible with the view that early life brain damage is responsible for an added proportion of left-handedness with a pathological origin. Left-handed post-meningitic children generally have worse neurodevelopmental outcome than non left-handed survivors.

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Chapter 2.3.

Association between a polymorphism in the promoter region of the Insulin-like Growth Factor I (IGF-I) gene and hand preference in women.

Manuscript based on this chapter:

Ramadhani MK, Grobbee DE, Rietveld I, Bots ML, van Duijn CM, Uiterwaal CSPM. Association between a polymorphism in the promoter region of the Insulin-like Growth Factor I (IGF-1) gene and hand preference in women. Submitted.

Summary

Background:
Left-handedness has been related to breast cancer and shared determinants rather than a direct link have been suggested to underlie this association. In particular, intra-uterine exposure to high levels of testosterone or other sex hormones have been suggested to collectively promote left-handedness and influence breast cancer risk. In women, IGF-1 levels are strongly associated with androgen levels, and IGF-1 has been related to breast cancer. Consequently, IGF-1 might be associated with left-handedness and similarly partly explain its association with breast cancer. We explored whether left-handedness of young adult women is associated with a functional polymorphism in the promoter region of IGF-1.

Subjects and Methods:
299 young adult women aged 26-31 years who participated in the Atherosclerosis in Young Adults (ARYA) birth cohort were included. Detailed handedness data were obtained using the validated Edinburgh questionnaire. Medical history and lifestyle information were assessed by questionnaires. Subjects were classified based on the carriership of CA repeats. Data were analysed by using t-test, chi square test and logistic regression analysis.

Results:
Using the most common 192-bp and 194-bp alleles with the highest associated IGF-1 levels as the reference, having long alleles (>194-bp) was inversely related to left-handedness (OR 0.3, 95% CI 0.1 to 0.7). Compared to right-handed women, left-handed women were more likely to be homozygous for 192-bp, less likely to be heterozygous 192-bp, and more likely to be non carrier of 192-bp ($p = 0.035$).

Conclusion:
In conclusion, left-handed women appear to have a shifted allele distribution in the promoter region of the IGF-1 gene compared to right-handed women. Subgroups of left-handed women may therefore have IGF-1 genes that are compatible with higher circulating IGF-1 levels.

Introduction

Left-handed women are at a higher risk of breast cancer than their non left-handed counterparts.[1;2] There are two main theories that have driven studies of an association between handedness and breast cancer.[2] First, there is a hypothesis stating that exposure to high levels of intra-uterine sex hormone levels such as testosterone may promote left-handedness.[3] Second, there is a theory that an intra-uterine environment characterized by exposure to high levels of sex hormones increases the risk for breast cancer in later life.[4]

It was shown recently that circulating sex hormones in women, particularly androgens, are closely positively related to Insuline-like Growth Factor -1 (IGF-1) levels in women.[5] On a different note, there is evidence that prenatal treatment with testosterone induces intra-uterine growth retardation and postnatal catch-up growth particularly in female sheep, a process that might be mediated by IGF availability.[6] In a longitudinal study of rhesus monkeys, IGF-1 was shown to play a major role in infant growth.[7] Moreover, children with a history of intra-uterine growth retardation and subsequently impaired to show catch-up growth had lower IGF-1 levels than their counterparts that did show catch-up growth.[8] Thus, if intra-uterine exposure to high levels of testosterone plays a role in the association between left-handedness and breast cancer, factors that determine the availability of IGF-1 levels may be directly involved. Variation in the gene encoding for IGF-1 may be one such factor.

There is an established relation between serum levels of IGF-1 and breast cancer, with higher circulating levels of IGF-1 conferring a higher risk of premenopausal breast cancer.[9] IGF-1 is a major mediator between growth hormone and growth throughout fetal and childhood development,[10] and may act as the missing link between intrauterine exposures of steroid hormones and breast cancer.[11] Recent studies show the importance of growth and body size in the pathogenesis of breast cancer.[12]

There have been few studies on gene polymorphisms of IGF-1 in relation to breast cancer of which just one small study showed an association.[9] However, these studies largely included post-menopausal women whereas IGF-1 levels have been particularly linked to pre-menopausal breast cancer.[9]

We previously reported the presence of an association between left-handedness and premenopausal breast cancer which may reflect a common origin in early life exposure to high sex hormone levels. To our knowledge there is no published evidence on a relation between handedness

and IGF-1 levels or IGF-1 gene polymorphisms. We examined whether a polymorphism in the promoter region of IGF-1 is associated with female handedness.

Subjects and Methods

Study design and population

The Atherosclerosis Risk in Young Adults (ARYA)-study comprises two birth cohorts of young adults, who were born in or around the two Dutch cities Utrecht and The Hague. The original aim of the cohort was to study the early determinants of cardiovascular diseases. The present analysis is restricted to the Utrecht cohort since handedness was only measured in this cohort. The Utrecht cohort includes 749 young adults born between 1970-1973, who attended secondary school in the city of Utrecht in the Netherlands and of whom the original medical records from the Municipal Health Service were available. Details on the rationale and design of the ARYA-study have been described elsewhere.[13]

Briefly, 4207 subjects had complete charts with birth weight and adolescent blood pressure. These were invited in writing but 2191 did not respond at all, 726 letters were undeliverable, 416 refused, 36 did not participate for logistic reasons, 18 had other reasons. 820 out of 4207 (19.5%) were willing to participate initially, but of these 14 were excluded because of pregnancy, and 57 withdrew secondarily. This left 749 out of 4207 (17.8%) who actually participated and whom we sent the Edinburgh handedness questionnaires,[14] 4 years after the initial inclusions. Of 749, the response rate was 76% (567/749), with the remaining 24% failing to respond or was untraceable after 2 attempts of mailings or callings. Of 567 participants who responded to the handedness questionnaires, there were 299 women, of whom 296 had complete IGF-1 genotype data.

Handedness measurement

Handedness was measured using the Oldfield (Edinburgh) handedness questionnaire.[14] There were 10 items of questions about hand preference while performing certain tasks and 2 about foot and eye preference, including: writing, drawing/painting, throwing, using scissors, using a tooth brush, using a knife without fork, using a spoon, using a broom, striking a match, opening a box, kicking, and looking with one eye.

As reported associations between handedness and breast cancer pertained to manual performance only,[1;2] we restricted our analysis to the 10 items asking for manual preference. Thus,

the laterality quotient (LQ) for hand preference was calculated from the first 10 items, using formula LQ = (cumulative right - cumulative left)/ (cumulative right + cumulative left) * 100. The LQ score ranges from -100 to 100. To obtain a dichotomous handedness classification (left or right) with sufficient contrast, we used the first tertile (left-handed) and the third tertile (right-handed). Due to the expectedly negative skewedness of the LQ data (see figure 1), with the majority of the subjects having the value of 100, the numbers of subjects across the tertiles were not equal. The LQ cut off value of the 33.3% left-lateralised percentile was 80 and the 66.7% right-lateralised percentile was 100. There were 95 subjects with LQ < 80 and 144 subjects with LQ = 100.

IGF-I genotype

Participants' genotypes were determined by polymerase chain reaction using oligonucleotide primers designed to amplify the polymorphic cytosine-adenine (CA) repeat 1 kb upstream of the human IGF-I gene.[15] The reaction was carried out in a final volume of 10 μl containing 50 ng of genomic DNA obtained from peripheral blood cells, 0.5 nmol/l forward primer (5'-ACCACTCTGGGAGAAGGGTA-3'), 0.5 nmol/l reverse primer (5'-GCTAGCCAGCTGGTGTTATT-3'), 0.25 nmol/l 2'-dNTP, 2.2 mmol/l Mg/Cl_2, 0.01% W1 (Gibco BRL), and 0.4 Taq DNA polymerase (Gibco BRL). Polymerase chain reaction was performed in 384 well plates (94°C 10 min; 35 polymerase chain reaction cycles 30 s at 94°C, 30 s on 55°C, and 30 s on 72°C; 72°C 10 min; 4°C hold). Forward primers were labeled with FAM, HEX, or NED to determine the size of polymerase chain reaction products by autosequencer (ABI 3100, POP4, filter set D, collecting time array 36 cm 7 s, peak-height between 100 and 2000, each lane containing three samples). The size of the polymerase chain reaction products was determined in comparison with internal ROX 500-size standard (Perkin Elmer).

Based on genotype, we classified the participants in two ways. In the earlier work by Vaessen, et al,[16] it was shown that the highest circulating level of IGF-1 may be found in homozygous 192-bp (wild type allele). The heterozygous for 192-bp have lower plasma IGF-1 levels, followed by the non carriers. More recently, Rietveld, et al[17] suggested that the second most frequent allele (194-bp) is also related to the highest production of IGF-1. The first classification consisted of three groups, according to the carrier status of the 192-bp polymorphism: 1) homozygous carriers; 2) heterozygous carriers; and 3) non-carriers. The second classification was based on the carriership of long alleles (>194-bp).

Data analysis

Characteristics of the ARYA-study participants are summarized as means with standard deviations for continuous data and as percentages for dichotomous or categorical variables. To test for group differences, we used the Chi square test or the t-test when appropriate.

The Chi square test and logistic regression were used to study the relation between handedness as dichotomous variable based on LQ and IGF-1 allele combinations in women. Logistic regression models were also used to adjust associations between allele combinations and handedness for baseline differences between left and right-handed women that might be possible confounding factors. To study the relationship between handedness and allele distributions, we used logistic regression analysis.

The results are expressed as odds ratios (OR) with corresponding 95% confidence intervals and intervals not including 1 ($p<0.05$) were considered statistically significant. Data were analysed using the SPSS for Windows statistical package (version 12, SPSS Inc., Chicago, IL, U.S.A.).

Results

Of 296 women who were included in the analysis, the left-handed women were somewhat younger (0.2 years, $p = 0.09$), bigger (1.3 kg/m^2 higher BMI, $p = 0.07$) and they smoked somewhat less ($p = 0.03$) than the right-handed women (table 1). Table 2 shows the allele distribution of the IGF-1 promoter polymorphism by handedness.

There was a statistically significant shift of distribution of homozygosity, heterozygosity and non-carriership for the 192-bp allele among the left-handed as compared to the right-handed (table 3). Among left-handed women, there were 4.6% more 192-bp homozygous, 10% more 192-bp non-carriers and 14.6% less 192-bp heterozygous individuals than among the right-handed women, respectively. Combining 192-bp and 194-bp alleles for homozygosity, heterozygosity and non-carriership showed exactly the same shift (of pattern) in distribution among left-handed but not statistically significant (data not shown). Table 3 also shows that much fewer left-handed women were carriers of long alleles (> 194 bp): odds ratio = 0.26, 95% CI 0.11 to 0.61, $p = 0.002$. Logistic regression showed that the unadjusted association did not change after adjustment for age, smoking and BMI that were each different between left-handed and right-handed women: adjusted odds ratio

= 0.27, 95% CI 0.11 to 0.64, p=0.003. The findings were substantiated with an additional analysis of allele frequency as the unit of observation.

Table 1. General characteristics of the women in the ARYA study (n = 296).

Variables	1st tertile LQ / Left-handed (n = 95)	3rd tertile LQ / Right-handed (n = 144)	P-value
Age (years)	28.3 (0.9)	28.5 (0.9)	0.09
BMI (kg/m^2)	25.8 (5.9)	24.5 (4.7)	0.07
Waist Hip Ratio	0.8 (0.1)	0.8 (0.1)	0.95
Height (cm)	170 (6)	171 (6)	0.09
Weight (kg)	74.3 (17.8)	71.6 (14.6)	0.16
Education (%)			0.48
Low	9.7	8.4	
Middle	53.1	61.1	
High	37.2	30.5	
Smoking status (%)			0.03
Never	58.9	42.8	
Past	15.8	16.6	
Current	25.3	40.7	
Alcohol intake (%)			0.96
Never	29.9	31.6	
Mild (1-8 days/month)	54.2	53.7	
Moderate (9-20 days/month)	13.9	11.6	
Heavy (>21 days/month)	2.1	2.1	
Birth Weight (gram)	3338 (618)	3343 (567)	0.95
Gestational age (weeks)	39.9 (1.3)	39.8 (1.5)	0.59

Values are mean (standard deviation) unless otherwise indicated. LQ = laterality quotient (measured from 10 items of Edinburgh questionnaire).

Using logistic regression with 192 bp and 194 bp alleles as the reference category, short alleles (< 192 bp) were associated with a statistically non-significant 40% increased chance for left-handedness (OR = 1.4 (95% CI 0.8, 2,7; p=0.27) while long alleles (> 194 bp) were associated with a statistically significant 70% lower chance for left-handedness (OR 0.3 (95% CI 0.1, 0.7; p=0.003).

Table 2. The allele distribution of the IGF-I promoter polymorphism by handedness based on LQ

Length PCR products		Total (n = 478)	1st tertile LQ / Left-handed (n = 190)	3rd tertile LQ / Right-handed (n = 288)
198-bp	Long alleles	11 (2.3)	2 (1.1)	9 (3.1)
196-bp		31 (6.5)	5 (2.6)	26 (9.0)
194-bp	Common alleles	97 (20.3)	47 (24.7)	50 (17.4)
192-bp (z)		297 (62.1)	115 (60.5)	182 (63.2)
190-bp	Short alleles	32 (6.7)	17 (8.9)	15 (5.2)
188-bp		8 (1.7)	3 (1.6)	5(1.7)
186-bp	Other rare alleles	2 (0.4)	1 (0.5)	1 (0.3)
176-bp				

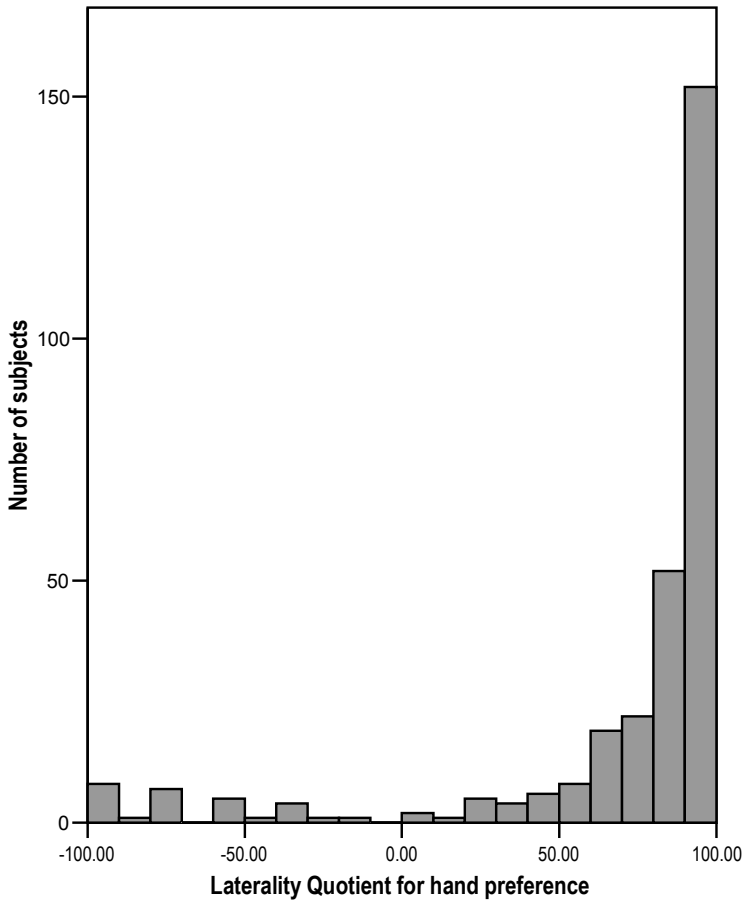
Values are n (%). z = wild type allele.
LQ = laterality quotient (measured from 10 items of Edinburgh questionnaire).

Table 3. Handedness of women by categorization of IGF-I promoter polymorphism

Categorization	Left-handed (%)	Right-handed (%)	p-value (X^2)
Category 1			
Homozygous of 192-bp	42.1	37.5	0.035
Heterozygous of 192-bp	36.8	51.4	
Non carrier of 192-bp	21.1	11.1	
Category 2			
Carrier of long alleles (>194-bp)	7.4	23.6	0.001
Non carrier of long alleles (>194-bp)	92.6	76.4	

Handedness measured from LQ = laterality quotient (left-handed: 1st tertile of LQ; right-handed: 3rd tertile of LQ)

Figure 1. Distribution of the Laterality Quotient for hand preference



Discussion

Our results show a distribution shift of a polymorphism in the promoter region of the IGF-1 gene in left-handed as compared to right-handed women. Subgroups of left-handed women have IGF-1 genotypes compatible with higher circulating IGF-1 levels.

To appreciate these findings, some methodological issues need to be addressed. The study was originally designed for other research questions notably on early determinants of CVD. Therefore, it is unlikely that the reasons of unresponsiveness of women who did not answer our handedness questionnaires were related to both handedness and IGF-1 promoter polymorphism.

Handedness was measured using the Oldfield handedness questionnaire,[14] which has been used extensively in previous studies on hand preference and diseases. Classification of handedness was done by using the extreme tertiles of the continuous variable (Laterality Quotient) derived from this questionnaire, in order to minimize the misclassification of participants with mixed handedness and thus enlarge the contrast between the left and right-handed group. Consequently however, the prevalence of what we designate left-handedness in our study is not solely based on manual preference and is therefore higher than one would expect for a dichotomous classification in presence or absence of manual left-handedness. The Oldfield handedness questionnaire ensured that handedness was measured from various tasks, which expectedly is more accurate than simply asking one's hand preference.

The classifications of the polymorphism were based on our own prior findings about its functionality. A recent review of studies linking this polymorphism to levels of IGF-1 in serum showed inconsistent results.[9] In some reports homozygosity of the wild type allele (192-bp or 19 repeat CA) was linked to the highest production of IGF1 in men and women, but other studies showed an inverse relation or no relation at all. Among the more recent and largest of these studies by our own group it was found that the 192-bp and 194-bp repeats in the IGF-1 promoter gene are related to the highest production of IGF-1, while the long alleles (> 194-bp) are related to the lowest production.[17]

We have found more homozygous 192-bp and non-carrying 192-bp left-handed and fewer heterozygous 192-bp left-handed than right handed women. Moreover, fewer left-handed women had long alleles than right-handed women. These associations did not seem to be explained by possible confounders in our study. In view of our earlier findings on functionality[17] this could mean that some left-handed women have a genetic background associated with higher IGF-1 production than right-handed women. At the same time, some (other) left-handed women would have a genetic background associated with lower IGF-1 production than right-handed women. The motive to explore the relation between the IGF-1 genes and handedness was because it could offer an explanation for the observed increased risk of breast cancer in left-handed women. If our finding that subgroups of left-handed women have a propensity for more common (in the general population) alleles and less long alleles is confirmed in other studies, this functional genetic background may help explain why some left-handed women should be at a higher risk for premenopausal breast cancer.[1] High IGF-1 levels have been related to premenopausal breast cancer,[9] but not to postmenopausal breast cancer.[18]

As far as we know this is a first report on a possible association between this IGF-1 polymorphism and female handedness. The mechanisms behind this association are unknown. Possibly, a genetic blueprint associated with higher IGF-1 levels may induce left-handedness for instance through a relative over-growth of the right hemisphere compared to the left hemisphere during periods of early fetal brain development. Indeed, genes and growth factors have been shown associated with somatic asymmetry through affecting certain aspects of brain development, such as with fibroblast growth factor.[19] Thus, exposure to high levels of steroids, say testosterone, in gestation would induce left-handedness preferentially in subgroups with high IGF-1 producing alleles.

Despite the fact that such mechanisms are speculative, we do feel that our findings are sufficiently suggestive to warrant further confirmation. Our study was relatively small for robust estimation of associations between polymorphisms and handedness and larger scale studies should allow for the collection of more detailed knowledge of this association. Moreover, a challenge is to find whether our association, IGF-1 polymorphism and handedness, might be underlying relations between handedness and later life chronic disease. Consequently, our findings on this IGF-1 gene polymorphism as a possible explanation for an association between handedness phenotype and chronic disease, such as breast cancer, should be put to the test in future studies.

In conclusion, left-handed women may have a shifted allele distribution in the promoter region of the IGF-1 gene as compared to right-handed women. This shift may indicate that subgroups of left-handed women have IGF-1 genotypes that are compatible with higher circulating IGF-1 levels.

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Chapter 3.

Left-handedness and later life health outcomes

Chapter 3.1.

Innate left-handedness and breast cancer risk.

Manuscript based on this chapter:

Ramadhani MK, Elias SG, van Noord PAH, Grobbee DE, Peeters PHM, Uiterwaal CSPM. Innate left-handedness and risk of breast cancer: case-cohort study. *BMJ* 2005;331(7521);882-3.

Summary

Background:
Left-handedness is a possible marker of intrauterine exposure to steroid hormones and is suggested to be related to the risk of breast cancer. Evidence for such an association is currently scarce.

Subjects and Methods:
We studied the association between innate handedness and breast cancer risk in a cohort of 12,178 middle-aged Dutch women participating in a breast cancer-screening project. During 16 years of follow-up, 426 new breast cancer cases were identified. The association between innate handedness and breast cancer risk was analyzed by a case-cohort approach, in which a random sample of 1,500 women was used to represent person-years lived in the entire cohort.

Results:
Of the random sample, 11.5% reported to be left-handed in early childhood. The risk for breast cancer was 39% higher in the left-handed group (hazard ratio (HR) 1.39; 95% confidence interval (CI) 1.09 to 1.81). The risk was 2.41 when the cancer was premenopausal, but there was no excess risk for postmenopausal cancers. We found an excess risk in left-handed women with a BMI $\leq$ 25 kg/m2 (HR 1.62; 95% CI 1.17 to 2.24 – *P* for interaction between handedness and BMI: 0.07), as well as in women who gave birth to at least one child (HR 1.58; 95% CI 1.19 to 2.11 – *P* for interaction between handedness and parity: 0.02), but not in those whose BMI was >25 kg/m^2 and nulliparous. Adjustment for potential confounders did not change these results. Handedness seemed not to be associated with the laterality of breast cancer.

Conclusion: These results support the hypothesis that left-handedness is related to increased breast cancer risk.

Introduction

Breast cancer remains a major health threat to women worldwide. Over the last decades, incidence rates of breast cancer have been increasing in both northern Europe, in northern America, and in developing countries, while the etiology of breast cancer remains partly elusive.[1]

One of the established risk factors of breast cancer is a high concentration of circulating sex hormones.[2] While most studies focus on reproductive factors reflecting adult hormonal environment, other studies indicate that intrauterine levels of sex hormones may also influence breast cancer risk later in life.[3] This intrauterine hormonal milieu, particularly exposure to testosterone and possibly to estrogens and progesterone, has also been proposed to play a role in cerebral lateralization of the fetus.[4] Handedness represents the most apparent of cerebral lateralization indicators, and may therefore be a marker of the intrauterine hormonal milieu.[4] Given this putatively shared hormonal origin, handedness might be associated with breast cancer risk.

Studies on handedness and breast cancer risk are scarce and inconclusive, and there are no follow up studies. An increase in breast cancer risk has been associated with left-handedness,[5] and with reversed cerebral asymmetry.[6] Case control studies suggesting that left-handedness is not[7] or inversely[8] related to breast cancer risk may be biased due to unaccounted confounding by age. Left-handedness has been related with left-sided breast cancer,[7] even though a more recent study suggested that there is no such relation.[5]

Whether left-handedness is related to disease and more specifically breast cancer risk is still open for debate. We studied the effect of handedness on breast cancer incidence and breast cancer laterality in a large population-based prospective cohort of middle aged healthy women with 16 years follow up time.

Subjects and Methods

Study population

In 1974, the DOM (Dutch acronym: Diagnostisch Onderzoek Mammacarcinoom) cohort was started in Utrecht, The Netherlands, and its surrounding municipalities, to study the benefits of early detection of breast cancer by mammographic screening.[9] Until 1986, a total of 55,519 women enrolled in four sub-cohorts, based on birth date, recruitment date, and questionnaires content: the DOM-1 (birth date: 1911-1925), DOM-2 (1926-1931), DOM-3 (1932-1941) and DOM-4 (1942-1945)

cohorts. The present study pertains to 12,178 women who were recruited in the DOM-3 cohort between 1982 and 1985 (participation rate 40%). These women filled out questionnaires about reproductive history, demography and lifestyle habits and only in this sub-cohort were women asked about their innate hand preference: "Are you left or non-left-handed by birth?". Trained assistants took anthropometric measures before mammography screening. Body mass index (BMI) was calculated as body weight in kg divided by the square of body height in meters. From the questionnaires, we derived the breast cancer risk factors: smoking status (never, past, current smoker), age at recruitment, age at menarche, parity status (nulliparous, parous), family history of breast cancer (mother, sister), and socio economic status (SES) based on type of health insurance: private (higher status), civil servant (intermediate status), sick fund (lower status) that might confound the relation between handedness and breast cancer.

Baseline questionnaire information on age at the last known menstruation and menopausal status (pre and post-menopause) was taken by asking whether the menses had ceased for more than 12 months and, if so, when it last occurred. DOM-3 participants were offered one screening at a relatively young age when not all had reached menopause. Those still pre-menopausal between 1982 and 1986 received additional questionnaires regarding their menopausal status until 1995. Response was about 80% at each mailing. In 1992, special efforts by additional questionnaires and by telephone were made to complete follow-up.[10]

Linkage of the total cohort with the DOM-project's own registry (from 1974) and the regional cancer registry (from 1989 onwards) provided all new invasive breast cancer cases that occurred in the total cohort until January 1st 2000. As the active follow-up for adequate information regarding the person-years lived in the total cohort is costly and time-consuming, vital status was ascertained in a random selection of 1,500 women from the total DOM-3 cohort. These women were followed for movement out of the catchment's area through contacts with the regional municipality registries until January 1st 2000. The accrued person-years of follow up of these 1,500 women were used to calculate person-years lived in the entire cohort (case-cohort design).[11]

Within the total DOM-3 cohort, there were 458 new breast cancer cases during the follow up period. Women were excluded from the analyses if there was no data available on innate handedness or other covariates. From the case-group, 2.2 % (10/458) had missing data on innate handedness, which was 2.0% (30/1,500) for the women in the random sample, and, for comparison, 2.2% (269/12,178) for the entire cohort. Similarly, 4.8% (22/458) of the case-group had missing data on covariates, which was 2.9% (44/1,500) of the random-sample and, again for comparison, 3.0%

(365/12,178) of the entire cohort. An additional 6 women were excluded for a diagnosis of breast cancer before recruitment (prevalent cases), leaving 1,426 women from the random sample available for analyses together with 426 breast cancer cases.

The case-cohort design was first introduced by Miettinen[12] and later extended to a failure time analysis design by Prentice.[13] Absolute incidence rates of breast cancer were calculated using person years derived from the random sample, which were then extrapolated to the whole cohort. Incidence rates were adjusted for age by using direct standardization with the total random sample as the standard. Furthermore, the proportion of breast cancer risk attributable to left-handedness was calculated. To assess the relation between handedness and the incidence of breast cancer, weighted Cox regression analysis was used as described by Barlow et al.[11] In the case-cohort design the standard errors of incidence estimates need to be corrected by a weighting scheme.[11] The weighting scheme proposed by Prentice was used as it estimates best resemble those from a full-cohort analysis.[11] Follow up time started from the inclusion onwards and ended at the date of primary invasive breast cancer diagnosis (event). Women who remained free of cancer during the observation period were either censored at date of movement, date of death or at January 1st 2000, whatever occurred first. Analyses were performed with SAS (version 8.2, SAS Institute Inc., NC, USA) by use of a macro (available at http://lib.stat.cmu.edu/general/robphreg) that computes the weighted estimates together with a robust standard error, from which we calculated 95% confidence intervals (CI). The proportionality of the hazards over time was evaluated with log minus log plots, and was found to be justified.

Uni- and multivariate models were run that considered various breast cancer risk factors as potential confounders. Continuous variables were introduced as such in the multivariate models and for categorical variables dummies were created. Additional analyses were performed to assess effect modification by BMI at recruitment (below or above 25 Kg/m^2), as a marker for growth, and by parity (nulliparous or parous). Furthermore, the relationship between handedness and breast cancer lateralization was analyzed. Women with bilateral breast cancer (5%) contributed both to the analyses on left and right-sided breast cancer. When analyzing the relation between handedness and cancer in the left breast, the women with cancer in the right breast in the sub cohort were considered as controls (censored at the time they died, at loss to follow up, or January 1st 2000, whatever occurred first) and the ones outside the sub cohort were excluded. The opposing applied when analyzing the cancer in the right breast.

Hazard ratios (HR) are reported with corresponding 95% confidence intervals (CI), and corresponding p-values with a cut-off for statistical significance of 0.05.

Results

At the end of follow up in January 2000, 84% of the random sample of 1,426 women was free from breast cancer, 3% had died, 7% had migrated from the region, 2% was lost to follow up, and 4% had been diagnosed with breast cancer during follow up period. A total of 21,508 person years were accrued in the random sample, with a median time of follow-up of 192 months (16 years). Extrapolated (taken into account the sampling fraction), 172,541 person years were accrued in the total cohort, during which 426 women were diagnosed with primary invasive breast cancer (breast cancer incidence rate: 2.5 per 1,000 person years).

11.5% of the random sample reported to be left-handed in early childhood (similar to the frequency in the whole population). As shown in table 1, most baseline characteristics and breast cancer risk factors were similar between left- handed and non-left-handed women, except that left-handed women were slightly older, more often had a positive mother/sister's history of breast cancer, and were more often nulliparous.

The left-handed group had an age adjusted breast cancer incidence rate of 2.8 per 1,000 person years compared to 2.1 per 1,000 person years in the non-left-handed group.

Table 2 shows that, left-handed women had a 39% higher risk for breast cancer than non-left-handed women. The risk was 2.41 when the cancer was premenopausal (diagnosis before reported onset of menopause or, if menopausal data were unavailable, diagnosis at age <51 years), but there was no excess risk for postmenopausal cancer. Adjustment for risk factors of breast cancer, such as age at recruitment, SES, BMI, smoking status, age at menarche, parity status, family history of breast cancer, age at the last known menstruation and menopausal status hardly affected the overall association between left-handedness and incidence of breast cancer.

Table 1. Baseline characteristics of middle age women according to innate handedness

	Left-handed (n=165)	Non-left-handed (n=1,261)
Age at recruitment (years)	47.4 (2.9)	46.9 (3.0)
Height (cm)	165.2 (6.0)	165.2 (5.8)
BMI (kg/m^2)	25.3 (4.1)	24.7 (4.0)
SES (%)		
Low	58.8	62.8
Middle	14.5	10.2
High	26.7	27.0
Smoking Status (%)		
Never	52.7	53.8
Past	12.1	12.1
Current	35.2	34.0
Mother diagnosed with breast cancer (%)	6.7	4.9
Sister diagnosed with breast cancer (%)	4.8	3.0
Age at menarche (years)	13.4 (1.6)	13.6 (1.5)
Age at birth of first child (years)	25.8 (3.8)	25.8 (3.8)
Number of alive born children (%)		
0	21.8	10.9
$\geq$ 1	78.2	89.1
Age at the last known menstruation (years)	47.7 (5.1)	48.4 (5.1)
Menopause (%)	84.8	80.3

Values are mean with the standard deviations unless otherwise indicated
BMI: body mass index; SES: socio economic status

Table 2. Association between handedness and incidence of breast cancer in study participants followed up for 16 years.

Innate handedness	Cases	Estimated person years	hazard ratio (95% Confidence Interval)	
			Crude	Adjusted†
Total				
Non left-handed	361	153,422	reference	reference
Left-handed	65	19,119	1.39 (1.09, 1.81)	1.32 (0.99, 1.76)
Premenopausal breast cancer				
Non left-handed	57	32,113	reference	reference
Left-handed	15	3,329	2.41 (1.35, 4.30)	2.20 (1.15, 4.20)
Postmenopausal breast cancer				
Non left-handed	257	127,426	reference	reference
Left-handed	39	17,665	1.12 (0.80, 1.57)	1.05 (0.75, 1.48)
Body mass index $\leq$ 25 Kg/m^2				
Non left-handed	217	95,964	reference	reference
Left-handed	45	11,332	1.62 (1.17, 2.24)	1.59 (1.15, 2.20)
Body mass index > 25 Kg/m^2				
Non left-handed	144	57,458	reference	reference
Left-handed	20	7,787	1.05 (0.67, 1.66)	1.04 (0.65, 1.64)
Nulliparous				
Non left-handed	61	16,486	reference	reference
Left-handed	9	3,759	0.68 (0.35, 1.32)	0.70 (0.36, 1.35)
Parous				
Non left-handed	300	136,936	reference	reference
Left-handed	56	15,360	1.58 (1.19, 2.11)	1.59 (1.18, 2.13)

The non-left-handed group was the reference group.
74 random sample participants and 32 cases with missing data on covariates or prevalent cases were excluded from these analyses.
Fifty eight breast cancer cases were not analysed as premenopausal or postmenopausal breast cancer, because of the unavailability of menopausal information and the age at diagnosis was 51 to 55 years.
*The number of person-years (lived in the total cohort) is extrapolated from the random sample.
†Adjusted: for socioeconomic status, age, height, body mass index (except in body mass index specific analysis), smoking status, history of breast cancer in mother or sister, age at menarche, parity status (except in parity specific analysis), all at baseline; adjusted for age at last known menstruation and menopausal status during follow up (except for outcome of premenopausal or postmenopausal breast cancer).

Birth weight, like handedness, is another presumed indicator of intrauterine environment.[14] As an association between high birth weight and increased breast cancer risk was previously found to be modified by childhood growth.[15] Moreover, testosterone levels were shown to be lower in women with higher BMI (compared to lower BMI) and in parous women (compared to nulliparous women).[16] Therefore, it was decided in the present analysis to evaluate whether the association between left-handedness and breast cancer risk was modified by current BMI and parity. In table 2, it is shown that breast cancer risk was only increased in lean or normal weight left-handed women (BMI $\leq$ 25 kg/m^2; HR 1.62, 95% CI 1.17 to 2.24), or in parous women (HR 1.58; 95% CI 1.19 to 2.11), as hazard ratios among the overweight or nulliparous women were close to or lower than 1 (tests for interaction: p = 0.07 for handedness and BMI as a continuous variable and p = 0.02 for handedness and parity).

The effect of left-handedness was comparable for tumors of the right (HR 1.28; 95% CI 0.87 to 1.91) and tumors of the left breast (HR 1.33; 95% CI 0.90 to 1.97).

Discussion

In this large prospective cohort study of middle-aged women, we showed that left-handedness is associated with increased breast cancer risk. This relation was largely confined to women with BMI $\leq$ 25 at baseline and to parous women.

To appreciate these findings, certain features of the study need to be addressed. The participation rate in our cohort was 40%. Our population may be healthier compared to the general population, as women who voluntarily join screening programs are more likely to have healthier lifestyles and to be higher educated.[17] However, there is no reason to assume that the studied relationship would be different between women who did and did not participate, thus selection bias is not likely to have occurred.

As the focus in our cohort was originally not on cerebral lateralization we had data on hand preference only but not other indicators of cerebral lateralization, which may introduce misclassification.[4] Expectedly however, such misclassification, if any, will have led to underestimation of the association, as it is most likely unrelated to the association between handedness and breast cancer. The prevalence of innate left-handed people in our population is similar to other reports.[18] We adjusted for several known breast cancer risk factors,[19] that might confound the relation between handedness and breast cancer. Some of these risk factors were shown related to handedness

preference as left-handed women had menarche and menopause at younger age than their counterparts and had higher infertility risks than right-handed women.[4;20] As there were no material changes in the hazard ratios after adjustments, we conclude that these factors seem to be neither confounders nor intermediate components of the causal pathway linking handedness to breast cancer.

We know of only five published case-control studies that have addressed handedness and breast cancer risk. Age-related bias is a long recognized hazard when evaluating handedness and morbidity or mortality. In four of these studies,[8;21-23] insufficient account was taken of differences in age distributions,[5;24] likely resulting in biased observations of lower frequencies of left-handedness in older case groups. The most recent case control study has dealt with this issue by matching the cases and controls by 5-years age strata.[5] That study reported a modest effect only in postmenopausal women, whereas in the current study it was confined in premenopausal women. Our cohort is a birth cohort with a relatively limited age range (39 to 52 years) and a virtually identical age distribution across the left-handed and non-left-handed group. Indeed, adjustment for age did not change our findings.

Genuine handedness reportedly is influenced by genetics, birth trauma, and other perinatal factors, but intrauterine hormone exposure may also play a role.[4] A new insight is that lateralized behavior (arm movement) occurs early in pregnancy,[25] possibly even before cerebral lateralization, and will last until later age. This gives support to either an early intra uterine influence playing a major role in determining handedness, or that genes directly determine left-handedness. Findings in animals have suggested that increased intrauterine exposure to certain steroid hormones (testosterone, progesterone, or oestradiol) has masculinising effects on neural organization.[4] It has been shown that fetal exposure to elevated levels of intrauterine testosterone or DES (a synthetic estrogen) is associated with atypical patterns of cerebral asymmetry, as evidenced by shifts in the animals' usual postural asymmetry.[4] Equivalent shifts in cerebral patterns and postural asymmetry (from right-handedness to left-handedness) possibly occur in humans as an effect of increased exposure to or sensitivity to intrauterine steroid hormones.[4] The finding of an increased frequency of left-handedness among women exposed in utero to DES supports this hypothesis.[26] Furthermore, young women who were diagnosed with congenital adrenal hyperplasia, a condition associated with high prenatal testosterone exposure, are more likely than non-affected sister controls to be left-handed.[27]

During pregnancy, maternal testosterone is converted to oestradiol by aromatase (a cytochrome p-450 enzyme) in the placenta and in fetal neural tissues, thus distinguishing the effects of specific intrauterine hormones may be difficult.[4] The connection between hand preference and breast cancer risk may lie in a common origin of intrauterine hormonal exposure. Indeed, in one small study among right handed women, computerized tomography[6] showed that atypical patterns of brain asymmetry, possibly reflecting intrauterine hormonal exposures, are more frequent among breast cancer cases, relative to non-cases.

In post-menopausal women, testosterone not only independently increases the risk of breast cancer but also through its changes into oestradiol by aromatase.[16;28] High intrauterine testosterone exposure may increase later life sensitivity to this hormone, by mechanisms still not fully understood.[4]

Both handedness[4] and birth weight[29] are considered surrogate measures for intrauterine hormonal environment. For several reasons we have looked at effect modification by BMI. First, childhood growth was shown to modify the effect of birth weight on breast cancer incidence.[15] Second, there was an observed increased left-handedness in short stature children, suggesting a common hormonal influence.[30] Third, testosterone levels were shown lower in women with higher BMI (compared to lower BMI) and in parous women (compared to nulliparous women).[16] Unexpectedly, we found the effect of handedness only in lean or normal weight for height women and parous women. The biological explanation of this finding remains highly speculative, but lean or parous women may be (perhaps genetically) more sensitive to the effect of the hormone(s) that cause both left-handedness and breast cancer. Alternatively, it may be that among the obese (or nulliparous women), the effect of high BMI (or null parity)[31] on outcome has such an overriding effect that the effects of handedness are undetectable. It was suggested that left-handed women may have lower fertility[4] and null parity is a well-known risk factor of breast cancer.[31] It is possible that parity, as an indicator of adult hormonal condition, may have an interaction with intra uterine hormonal milieu (represented by innate handedness). That is, left-handed women who are infertile as adults may have bigger risk to develop breast cancer, compared to left-handed fertile women.

Breast cancer is more common in the left breast[32] which may reflect the tendency of women to have bigger left breasts than right breasts.[7] Our finding is in line with previous observations[5] that left-handedness is not related to laterality of breast cancer.

Our findings do indicate that the origins of cerebral lateralization play a role in the etiology of breast cancer. The fact that it was only confined to premenopausal women is compatible with left-

handedness being a marker of constitutional risk rather than of environmental risk as with postmenopausal breast cancer.

In conclusion, our findings in a large prospective cohort study provide evidence for a substantially increased breast cancer risk among left-handed women. Although still speculative, our results are in agreement with the hypothesis that left-handedness and breast cancer originate from a common intrauterine hormonal cause.

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Chapter 3.2.

Left-handedness, depression and disease proneness.

Manuscript based on this chapter:

Ramadhani MK, Uiterwaal CSPM, Grobbee DE, Neeleman J, Burger H. Left-handedness, depression and disease proneness. Submitted

Summary

Background:
Left-handedness is a possible marker of an intrauterine adverse environment and is suggested to be related to various risks of psychological and physical diseases. Evidence for an association between handedness and psychiatrical symptoms among healthy adult individuals is currently scarce.

Subjects and Methods:
We studied the association between hand preference and psychological distress and diagnosed depression, as well as self reported treated disease and disease proneness, in a cohort of 1093 middle-aged Dutch participants in a follow up cohort. These associations were studied by (multinomial) logistic regression analysis.

Results:
Of the total cohort, 10.9% reported to be left-handed. Left-handers had a higher risk of psychological distress in adult life (OR 1.8, 95% CI 1.1 to 2.9), a diagnosis of moderate depression (OR 2.3, 95% CI 1.0 to 5.4), for higher perceived disease proneness (OR 2.2; 95% CI 1.1 to 4.5) and to have >2 treated chronic illnesses (OR 1.8, 95% CI 0.9 to 3.6), than right-handers. The results remained after adjusting for age and gender.

Conclusion:
These results support the hypothesis that left-handedness may be related to proneness to psychological distress and physical diseases.

Introduction

Approximately 10% of the population is left-handed.[1] There is an increasing body of evidence in support of a genetic basis of hand preference,[1-3] but there are many observations to suggest a role for early life adverse environmental influences as well.[4,5]

Handedness may be used as a marker of cerebral lateralization of functions, as exemplified by the close association between right-handedness and left-hemisphere speech mediation.[6,7] Non right-handedness has been reported to be related to a variety of psychiatric disorders, such as schizophrenia,[8] particularly in males[9] and borderline personality disorder.[10] Also, there have been observations on a relationship between left-handedness and depression, especially in males.[11] Antenatal maternal anxiety has been linked to increased mixed-handedness[12] and also in animal studies maternal anxiety has been linked to certain neurobehavioral problems in the offspring.[13]

Although there have been reports on associations between handedness and overt psychiatric disease, much less is known about associations with psychiatric symptoms in healthy individuals. We aimed to study the link between left-handedness and perceived stress and diagnosed depression in adulthood as well as its link with self reported disease and disease proneness.

Subjects and Methods

Patients and methods

The data presented in this study are obtained from a sub sample of participants of the PREVEND study.[14] The PREVEND study (Prevention of REnal and Vascular ENdstage Disease) was designed to investigate the natural course of microalbuminuria and its relation with renal and cardiovascular disease in the general population. The study cohort is formed by male and female inhabitants aged 28 to 75 years of the city of Groningen, the Netherlands. These inhabitants were asked to send in a morning urine sample. A sample population consisting of all subjects with an albumin concentration of more than 10 $mg.l^{-1}$, together with a randomly selected sample of the remainder of the population (morning urine albumin excretion < 10 $mg.l^{-1}$) made two visits to an outpatient clinic. Between 1997 and 1998, 8592 participants were included into PREVEND and had baseline measurements. Between 2001 and 2003 these participants were re-invited for physical examination and the assessment of neuroticism, non-psychotic psychiatric symptoms, medication use, and chronic and acute somatic complaints.

Part of this PREVEND cohort was approached for more extensive measurements of psychosocial and psychiatric health in the context of the Study of Allostatic Load as a Unifying Theme (SALUT) study. In the selection of SALUT participants, the original over sampling of high urinary albumin concentration in PREVEND was annulled, such that the SALUT sample was again representative of the general population. The analyses for the present study included all 1093 subjects who finalized these additional SALUT measurements (response rate 40%).

Measurements

Demography, birth, childhood, and handedness

Data on birth and childhood were obtained using sections of questionnaires used in earlier large scale epidemiological studies.[15-17] These sections included a question 'Are you right or left-handed?'. Demographic status, i.e. age, gender and educational attainment were assessed using the General Aptitude-Test Battery.[18]

Neuroticism (EPQ-12)

Neuroticism was measured using the 12-item neuroticism subscale of the EPQ-R, a revised shortened version of the Eysenck Personality Questionnaire (EPQ),[19] which has been translated into the Dutch language.[20,21]

Psychological distress (SCL-8) and depression

Psychological distress was measured using an 8-item shortened version of the Hopkins Symptoms Checklist (SCL-8),[22] which has been translated into Dutch.[23] The scale was modified such that the items were rated on a five-point scale instead of a four-point scale, i.e. not at all, mild, moderate, relatively severe, and very severe. SALUT participants further underwent the Composite International Diagnostic Interview (CIDI), a structured diagnostic interview that generates ICD-10 and DSM-IV diagnoses.[24] This interview was focused on the CIDI sections measuring somatisation, anxiety, depression and mania.

Disease-accident proneness and self reported illnesses

The question about disease proneness was taken from the RAND 36-Item Health Survey 1.0,[25-27] which has been translated into Dutch.[28] The question about accident proneness was not taken from an existing checklist. Participants were asked to respond on a five-point scale (very much disagree,

disagree, indecisive, agree, very much agree) to the following sentence: "I get sick easier than other people" and "I have more often accidents (including small accidents) than others".

Physical and mental health was measured using self-report questionnaires for chronic and acute disease assessment.[29-30] The participants were offered a list of chronic illnesses and common complaints reported to general practitioners, and were asked to choose from the list the chronic illnesses they had been treated for, and acute complaints during the last month.

Data analysis

Descriptive statistics were calculated by handedness. Multinomial and binary logistic regression models were used to quantify the association between handedness (independent variable) and the various psychological categorical outcome measures (dependent variable). For instruments yielding total scores, distribution based categories were made, such as tertiles for SCL8-scores and the median for EPQ-12-scores. Univariate associations were calculated as odds ratios with 95% confidence intervals (95%CI). Subsequently, the same (multinomial) logistic regression models were used to provide odds ratios adjusted for possible confounders. Analyses were performed with SPSS, version 12.0.2 for Windows.

Results

Of 1093 participants included in our study, 10.9% were left-handed. The left-handed group was slightly younger, showed an amply threefold chance of having been born with assisted delivery and a considerably higher proportion of treatment in an incubator as neonates as compared to the right-handed group (table 1).

Table 1. General characteristics based on present handedness (N=1093)

	Left-handed (119, 10.9%)	Right-handed (974, 89.1%)	p-value
Mean (sd) age (years)	51.7 (11.2)	53.3 (11.4)	0.136
Gender (%):			
Male	44.5	46.7	0.697
Female	55.5	53.3	
Education (%):			
Low	33.9	30.9	0.730
Middle	15.2	17.6	
High	50.9	51.6	
Birth weight < 2500 g (%)	5.9	5.4	0.820
Birth assisted with vacuum/forceps (%):	9.4	3.0	0.004
Treated in incubator as neonates (%)	6.3	2.6	0.040
Having been breast-fed (%)	5.9	5.4	0.820

Tests used: t-test and chi square

In table 2, it is shown that left-handers had a higher risk of an SCL8 score in the second tertile or above than right–handers. Adjusting for gender and age did not change the results. (p for trend = 0.03, adjusted for age and gender). Using “no depression” as the reference category, left-handers had a 2.3 times higher chance for moderate depression than right-handers. Adjustment for age and gender did not change the magnitude of the association but it did lose its statistical significance. There was a slight insignificant increase in chance for left-handers to be diagnosed with mild depression. There was no association between handedness and neuroticism.

Table 2. Handedness and stress/depression (N=1093)

Dependent variables	Left-handed (119)	Right-handed (974)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
SCL8 score (psychological distress)				
1st tertile	22.0	31.2	-	-
2nd tertile	33.9	33.9	1.4 (0.8, 2.4)	1.4 (0.8, 2.3)
3rd tertile	44.1	35.0	1.8 (1.1, 2.9)	1.7 (1.0, 2.8)
EPQ-12 score (neuroticism)				
0 - 1	44.2	48.9	-	-
2 or higher	55.8	51.1	1.2 (0.8, 1.8)	1.2 (0.8, 1.8)
Major depressive disorder, single episode (CIDI)				
No depression (%)	84.9	88.6	-	-
Mild depression (%)	7.6	5.9	1.3 (0.6, 2.8)	1.4 (0.6, 2.8)
Moderate depression (%)	5.9	2.7	2.3 (1.0, 5.4)	2.2 (0.9, 5.1)
Severe depression (%)	1.7	2.9	0.6 (0.1, 2.6)	0.6 (0.1, 2.5)

*adjusted for gender and age

Table 3 demonstrates that left-handed participants considered themselves to be more prone to diseases than right handed participants. With no self reported illnesses (see table legend) as the reference category, the left-handers had a higher risk of having more than two treated chronic illnesses. Adjustment for age and gender did not change this result. Left-handed participants reported less acute complaints, although not statistically significant. The lower risk for acute complaints among left-handers was not explained by the higher risk for chronic treated illnesses, as the association between left-handedness and chronic treated illnesses remained virtually unchanged after adjusting for acute complaints (1-2 disease versus no disease OR =1.1, 95% CI 0.7 – 1.7; >2 diseases versus no diseases OR = 2.1, 95% CI 1.0 – 4.4).

Table 3. Handedness and disease proneness (N=1093)

Dependent variables	Left-handed (119)	Right-handed (974)	Unadjusted OR (95% CI)	Adjusted OR (95% CI)*
Do you consider your self to be more prone to diseases?				
No (%)	77.3	85.3	-	-
Indecisive (%)	14.3	10.5	1.5 (0.9, 2.6)	1.5 (0.8, 2.6)
Yes (%)	8.4	4.2	2.2 (1.1, 4.5)	2.2 (1.1, 4.5)
Chronic illnesses (treated) in the last 12 months (%)				
No illness	62.2	64.4	-	-
1 – 2 illnesses	29.4	30.7	1.0 (0.6, 1.5)	1.1 (0.7, 1.6)
> 2 illnesses	8.4	4.9	1.8 (0.9, 3.6)	1.9 (0.9, 4.0)
Number of acute complaints (often) (%)				
No complaints (1st quartile)	29.4	21.0	-	-
1 – 2 complaints (2nd quartile)	27.7	31.0	0.6 (0.3, 1.0)	0.6 (0.4, 1.1)
3 – 4 complaints (3rd quartile)	21.0	21.6	0.7 (0.4, 1.2)	0.7 (0.4, 1.2)
5 – 17 complaints (4th quartile)	21.8	26.4	0.6 (0.4, 1.1)	0.6 (0.3, 1.0)

* adjusted for gender and age
included in chronic diseases: asthma/COPD, sinusitis, ulcus pepticum, severe bowel diseases (> 3 months), gall blader infection/stones, liver diseases, prolaps uteri, thyroid diseases, persistent back diseases (HNP), arthrosis, rematoid arthritis of hand/feet, other chronic reuma, myocardial infarction, diabetes, epilepsy, vertigo/falling, migraine, severe skin diseases, malignancy, multiple sclerosis, Parkinson, ME, blindness/severe impaired sight, deafness/severe impaired hearing, injury due to trauma, hypertension, other diseases.
included in acute complaints: sneezing, clogged nose, coughing, rhinitis, influenza, ear infection/pain, sore throat, dyspnoe, fever, eczema, itchy, HSV1, nausea with or without vomiting, heartburn, obstipation, diarrhoea, enteralgia/stomach ache, headache, tennis elbow/RSI, vertigo, severe fatigue, small accident.

As birth trauma has been reported a cause of left-handedness, we evaluated whether differences in delivery and neonatal treatment between left and right handed participants (see table 1) could explain the findings in tables 2 and 3. Adjustment for differences in rates of vacuum or forceps assisted birth or rates of incubator treatment as neonates did not have a material influence. Analysis on handedness and accident proneness showed no association (Chi-square 0.48, df 4, p=0.98).

Discussion

Our study of healthy adults shows associations between being left-handed and more psychological distress, more diagnosed moderate depression, and higher disease proneness. In addition, a trend towards the presence of more treated chronic illnesses was observed in left-handed subjects.

Before further discussing our findings, some methodological aspects need to be addressed. We confirmed in a fairly large cohort of adults associations between left-handedness and psychopathology in the anticipated direction. However, these were not present for all instruments, leading us to conclude that these associations, although in our view genuine, are not very strong. This may partly be due to determinant and outcome measurement misclassification. In our study, hand preference was measured by a simple question whether participants were right or left-handed, while hand preference is only one component of cerebral motor lateralization. Such misclassification may have led to underestimation of effects. Furthermore, our participants had an average age of around 50 years and such generations may have experienced social pressure against particularly left-hand writing with adverse psychological consequences as a result. This would have yielded overestimation of effects. In a recent study in 1277 older individuals, there was no association between left hand writing per se and psychosocial outcome, while subjects reporting a hand preference switch, particularly when unsuccessful, did have lower quality of psychosocial and physical well-being.[31] Thus, while in Western societies social pressure against left-handedness has decreased in young persons, it cannot be excluded that in older persons forced switching of hand preference induces psychosocial problems rather than the other way around. However, in our data the associations between handedness and outcome, e.g. SCL-8, did not clearly depend on age (data not shown) leading us to conclude that forced hand preference switching may not have played a major role in our findings.

Perhaps the most frequently observed relation between cerebral lateralization and psychopathology is with psychosis, particularly schizophrenia. A recent meta-analysis of over 40 studies on handedness and schizophrenia showed that all atypical handedness patterns, such as left-handedness and mixed handedness, were more prevalent among schizophrenia patients.[32] Observations on handedness or rather cerebral lateralization and particularly schizophrenia have led to various theories about the exact nature of the association. Genetic and early life environmental influences, such as brain damage, are suggested to underlie the handedness and schizophrenia association. There are several associated theories suggesting an autosomal genetic locus,[33-35] but

there is also evidence supporting a genetic locus on the X-chromosome.[36] However, associations with handedness may not be specific to schizophrenia and indeed such associations have been found with other psychiatric outcomes. This may partly be due to the observation that (genetic) determinants of schizophrenia also play a role in other psychiatric disease leading some researchers to conclude that schizophrenia as a diagnostic phenotype may be too narrow.[37] Alternative to genetic origins of the association, birth complications have long been recognized to be associated with later life psychosis[38-39] and may explain associations with handedness.[40] As the personality trait neuroticism is considered a marker of continuous vulnerability to psychopathology, we did not expect to see virtually no association with left-handedness.[41] From this observation we conclude that left-handedness is a risk factor for the depressed state, rather than for an unfavourable personality trait. Another observation that seems hard to rhyme with the other observations is that the prevalence of frequent acute complaints in the left-handed group was lower. We did exclude the possibility that having reported more chronic treated illnesses was directly related to less reporting of acute complaints. We have currently no explanation for lower acute complaint rates among left-handers.

The evidence on a relationship between gestational stress and brain morphology and behavior in offspring has been elaborately reviewed by Weinstock.[42] From human and particularly animal studies Weinstock proposes that indeed maternal stress in gestation may, through various hormonal systems, have later life consequences for the offspring such as depressive symptoms or schizophrenic symptoms. Studies in Rhesus macaques and in humans show a relation between (stress) hormone levels or stress reactivity and hand preference.[43-45] Maternal stress in gestation may also be associated with hand preference in the offspring. In the ALSPAC study among 7,431 mother-child pairs, it was recently shown that maternal anxiety at 18 weeks of gestation was associated with a 23% higher risk for mixed-handedness by maternal report scale of the child at 42 months post partum, independent of parental handedness, obstetric and other antenatal risks, and postnatal anxiety.[12] Thus, hand preference may indeed be a marker for an adverse intrauterine environment through maternal stress and later life psychosocial consequences.

Our findings with regard to self-reported disease are in agreement with recent findings showing young non-right handed students to report more specific health problems.[46] This may be through a direct link between psychological well being and physical illnesses.[47-48] Stress during certain periods of life is related to certain hormonal changes, typically cortisol, which through altered physiological functions may later increase the risk of certain chronic illnesses.[47-49]

In recent decades, there is a growing interest into early life origins of diseases in adulthood. The fetal and infant origin of adult diseases hypothesis[50] may reflect comparable mechanisms leading to both left-handedness and increased morbidity or mortality in later life.[51] As an adverse environment in utero may lead to higher morbidity due to certain adult diseases,[52] left-handedness may similarly be associated with increased incidence of adult illnesses.

In summary, our data lend support to the hypothesis that cerebral lateralization is related to several aspects of psychological well-being in the general population. It seems possible that handedness is a marker for general vulnerability to psychosocial and physical problems. Our findings suggest that left-handedness is related to proneness to psychological distress, depression and physical diseases.

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Chapter 3.3.

Innate handedness and disease specific mortality in women.

Manuscript based on this chapter:

Ramadhani MK, Elias SG, van Noord PAH, Grobbee DE, Peeters PHM, Uiterwaal CSPM. Innate handedness and diseases specific mortality in women. Submitted.

Summary

Background:
Left-handedness has been reported to be associated with reduced life expectancy but the evidence is not conclusive.

Subjects and Methods:
The association between innate handedness and overall as well as cause specific mortality was studied in a cohort of 12,178 middle aged Dutch women followed for almost 13 years from 1982. The relation between innate handedness and mortality was analyzed using Cox regression in a case-cohort approach, in which a random sample of 1,500 women was used to adequately represent person-years under observation for the entire cohort.

Results:
During a median follow-up of 12.6 years, 252 women died. Hazard ratios comparing left-handed women to non left-handed women were 1.4 for all-cause mortality (95% confidence interval (CI) 0.9, 2.0) , 1.7 for total cancer mortality (95% CI 1.0, 2.7), 2.0 for breast cancer mortality (95% CI 0.8, 4.6), 4.6 for colorectal cancer mortality (95% CI 1.5, 14.3), 1.3 for cardiovascular diseases mortality (95% CI 0.5, 3.3), and 3.7 for cerebrovascular mortality (95% CI 1.1, 12.1) after adjusting for potential confounders (socio-economic status, age, body mass index and cigarette smoking status at study recruitment).

Conclusion:
Left-handedness is associated with higher mortality in women.

Introduction

Approximately 10 percent of the population is left-handed.[1] There is an increasing body of evidence in support of a genetic basis of hand preference,[1;2] but there are many observations to suggest early life environmental influences as well.[3;4] One of the prevailing explanations is that it stems from an adverse prenatal environment, such as excessive exposure to testosterone, which influences brain lateralization.[3]

In recent decades, there is a growing interest into early life origins of adult chronic diseases. The fetal and infant origin of adult diseases hypothesis[5] may include mechanisms leading to both left-handedness and increased morbidity and mortality in later life.[6] As an adverse environment in utero may lead to higher mortality due to certain adult chronic diseases,[7] left-handedness may similarly be associated with reduced survival. Indeed, left-handers are reported underrepresented in the older age groups, although such findings are still much debated.[8;9]

Several studies[8;10-17] have attempted to explain the decreased number of left-handed among elderly people through a modification hypothesis, meaning cultural or social pressure against left-handedness, or an elimination hypothesis, meaning higher mortality rates among the left-handed group. The modification hypothesis could not explain the reduced number of left-handed people in the elderly group.[8] The elimination hypothesis has been studied to some extent, but the results are largely inconsistent.[9] Two studies reported a relation between left-handedness and increased mortality,[8;13] whereas two others found left-handedness to be associated with a survival benefit.[10;16] However, many studies did not find any relation.[11;12;14;15;17;18] Most of these studies used cross-sectional designs, cohorts with short follow up, or very selective groups of participants.

We were able to explore the relation between handedness and cause specific mortality further in a large prospective, population based, cohort study of middle-aged women.

Subjects and Methods

In 1974, a population based project was started in Utrecht, The Netherlands, and its surrounding municipalities, to study the early detection of breast cancer by mammographic screening.[19] The project was called DOM-project (Dutch acronym: Diagnostisch Onderzoek Mammacarcinoom). Within the DOM-project, there were four sub-birth cohorts, but only women who participated in the DOM-3 cohort (birth date 1932 – 1941) were asked by questionnaire about their handedness. Participation in

the DOM-3 cohort was about 40 percent from all invited women, and a total of 12,178 women were recruited from 1982-1985.

These women filled out extensive questionnaires about reproductive history, demography and lifestyle habits. Women were asked about their innate hand preference: “Are you left or non left-handed by birth?”. Of all women, 269 (2 percent) did not answer the question about handedness, and these were excluded from the analyses. Trained assistants took anthropometric measures before mammography screening. Body mass index (BMI) was calculated as body weight in kg divided by the square of body height in meters. From the questionnaires, we derived some potential confounders such as cigarette smoking status (never, past and current smoker), age at recruitment, and socio-economic status (SES), which was based on type of health insurance: private (higher status), civil servant (intermediate status), sick funds (lower status).

Information on date and cause of death was actively passed through to the DOM-project registration office by the regional municipalities and general practitioners until January 1996. However, date and destiny of emigration of study participants were not actively monitored, hampering precise measurement of person-years lived under observation in the entire cohort. As the active assessment of complete follow-up information - necessary for obtaining adequate information regarding loss to follow-up for the whole cohort - is costly and time consuming, we randomly selected a sample of 1,500 women (12 percent) of the DOM-3 cohort. For this random sample we ascertained emigration data and vital status through regional municipality registries until January 2000, but for the current analysis follow-up was truncated at January 1996. As these women were randomly selected, their accrued person-years of follow up were used to represent person-years lived in the entire cohort (case-cohort design).[20]

Within the cohort, 252 women died during the follow-up period until January 1996. Causes of death were classified by the International Classification of Diseases, ninth revision (ICD9) criteria. We investigated effects on total mortality, but also on disease-specific mortality: total cancer (ICD9: 140-209), breast cancer (ICD9: 174), colorectal cancer (ICD9: 153-154), total cardiovascular diseases (CVD; ICD9: 390-459), cerebrovascular diseases (ICD9 430-438), and other causes. More detailed analyses on cause-specific mortality, such as other types of cancer or ischemic heart disease, were hampered by the increasingly smaller number of cases for other causes of death.

Women were excluded from the analysis if there was no data available on innate handedness or other covariates. From the women who died (cases), 11/252 (4.4 percent) had missing data on innate handedness, which was 30/1,500 (2.0 percent) for the women in the random

sample, and, for comparison, 269/12,178 (2.2 percent) for the entire cohort. Similarly, 1/252 (0.4 percent) of the case group had missing data on covariates, which was 4/1,500 (0.3 percent) for the random sample and, again for comparison, 28/12,178 (0.2 percent) for the entire cohort. An additional 11 women were excluded from the random sample as they were lost to follow up immediately following examination, leaving 1,455 women from the random sample available for the analyses together with 240 cases. The ethical approval for the DOM study was granted by the ethical committee of the University Medical Center Utrecht.

The case-cohort design was first introduced by Miettinen[21] and later extended to a failure time analysis design by Prentice.[22] Age-adjusted absolute incidence rates were computed using direct standardization, with the use of the random sample population as the standard. For these analyses, the person-years lived in the random sample were extrapolated to the total cohort.

To assess the relation between handedness and mortality (overall and specific causes), we used weighted Cox regression analysis. The methods for such analysis are similar to standard Cox regression, and have previously been described by Barlow et al.[20] In the case-cohort design the standard errors of risk estimates need to be corrected by a weighting scheme.[20] We chose to use the weighting scheme proposed by Prentice as it was found to provide estimates that best resemble those from a full-cohort analysis.[20] Follow up time started from the inclusion onwards (between 1982 and 1985) and ended at the date of death. Women who remained alive (or died of other causes when a specific cause was under investigation) during the observation period were censored at date of movement, date of death or at January 1, 1996, whatever occurred first.

Analyses were performed with SAS (version 8.2, SAS Institute Inc., NC, USA) by use of a macro (available at http://lib.stat.cmu.edu/general/robphreg) that computes the weighted estimates together with a robust standard error, from which we calculated 95 percent confidence intervals (CI). The proportionality of the hazards over time was evaluated with log minus log plots. As cohort members where relatively healthy at recruitment, log minus log plots showed that mortality hazards were virtually identical between left- and non-left-handed women for the first five years after recruitment, and started to deviate from each other afterwards to reach parallelism. Therefore, to fully fulfil the proportional hazard assumption, we also analysed the data excluding the first five years of follow up. For reasons of limited numbers of events, this analysis was restricted only to overall mortality.

Uni and multivariate models were run that considered potential confounders. Continuous variables were introduced as such in the models and for categorical variables dummies were created.

Hazard ratios (HR) are reported with corresponding 95 percent confidence intervals (CI), and corresponding p-values with a cut-off for statistical significance of 0.05.

Results

At the end of follow up, 89.6 percent of the random sample of 1,455 women were still alive, 2.6 percent had died, 6.5 percent had migrated from the region, and 1.4 percent were lost to follow up. A total of 17,567 person years were accrued in the random sample, with a median time of follow-up of 151 months (12.6 years). Extrapolated (taken into account the sampling fraction), 143,521 person years were accrued in the total cohort, during which 240 women died (overall mortality rate: 1.7 per 1,000 person years).

Of the study population, 11.5 percent reported to be left-handed in early childhood. Baseline characteristics according to innate handedness are presented in table 1. Left and non left-handed women did not materially differ in age, BMI, SES, and smoking habits.

The left-handed group had a crude mortality rate for all causes of 2.3 (adjusted for age by direct standardization: 2.1) compared to 1.5 (adjusted for age: 1.6) per 1,000 person years in the non left-handed group.

Table 2 shows that, after adjustment for age, SES, BMI and cigarette smoking status, left-handed women had a 1.36 times higher risk of dying from all causes than non left-handed women, although this was not statistically significant. The adjusted HR (total mortality), after excluding the first five years of follow up time was 1.58 with 95% CI 1.03 to 2.42.

With regard to cancer mortality, left-handed women had a 1.65 times higher risk of dying from any type of cancer (95% CI 1.03 to 2.65), a 4.64 times higher risk of dying from colorectal cancer (95% CI 1.47 to 14.29), and a 1.95-fold higher risk of dying from breast cancer (95% CI 0.83 to 4.56), although the latter was not statistically significant. Handedness was not significantly associated with overall cardiovascular mortality (HR 1.34, 95% CI 0.54 to 3.33), but left-handed women had a 3.69 times higher risk of dying from cerebrovascular diseases than non left-handed women. Left-handedness was not associated with death due to other causes of death than the above-mentioned.

Table 1. Characteristics at study recruitment (1982-1985) of the random sample (n = 1,455 women) from the DOM-3 cohort at age 41-53 years according to innate handedness, Utrecht, the Netherlands, 1982-1996

	Left-handed (n=168)	Non left-handed (n=1,287)
Age at recruitment (years)*	47.5 (41.6, 53.1)	46.9 (41.1, 53.1)
Age at the end of follow up (years)*	58.8 (45.8, 64.0)	58.5 (42.8, 64.0)
BMI (kg/m^2)	25.3 (4.0)	24.8 (4.0)
SES (n, %)		
Low	98 (58.3)	810 (62.9)
Middle	24 (14.3)	132 (10.3)
High	46 (27.4)	345 (26.8)
Cigarette smoking status (n, %)		
Never	88 (52.4)	692 (53.8)
Past	20 (11.9)	158 (12.2)
Past < 20 per day	16 (80.0)	137 (86.7)
Past > 20 per day	4 (20.0)	21 (13.3)
Current	60 (35.7)	437 (34.0)
Current < 10 per day	25 (41.7)	203 (46.5)
Current 11 - 20 per day	21 (35.0)	144 (33.0)
Current > 20 per day	14 (23.3)	90 (20.5)

Values are mean with the standard deviations unless otherwise indicated
* median (minimum, maximum)
BMI: body mass index; SES: socio-economic status

Table 2. Innate handedness and mortality risk of women followed up for ± 13 years from age 41-53 years, Utrecht, the Netherlands, 1982-1996

	Deaths		Hazard ratio	95% Confidence interval
	Left-handed (16,985 PY)	Non left-handed (126,509 PY)		
All causes mortality	37	203		
Crude			1.43	0.98, 2.09
Adjusted			1.36	0.92, 2.01
Total Cancer mortality	25	113		
Crude			1.74	1.10, 2.74
Adjusted			1.65	1.03, 2.65
Breast cancer mortality	7	27		
Crude			2.04	0.88, 4.69
Adjusted			1.95	0.83, 4.56
Colorectal cancer mortality	5	8		
Crude			4.87	1.57, 15.05
Adjusted			4.64	1.47, 14.29
Total Cardiovascular diseases mortality	6	33		
Crude			1.43	0.59, 3.45
Adjusted			1.34	0.54, 3.33
Cerebrovascular diseases mortality	4	9		
Crude			3.48	1.06, 11.39
Adjusted			3.69	1.12, 12.14
Other causes mortality	6	57		
Crude			0.83	0.35, 1.93
Adjusted			0.80	0.34, 1.88

Adjusted for socio-economic status (low, middle, high), body mass index (kg/m^2), age at study recruitment (years), cigarette smoking status (never, past, current). Other causes: hepatitis, liver cirrhosis, metabolism disorder, meningitis, respiratory tract diseases, renal failure, injury. PY, person years extrapolated from the random sample.

Discussion

In this cohort of middle-aged women, we showed that innate left-handedness is associated with increased mortality, particularly from cancer and cerebrovascular diseases. To appreciate these findings, certain features of our study need to be addressed. The participation rate in our cohort was 40 % and women were selected from participants in a breast cancer screening project. Our population may be healthier compared to the general population, as women who voluntarily join screening programs are more likely to have healthier lifestyles and to be higher educated.[23] However, there is no reason to assume that the relationship between handedness and mortality would differ between women who refused or agreed to participate in the DOM-3 project, thus selection bias is not likely to have occurred.

A limitation of the present study is the low number of cases, which prohibited more detailed analyses on left-handedness and cause-specific mortality. However, the confidence intervals show that the numbers still allowed for meaningful detection of associations. Another limitation is that we could not study the effects of handedness in men, a group with a higher percentage of left-handedness.[1]

Measuring handedness using one question about writing hand or self-assessment may introduce misclassification.[3] As our focus was originally not on handedness, we only asked for innate hand preference. However, this method would only introduce underestimation of the results since this misclassification is likely to be at random (non differential) and unrelated to the association between handedness and mortality. The proportion of innate left-handed people in our population is similar to previous studies.[1]

The data on mortality was obtained from active follow up through regional municipalities and general practitioners, and we therefore believe it to be largely complete. The observed mortality rate in our cohort (2.3 per 1000 person years) is lower than what would be expected from data from the general Dutch population (3.4 per 1000 person years) based on mortality data from the Netherlands Central Bureau of Statistics in 1996 (www.cbs.nl). This probably reflects better average health among our participants.

We could think of only a limited number of possible confounders, in the sense of external factors biasing associations between handedness and mortality. Previously, it has been shown that age, BMI, SES, and cigarette smoking status, each are independent risk factors for mortality (overall and also for some specific cause of death)[24;25] and some may also be related to hand preference.[3]

We adjusted for these factors (obtained at study recruitment) despite the fact that the left and non left-handed women only showed very minor differences with regard to these variables. Consequently, there were no material changes in the hazard ratios after adjustments leading us to conclude that in our data these factors seem to be neither confounders nor intermediate components of the causal pathway linking handedness to mortality.

There have been conflicting results in studies on the relationship between handedness and mortality. A study about handedness and mortality performed by Halpern and Coren,[13] stated that left-handed people have a 9 years shorter life expectancy than their right-handed counterpart. Earlier studies,[8] which were widely criticized, found a significantly shorter life span in the left-handed group. More recent studies found no difference in mortality between the left and non left-handed[11;12;14;15;17] except for injury related death,[11;15] or even longer survival for the left-handed.[10;16] These studies used present handedness with various methods to measure handedness, specific age and sex group or shorter follow up time. The use of present handedness, especially in older generations, would introduce misclassification of many innate left-handers as right-handers, which could underestimate the true relationship between handedness and mortality. Present handedness may be highly influenced by societal pressure towards right-handedness.

Even though left-handedness has been related to a higher occurrence of breast cancer in one case-control study[26] and recently in our own cohort study[27] and a lower occurrence of one type of brain tumor,[28] to our knowledge, no previous study has shown a relationship between left-handedness and mortality due to cancer. Left-handedness was not related to CVD mortality in previous studies.[10;12;18] We could not examine the effect of left-handedness to injury related mortality due to a very small number of such events in our population. However, our study supports the hypothesis that a decreased number of left-handed people among the elderly might be caused by elimination due to higher susceptibility to certain diseases or higher case fatality among the left-handed and not due to incapability to adapt in the non left-handed world leading to elimination by accident related death.

If our observations about handedness and mortality are true there may be a number of possible explanations. People who are exposed to an adverse environment during fetal life or birth may have certain defects in their system, including 'atypical' laterality (left-handedness),[3] disease susceptibility (inadequate immune system),[3] or may develop unhealthy lifestyles such as smoking or alcoholism.[29] However, we did not find clues that lifestyle differences between the handedness groups explained our findings. So far, the most plausible genetic theories of handedness come from

Annett et al[2] and McManus et al,[1] which, however, do allow for a marked contribution from non-genetic factors to affect hand preference. There is no evidence so far suggesting that genes involved in hand preference also act as an underlying factor for susceptibility for certain adult diseases.

In our study, left-handedness was related to a wide variety of major diseases in humans, diseases with some mutual and some completely different causal mechanisms. As breast cancer constitutes a majority of cancer deaths in our cohort, it probably predominates the relationship between left-handedness and cancer mortality, although not statistically significantly in this study, maybe due to lack of statistical power. It has been suggested that breast cancer may be initiated in utero by high exposure to steroid hormones[30] that may also cause left-handedness.[3] For cerebrovascular diseases, the link may be even less straightforward. Nevertheless, as left-handedness is also more common in extremely low birth weight babies[31] and low birth weight was suggested to be related to higher cerebrovascular mortality[32], the link might lay in the common intrauterine environment.[6]

In summary the results of our study among 12,178 women followed for 13 years support the view that left-handedness is associated with higher mortality, especially due to cancer and cerebrovascular disease.

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Chapter 4.

General discussion

Introduction

In this thesis we have revisited hand preference as a marker for early life pathology in epidemiologic studies. We have explored some putative pathological causes of left-handedness, and links between and possible substrates for left-handedness and later life health outcomes. In the following we will focus our discussion of the findings in this thesis in view of the premises that formed our starting point and that were summarized in the introduction of this thesis.

The left-handedness distribution: is it partly pathological?

Originally, the term pathological left-handedness was reserved for hypothesized instances where cerebral lateralization was hampered by brain damage.[1] In chapter 2 of this thesis, we have indeed found that some causes of brain damage, like intra-ventricular haemorrhage or meningitis, are associated with left-handedness. Thus, while handedness is likely predominantly explained by normal variation of genes that are directly involved in lateralization mechanisms, our findings certainly indicate that part of the laterality distribution is pathological. Furthermore, the genetic background underlying the handedness distribution may not be restricted to hypothesized 'lateralization genes'. We have found a functional polymorphism of the IGF-1 gene to be involved in left-handedness. Thus, genes that one would not expect to be involved in the mechanisms of lateralization may actually have an influence. For none of these causes of left-handedness, brain damage, genes or any other causes, can we assert that they are pathological in the sense of having later life adverse health consequences. However, some of these causes are certainly pathological in the sense of being induced by external noxious influences. Therefore, we propose to use the term pathological for left-handedness that can be explained by particular noxious causes, and that occurs irrespective of constitutional lateralization preference.

In our thinking about handedness and later life health consequences, a broad concept of pathological left-handedness is important. In our studies about later life health outcomes we were not able to a priori discern pathological left-handedness from non-pathological left-handedness. This inability has probably resulted in misclassification which generally renders associations more difficult to detect. Consequently, if we adopt the concept of pathological left-handedness having later life consequences, the associations that we did find may in reality be much stronger had we been able to discriminate pathology from physiology.

Handedness and later life outcomes: true or confounded?

We set out from circumstantial prior evidence on possible associations between handedness and later life outcomes. The sheer variety of associations that we encountered forced us to choose from two basic assumptions:

1. that handedness is a marker for biological processes that are so central to life that any disturbances should indeed have consequences in a variety of health areas,

or,

2. that this variety of associated health issues reflects confounding.

Although we obviously followed the first assumption, we still need to address the second. In this thesis, we have found associations between handedness and several indices of psychological well-being, breast cancer incidence, cause-specific mortality. While the choice of these subjects was inspired by published work of other researchers, our data lend further support to the presence of an association between left-handedness and a range of adverse health outcomes. In our analyses relating handedness to outcome we have attempted to address the issue of confounding to the best of our ability. Overall and irrespective of whether we studied psychiatry, breast cancer or mortality, we could not demonstrate material effects of adjustment for putative confounders. In each of these studies we have attempted to comprehensively consider and address confounders of the relation between handedness and outcome. The typical line of thought is whether there are factors that are known risk factors for the disease under study, that may have some relation with the determinant, in our case handedness, and that are no intermediate factor in the chain of events involving this determinant and this disease. Thus, full confounding would mean that an observed unadjusted association between handedness and outcome is not true but is completely explained by some external factor(s), confounders, and there just is no chain of events involving handedness and outcome. Proper adjustment would reduce such spurious association to its null value. In our studies, we have used risk factors for various studied diseases as putative confounders. Since we knew that these were risk factors for disease, we have consistently attempted to find out whether such risk factors could be confounders by tabulating them against categories of the determinant, handedness. Those factors that did show differences by categories of handedness were subsequently considered in multivariate analyses. While this approach to adjustment is common practice in epidemiology, the distinction between confounders and intermediate factors is not always straightforward. The distinction should be based on thorough medical knowledge about the association studied. From the

variety of outcomes that were associated with handedness, one of the plausible propositions about causality could be that left-handedness is a marker of several different causal processes. If true, this markedly complicates selection and measurement of actual confounders and their distinction from intermediate factors. There is little left apart from the approach we took in our analyses, that is; scroll in the analysis for putative confounders among known and measured risk factors for the disease under study.

Handedness: a cause or a proxy of causes?

If our findings concerning handedness and later life outcome reflect the truth, are unconfounded, we should still not discard the possibility that the sheer use of left hands in a world dominated by right-handers is the cause of associations, rather than some yet unknown underlying process. We know that left-handedness may have advantages that have contributed to its persistence throughout evolution.[2] We also know about disadvantages of being left-handed, such as inability to fully adapt to a right-handed designed world, leading to higher accident proneness.[3-5]

We observed an association between handedness and various indices of psychopathology among healthy adults. It is not inconceivable that the consequences of being left-handed or having to change hand preference as a result of social pressure leads to psychological distress.[6] However, in the case of left-handedness being the primary relevant exposure for let us say depression, it would no longer concern only a subgroup of pathological left-handedness but the total group or at least a large proportion of left-handedness. Thus, depression should then affect larger proportions of left-handers than can currently be shown. It is quite inconceivable how social pressure against left-handedness or just being left-handed itself could be associated with 'harder' psychopathology such as schizophrenia and mania.

Breast cancer is 5% more likely to occur in the left breast than in the right breast and particularly in young women, below the age of 45 years.[7] It is therefore for instance possible that right-handed women have some advantage as to the manual self-detection of breast nodes. However, such advantage would be of only minor impact and it would in our view be difficult to explain our findings with regard to left-handedness and pre-menopausal breast cancer through such presumed right-handed detection advantage.

The question is whether we should adopt the view that pathological left-handedness could be involved in causing all kinds of later life disease. For some disease the disturbed cerebral

lateralization itself could play a role in its origin, for instance in the case of schizophrenia or mania or other psychopathology.[8;9] Some of the associations may be predominantly based on erroneous genetic mechanisms as proposed by some.[9-12] Alternatively, for other diseases such as for instance breast cancer, it seems more likely that intra-uterine exposure to high hormone levels are both the cause of disturbed lateralization and early life setting of breast cancer risk.[13] In the latter case it is not lateralization itself but the underlying process that is of eventual interest. Disturbed lateralization would then just be a co-phenomenon to be used as a marker for the underlying process. Over viewing the results described in this thesis, we take the view that for most associations between handedness and later life outcome, pathological left-hand preference is a proxy for as yet unknown underlying processes.

Cerebral lateralization: is manual motor preference a good measurement?

This thesis was largely based on existing data. In the data on cerebral intra-ventricular haemorrhage and meningitis as possible causes of left-handedness, the outcome, handedness was in the original studies assessed as a component of the measurement of neurological development. The studies were not specifically designed for questions concerning cerebral lateralization. Therefore, such lateralization was predominantly measured using questions and observations about manual motor preference of these children only. In the studies on later life outcomes associated with handedness, breast cancer and mortality, handedness of participating women was inquired for at the start of the cohort, now some 20 years ago. The question about innate manual preference was at that time added to the battery of measurements for reasons immediately reflecting the hypotheses described in the present thesis. One of our co-authors, Dr. P.A.H. van Noord, was inspired in the early 1980's listening to a presentation of Dr. Norman Geschwind, a neurologist at Harvard University, in Boston, USA. Dr. Geschwind, who died in 1984, spoke at that time about a hypothesis that intra-uterine exposure to high steroid hormones, particularly testosterone, could induce excess left-handedness, a hypothesis that is now known as the Geschwind (Behan) Galaburda theory.[14] Obviously, hand preference was not the determinant of sole interest in our cohort. It can often not be avoided in large scale epidemiological cohort studies that choices in baseline determinant measurements have to be made. For that reason, cerebral lateralization was measured using only one question about innate manual motor preference. In the Atherosclerosis Risk in Young Adults (ARYA) study, we were able to

specifically add measurements of cerebral lateralization in a limited group of young women using a dedicated questionnaire to measure an array of manifestations of cerebral lateralization.[15]

Thus, in the majority of studies of this thesis we had to rely on a simple and crude measure of cerebral lateralization. This surely has been a source of lateralization misclassification. However, in spite of these inherent limitations the data nonetheless offer support for most of the associations we hypothesized at the start of our work. This further supports the view that the associations, if true, are strong and the current data have led to underestimations.

Cerebral lateralization: a view to the future

Decades after first postulating his fetal origins for adult disease hypothesis, Prof. David Barker very recently showed that those with low birth weight and rapid weight gain as children are at the greatest risk for coronary artery disease.[16] Indeed, while that hypothesis has met ferocious professional opposition from its first postulation onwards, it is now more generally accepted that early life adverse environments may influence chronic disease risk in later life.[17]

However, studies on associations between handedness and disease outcomes can still be subject to heavy dispute both among health professionals, the press, and the lay public. This is exemplified by the heavy public discussion of our findings relating left-handedness to pre-menopausal breast cancer (see appendix). Such discussion touches on anxiety deemed unnecessary,doubts about the plausibility of findings, doubts about practical usefulness, and doubts about validity. This is probably a reflection of the current inability to specify the general left-handedness that everybody can relate to, towards the pathological left-handedness that we think is the exposure of interest. This inability is highly comparable to having to resort to low birth weight as an imperfect and markedly misclassified measure for adverse intra-uterine environment in relation to later disease.[17] Each of us have one form of the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene, while ACE-inhibition is known for its role in hypertension lowering and cardiovascular disease protection.[18] Few would be much bothered about their particular individual polymorphism. Few also, would have expected this polymorphism to be associated with breast cancer risk, even though the investigators had a plausible a priori hypothesis about the mechanisms and presumed actions of Angiotensin II on angiogenesis and growth in breast tissue.[19] The scientific reasoning behind our research on cerebral lateralization and disease is not much different from studying birth weight and disease or genetic polymorphisms and disease. While

probably few would question the usefulness of studying genes in association with disease, the direct therapeutic applicability of genetic findings is likely to be as remote as for findings about cerebral lateralization and disease. Moreover, findings like these may have practical implications as disease risk indicators that are less remote. The relevance of revisiting pathological left-handedness is its potential source of knowledge to direct further etiological research.

Further research should concentrate on possible mechanisms behind the occurrence of what we define in this thesis to be pathological left-handedness. Such mechanisms might sometimes even be iatrogenic as suggested by the findings of increased left-handedness as a side effect of ultrasound scanning in gestation,[20] a suggestion not immediately discarded by the current leaders in epidemiology.[21] Research into the causes of pathological left-handedness, given our definition, will have to be of a pluriform nature. Advances in techniques for measuring hormonal systems, cerebral imaging, genetics, proteomics, and metabolomics should provide more clues as to what the exact mechanisms behind pathological cerebral lateralization are. Such mechanisms most likely exert their influence in early life. Research in obstetrics and paediatrics, in concert with basic and imaging sciences and epidemiology, will expectedly have to play a pivotal role in the further elucidation of such mechanisms. Such knowledge will allow for more focus on subgroups of left-handers that according to the findings in our thesis are at an increased disease risk as compared to their normal left or right-handed counterparts.

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Appendix

Some critical notes and authors' replies related to handedness and breast cancer published at BMJ.

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Ramadhani and colleagues present the results of the prospective study analysing the contribution of innate hand preference, amongst a number of other factors, to the development of breast cancer.[1] They conclude that there is an association between innate left-handedness and the development of pre-menopausal breast cancer. The mechanism by which this occurs has not been established, although the authors speculate that the origin of the association may lie in intrauterine exposure to steroid hormones. Rather than being used as evidence for causality, the authors should consider whether the link between both handedness and the incidence of breast cancer and a common third variable (in-utero sex hormone exposure) indicates that the apparent association is nothing more than a spurious correlation.[2] Until this has been addressed and controlled for, their conclusion that left-handedness is related to increased risk of breast cancer is not valid.

1. Ramadhani MK, Elias SG, van Noord PAH, Grobbee DE, Peeters PHM, Uiterwaal CSPM. Innate left-handedness and risk of breast cancer: case- cohort study. *BMJ* 2005;331:882-3.

2. Stigler SM. Correlation and causation: a comment. Perspect Biol Med 2005;48:S88-S94.

Authors' reply

Bloor argues that the association between handedness and breast cancer may be spurious because, if we understand it correctly, the mechanisms behind such association are not established.

First, it is important to take notice of the order of events. We did not first find an association and only then started speculating about mechanisms. As clearly indicated in our paper, there was first a hypothesis about in-utero influences, including exposure to steroids, and breast cancer risk.[1] Like the group that proposed this hypothesis,[2] we have used handedness as a putative marker for such early life influences. As we did find an association which is obviously not directly causal, we subsequently speculated that the original hypothesis might underlie it.

Second, it is important to distinguish speculations from conclusions. We speculated that our findings may support other researchers' hypothesis. Our conclusion is restricted to the observation that we did, cautious in our view, and without any reference to causality. The validity of our conclusion that left-handedness is related to increased risk of breast cancer does not depend on speculations about putative mechanisms, but on whether the observation is correct, unbiased. We will consider our findings spurious only if we are shown that flaws in our study design, conduct or analysis have led us to report an association that actually does not exist. So far, we have not encountered such critics.

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2. Titus-Ernstoff L, Newcomb PA, Egan KM, Baron JA, Greenberg ER, Trichopoulos D et al. Left-handedness in relation to breast cancer risk in postmenopausal women. *Epidemiology* 2000;11:181-4.

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Ramadhani et al. fail to tell us in their article what implications for the management of breast cancer (if any) their results will have. The incidence of left-handedness in the general population is about 11.5% (10- 11% of women and about 13% of men are left-handed).[1] The incidence for breast cancer is 1:8 over a lifetime for a women, 1:2,212 at the age of 30, 1:235 at the age of 40, 1:54 at the age of 50 and 1:23 at the age of 60.[2] Screening for breast cancer is currently available in the UK for females >50 years of age by means of mammography, an investigation not routinely performed under the age of 35. Given the small numbers of women actually effected by the results of Ramadhani et al.'s study it appears that it will not be necessary to invent a new screening tool for this "population at risk".

The study does further not mention any impact of family history on the results. Have the authors checked for hereditary breast cancer (BRCA1 and BRCA2 gene carriers) and familial breast cancer?

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Authors' reply

Lieske rightfully inquires about the implications of the association between left-handedness and breast cancer for the management of the disease. We did indeed avoid any reference to practical implications because we feel that our findings at present do not allow for such thinking. The main reasons are given below.

Our study was intended as an etiological study aiming at another scope of causes of breast cancer.[1] We used handedness as a possible marker of such causes. There have been other studies on the relation between hand preference and breast cancer which have not all been consistent. Therefore, we consider this research to be in the stage of confirmation rather than application.

Consequently to our intentions, our analysis was focused exclusively on hand preference as the determinant and breast cancer as the outcome, while accounting for other breast cancer risk factors as possible confounders. Our purpose was particularly not to assess the (additional) predictive value of hand preference in conjunction with other breast cancer predictors.[2] When thinking about selective screening for breast cancer there are many predictors to take into account for individual women, with an as yet unknown contribution of hand preference. As Lieske correctly indicates, the relation with left-handedness was with pre-menopausal breast cancer. The absolute risk for such cancer is low and although we did no formal analysis, we anticipate no role for handedness as a predictor of that risk to justify its use as a breast cancer risk screening tool. We did account for family history (in mother or sister) in the analysis as mentioned in the table legend of our paper and this did not influence our findings. We did not consider hereditary breast cancer, which accounts for only a small proportion of all cases of breast cancer. Specifically, we did not consider it a confounder in the association that we studied.

Summary

Investigations to explore human handedness have been conducted for ages. It is still uncertain what causes people to use the left or the right hand. Clues supporting the genetic origin of handedness have been accumulating and provide the best evidence. Nevertheless, in some populations there are added proportions of left-handedness that do not seem to be explained solely by genes. The term pathological left-handedness has emerged around 30 years ago, referring to some right-handers who became left-handed due to an early life brain insult. This thesis is an attempt to revisit pathological left-handedness, exploring some of its origins and possible later life health outcomes.

In this thesis, indicators of early life brain damage seemed to be related to a higher risk of becoming left-handed. Chapter 2.1 was based on a cohort of prematurely born children in an academic hospital in Utrecht, the Netherlands, who had been followed up for 8 years. As neonates, these children underwent serial cranial ultrasound (US) examinations to determine brain lesions. At school age (median age 8 years), these children were asked for their hand preference, as well as being observed while doing certain manual tasks. Children with severely abnormal findings had an increased chance to become left-handed compared to those with normal US findings: odds ratio (OR) 4.1, 95% confidence interval (CI) 1.6 to 10.0, p=0.003. Findings were mainly attributable to Intra Ventricular Haemorrhage (IVH). Children with left-sided IVH showed a higher chance for left-handedness compared to those without IVH (OR 4.4, 95% CI 1.7 to 11.3, p=0.002), whereas right-sided IVH did not. Furthermore, neonates with left-sided mild IVH (grade I and II) still showed an increased chance for left-handedness: OR 4.0, 95% CI 1.5 to 10.9, p=0.007. The results indicate that even a small intraventricular haemorrhage affecting the left side of the brain may induce left-handedness. This is likely related to the role of the subependymal germinal matrix in the developing brain.

In chapter 2.2, we investigated whether brain insults, such as bacterial meningitis, occurring in early childhood, may increase the chance of children to become left-handed. We studied the association between a meningitis severity score, derived from clinical and laboratory signs and symptoms which were previously reported to be predictive of more severe bacterial meningitis, and the chance of becoming left-handed. Furthermore, we also studied whether the left-handed children had a different neuropsychological, hearing, and motor skill performance at school age compared to the right- handed children. This study was performed in a cohort of Dutch children followed up for 7.4 years from the age when bacterial meningitis was first diagnosed until reaching school age (mean age 9.7). Fifteen percent were left-handed. Severity of childhood bacterial meningitis was related to left-handedness (OR 6.2, 95% CI 2.0 to 18.6 for those with a total severity score above the median

compared to those below). Compared to non left-handed children, left-handed children had a lower IQ (mean difference - 6.6, 95% CI -12 to -1.2), a lower vocabulary score of WISC-r (mean difference - 1.0, 95% CI -2.1 to 0), and a lower Beery score on visual-motor integration (mean difference – 4.9, 95% CI -10.1 to 0.4). Left-handed children also had more combined academic and behavioural limitations (OR 2.7, 95% CI 0.9 to 8.6), lower manual speed of the dominant hand (mean difference - 9 taps, $p < 0.05$) and better manual steadiness in the non-dominant hand (mean difference of contact's time -2.7 second, $p<0.05$). Our results support the role of early life brain damage in left-handedness. Left-handed post-meningitic children generally have worse neurodevelopmental outcome than non left-handed survivors.

In chapter 2.3 we studied whether a functional polymorphism in the promoter area of IGF-1 gene and left-handedness are related, as a possible explanation for associations found between handedness and breast cancer. This cross-sectional study was performed in a birth cohort of young adult women who were included from the Utrecht area. The polymorphism was determined at first inclusion in young adulthood and handedness was measured using the Edinburgh inventory 4 years after inclusion. Using the most common 192-bp and 194-bp alleles with the highest associated IGF-1 levels as the reference, having long alleles (>194-bp) was inversely related to left-handedness (OR 0.3, 95% CI 0.1 to 0.7). Compared to right-handed women, left-handed women were more likely to be homozygous for 192-bp, less likely to be heterozygous 192-bp, and more likely to be non carrier of 192-bp ($p = 0.035$). Left-handed women appear to have a shifted allele distribution in the promoter region of the IGF-1 gene compared to right-handed women. Subgroups of left-handed women may therefore have IGF-1 genes that are compatible with higher circulating IGF-1 levels.

In chapter 3.1, we studied the association between left-handedness and breast cancer incidence in middle age women. We used a cohort of a breast cancer screening program in Utrecht, the Netherlands. At inclusion, these women were asked for their innate hand preference and from then onwards they were followed for the occurrence of breast cancer for 16 years. Data on demography and reproductive history were obtained using a questionnaire at inclusion. Of the random sample, 11.5% reported to be left-handed in early childhood. The risk for breast cancer was 39% higher in the left-handed group (hazard ratio (HR) 1.39; 95% CI 1.09 to 1.81). The risk was 2.41 when the cancer was premenopausal, but there was no excess risk for postmenopausal cancers. We found an excess risk in left-handed women with a BMI $\leq$ 25 kg/m2 (HR 1.62; 95% CI 1.17 to 2.24 – *P* for interaction between handedness and BMI: 0.07), as well as in women who gave birth to at least one child (HR 1.58; 95% CI 1.19 to 2.11 – *P* for interaction between handedness and parity: 0.02),

but not in those whose BMI was >25 kg/m^2 and who were nulliparous. Adjustment for potential confounders did not change these results. Handedness seemed not to be associated with the laterality of breast cancer. These results support the hypothesis that left-handedness is related to increased premenopausal breast cancer risk.

In chapter 3.2 we investigated the association between left-handedness, depression, and diseases proneness. We used a cohort of adult men and women of whom at inclusion data on demography and handedness were assessed by questionnaires. Approximately 4 years afterwards, these participants were asked to fill out standardized questionnaires in the psychiatric domain and also data on CIDI (Composite International Diagnostic Interview) based depression were obtained. Of the total cohort, 10.9% reported to be left-handed. Left-handers had a higher risk of psychological distress in adult life (OR 1.8, 95% CI 1.1 to 2.9), a diagnosis of moderate depression (OR 2.3, 95% CI 1.0 to 5.4), for higher perceived disease proneness (OR 2.2; 95% CI 1.1 to 4.5) and to have >2 treated chronic illnesses (OR 1.8, 95% CI 0.9 to 3.6), than right-handers. The results remained after adjusting for age and gender. These results support the hypothesis that left-handedness may be related to proneness to psychological distress and physical diseases.

In order to investigate a question about left-handedness and mortality risk that has been much debated in the past, we used the same cohort that we used in chapter 3.1. These women were followed up for the outcome for almost 13 years from inclusion (chapter 3.3). During a median follow-up of 12.6 years, 252 women died. Hazard ratios comparing left-handed women to non left-handed women were 1.4 for all-cause mortality (95% CI 0.9 to 2.0) , 1.7 for total cancer mortality (95% CI 1.0 to 2.7), 2.0 for breast cancer mortality (95% CI 0.8 to 4.6), 4.6 for colorectal cancer mortality (95% CI 1.5 to 14.3), 1.3 for cardiovascular diseases mortality (95% CI 0.5 to 3.3), and 3.7 for cerebrovascular mortality (95% CI 1.1 to 12.1) after adjusting for potential confounders (socio-economic status, age, body mass index and cigarette smoking status at study recruitment).

The main results of the above studies are reviewed and discussed in chapter 4. Further discussion about the clinical relevance, public debates and suggestions for future research is also presented in this chapter. We have shown that, while handedness is likely predominantly explained by normal variation of genes that are directly involved in lateralization mechanisms, our findings do indicate that part of the laterality distribution is pathological. For none of these causes of left-handedness, brain damage, genes or any other causes, can we assert that they are pathological in the sense of having later life adverse health consequences. However, some of these causes are certainly pathological in the sense of being induced by external noxious influences. The sheer variety

of associations that we encountered may indicate that handedness is a marker for biological processes that are so central to life that any disturbances should indeed have consequences in a variety of health areas, or, that this variety of associated health issues reflects confounding.

From the variety of outcomes that were associated with handedness, one of the plausible propositions about causality could be that left-handedness is a marker of several different causal processes. For some disease the disturbed cerebral lateralization itself could play a role in its origin, for instance in the case of schizophrenia or mania or other psychopathology. Alternatively, for other diseases such as for instance breast cancer, it seems more likely that intra-uterine exposure to high hormone levels are both the cause of disturbed lateralization and early life setting of breast cancer risk. Overviewing the results described in this thesis, we take the position that for most associations between handedness and later life outcome, pathological left-hand preference is a proxy for as yet unknown underlying processes.

In conclusion, indicators of early life brain damage seemed to be related to a higher risk of becoming left-handed. Furthermore, being left-handed seemed to increase the risk of getting premenopausal breast cancer, psychological and physical distress, and mortality, especially due to cancer and cerebrovascular diseases. Studying handedness, which is only one aspect of overall laterality, is a complex process. The results of the studies in this thesis should be an inspiration for further research into the concept of pathological left-handedness.

Samenvatting

Onderzoek naar handvoorkeur van de mens bestaat al eeuwen. Het is nog steeds niet precies bekend wat precies de oorzaak is van het gebruik van de linker dan wel de rechter hand. De krachtigste aanwijzingen geven een genetische oorzaak voor handvoorkeur aan. Niettemin is in sommige populaties een deel van de linkshandigheid niet alleen maar op basis van genen te verklaren. De term pathologische linkshandigheid is ongeveer 30 jaar geleden voor het eerst gebruikt voor de theorie dat sommige rechtshandige kinderen linkshandig kunnen worden als gevolg van een hersenbeschadiging vroeg in het leven. Dit proefschrift is een poging om deze pathologische linkshandigheid verder uit te diepen door oorzaken en mogelijke uitkomsten later in het leven te bestuderen.

In dit proefschrift waren indicatoren van vroege hersenschaden gerelateerd aan een hogere kans om linkshandig te worden. Hoofdstuk 2.1 was gebaseerd op een cohort van prematuur geboren kinderen in een academisch ziekenhuis in Utrecht in Nederland, die waren gevolgd gedurende 8 jaar. Als neonaten ondergingen deze kinderen herhaalde ultrageluidonderzoeken van de hersenen om hersenbeschadigingen aan te tonen. Op schoolleeftijd (mediaan 8 jaar), werd deze kinderen gevraagd naar hun handvoorkeur alsmede geobserveerd terwijl zij bepaalde taken met de hand uitvoerden. Kinderen met ernstige afwijkende bevindingen hadden een toegenomen kans om linkshandig te worden vergeleken met kinderen met normale scans: odds ratio (OR) 4.1; 95% betrouwbaarheidsinterval (BI) 1.6 tot 10.0, p=0.003. De bevindingen konden vrijwel geheel worden toegeschreven aan intraventriculaire bloedingen. Kinderen met linkszijdige bloedingen hadden een hogere kans op linkshandigheid dat kinderen zonder bloedingen (OR 4.4; 95% BI 1.7 tot 11.3, p=0.002), terwijl rechtzijdige bloedingen geen verband hielden met linkshandigheid. Verder hadden neonaten met linkszijdige milde intraventriculaire bloedingen (graad I en II) een verhoogde kans op linkshandigheid: OR 4.0; 95% BI 1.5 tot 10.9, p=0.007. Deze resultaten geven aan dat een kleine intraventriculaire bloeding aan de linker zijde van de hersenen linkshandigheid kunnen veroorzaken. Dit heeft waarschijnlijk te maken met de rol van de subependymale germinale matrix in de zich ontwikkelende hersenen.

In hoofdstuk 2,2, hebben we onderzocht of hersenbeschadiging, met name bacteriële meningitis die ontstond op vroege kinderleeftijd, de kans op linkshandigheid kon verhogen. Wij bestudeerden het verband tussen een meningitis ernst score, afgeleid van klinische en laboratorium bevindingen en symptomen waarvan eerder werd gerapporteerd dat ze voorspellend zijn voor meer ernstige bacteriële meningitis, en de kans op linkshandigheid. Verder bestudeerden we of linkshandige kinderen verschillen presteerden op neuropsychologisch terrein, het gehoor en

motorische vaardigheden op de schoolleeftijd vergeleken met rechtshandige kinderen. Deze studie werd uitgevoerd in een cohort van Nederlandse kinderen die gevolgd waren gedurende 7.4 jaar vanaf de leeftijd waarop de bacteriële meningitis het eerst werd gediagnosticeerd tot op de schoolleeftijd (gemiddelde leeftijd 9.7 jaar). Vijftien procent was linkshandig. De ernst van de bacteriële meningitis was gerelateerd aan linkshandigheid (OR 6.2, 95% BI 2.0 tot 18.6 voor degenen met een totale meningitis ernst score boven de mediaan vergeleken met degenen onder de mediaan). Vergeleken met niet-linkshandige kinderen hadden linkshandige kinderen een lager IQ (gemiddeld verschil - 6.6; 95% BI -12 tot -1.2), een lagere vocabulaire score op de WISC-r (gemiddeld verschil -1.0; 95% BI -2.1 tot 0), en een lagere Beery score voor visueel-motorische integratie (gemiddeld verschil – 4.9; 95% BI -10.1 to 0.4). Linkshandige kinderen hadden ook meer gecombineerde leer- en gedragsproblemen (OR 2.7; 95% BI 0.9 tot 8.6), lagere snelheid van de dominante hand (gemiddeld verschil -9 tikken, $p < 0.05$), en betere vastheid in de niet-dominante hand (gemiddelde verschil in contact tijden -2.7 seconden, $p<0.05$). Onze resultaten ondersteunen een rol voor vroege hersenschade in linkshandigheid. Linkshandige kinderen die een bacteriële meningitis overleefden hebben een minder goede ontwikkeling dan niet-linkshandige kinderen die een dergelijke infectie overleefden.

In hoofdstuk 2.3 bestudeerden we of een functioneel polymorfisme in het promotor gebied van het IGF-1 gen gerelateerd is aan linkshandigheid, als een mogelijke verklaring voor verbanden die we vonden tussen handvoorkeur en borstkanker. Deze cross-sectionele onderzoek werd uitgevoerd in een geboortecohort van jonge volwassen vrouwen die waren geïncludeerd vanuit Utrecht en omgeving. Het polymorfisme werd bepaald bij de eerste inclusie op jong volwassen leeftijd en handvoorkeur werd gemeten met behulp van de Edinburgh schaal 4 jaar na inclusie. Bij gebruik van de meest voorkomende 192-bp en 194-bp allelen met de hoogst geassocieerde IGF-1 niveaus als de referentie, was het hebben van lange allelen (>194-bp) omgekeerd gerelateerd aan linkshandigheid (OR 0.3, 95% BI 0.1 tot 0.7). Vergeleken met rechtshandige vrouwen hadden linkshandige vrouwen een hogere kans om homozygoot voor 192-bp te zijn, minder kans om heterozygoot voor 192-bp te zijn, en meer kans om niet-drager van 192-bp te zijn ($p = 0.035$), Linkshandige vrouwen lijken een verschoven allelverdeling te hebben in het promotor gebied van het IGF-1 gen vergeleken met rechtshandige vrouwen. Subgroepen van linkshandige vrouwen zouden daarom IGF-1 genen kunnen hebben die compatibel zijn met hogere circulerende IGF-1 niveaus.

In hoofdstuk 3.1, bestudeerden we het verband tussen linkshandigheid en de incidentie van borstkanker in vrouwen van middelbare leeftijd. We gebruikten een cohort op basis van een

borstkanker screeningsprogramma in Utrecht, Nederland. Bij inclusie was aan deze vrouwen gevraagd naar hun aangeboren handvoorkeur en werden ze gevolgd op het ontstaan van borstkanker over een periode van 16 jaar. Demografische gegevens en gegevens over de reproductieve voorgeschiedenis werden verkregen met een vragenlijst bij inclusie. In een random steekproef was 11.5% gerapporteerd als linkshandig. Het risico op borstkanker was 39% hoger in de linkshandige groep (hazard ratio (HR) 1.39; 95% BI 1.09 tot 1.81). Het relatieve risico was 2.41 voor premenopausale borstkanker, maar er was geen toegenomen risico voor postmenopausale borstkanker. We vonden een verhoogd risico in linkshandige vrouwen met een body mass index (BMI) $\leq$ 25 kg/m2 (HR 1.62; 95% BI 1.17 tot 2.24 – *P* voor interactie tussen handvoorkeur en BMI: 0.07), alsmede in vrouwen die tenminste een kind kregen (HR 1.58; 95% BI 1.19 to 2.11 – *P* voor interactie tussen handvoorkeur en pariteit: 0.02), maar niet in vrouwen met een BMI >25 kg/m^2 en die nooit kinderen kregen. Correctie voor potentiële confounders veranderde deze resultaten niet. Handvoorkeur leek niet gerelateerd aan lateraliteit van de borstkanker. Deze resultaten ondersteunen de hypothese dat linkshandigheid is gerelateerd aan een verhoogd risico op premenopausale borstkanker.

In hoofdstuk 3.2 onderzochten we het verband tussen linkshandigheid, depressie en vatbaarheid voor ziekte. We gebruikten een cohort van volwassen mannen en vrouwen van wie bij inclusie demografische gegevens en gegevens over handvoorkeur beschikbaar waren uit vragenlijsten. Ongeveer 4 jaar nadien werd deze deelnemers gevraagd om standaard vragenlijsten in te vullen in het domein van de psychiatrie en verder werden gegevens over depressie verkregen met behulp van de CIDI (*Composite International Diagnostic Interview*). Van het totale cohort rapporteerde 10.9% linkshandig te zijn. Linkshandigen hadden een hoger risico op psychologische problematiek op volwassen leeftijd (OR 1.8; 95% BI 1.1 tot 2.9), op een diagnose van milde depressie (OR 2.3; 95% BI 1.0 tot 5.4), op een hogere ervaren ziektevatbaarheid (OR 2.2; 95% BI 1.1 tot 4.5) en op het hebben van > 2 behandelde chronische ziekten (OR 1.8; 95% BI 0.9 tot 3.6), dan rechtshandigen. Deze resultaten bleven bestaan na correctie voor leeftijd en geslacht. Deze resultaten ondersteunen de hypothese dat linkshandigheid gerelateerd kan zijn aan vatbaarheid voor psychoproblematiek en lichamelijke ziekten.

Om het verband tussen linkshandigheid en sterfterisico te onderzoeken, hetgeen in het verleden vaak onderwerp van veel debat is geweest, gebruikten we hetzelfde cohort dat is beschreven in hoofdstuk 3.1. Deze vrouwen werden gevolgd gedurende bijna 13 jaar vanaf inclusie (hoofdstuk 3.3). Gedurende een mediane follow-up duur van 12.6 jaar stierven 252 vrouwen. Hazard

ratios die linkshandige vrouwen vergelijken met rechtshandige vrouwen waren 1.4 voor sterfte door alle oorzaken (95% BI 0.9 tot 2.0) , 1.7 voor totale kanker sterfte (95% BI 1.0 tot 2.7), 2.0 voor borstkanker sterfte (95% BI0.8, 4.6) tot 4.6 voor colorectaal kanker sterfte (95% BI 1.5 tot 14.3), 1.3 voor sterfte aan hart- en vaatziekten (95% BI 0.5 tot 3.3) en 3.7 voor cerebrovasculaire sterfte (95% BI 1.1 tot 12.1) na adjustering voor potentiële confounders (sociaal-economische status, leeftijd, body mass index en roken bij inclusie).

De belangrijkste resultaten van de bovengenoemde studies worden besproken en bediscussieerd in hoofdstuk 4. Verdere discussie over de klinische relevantie, publieke debatten en suggesties voor toekomstig onderzoek worden ook in dat hoofdstuk gegeven. Wij hebben laten zien, terwijl handvoorkeur vooral wordt bepaald door normale variatie in genen die direct zijn betrokken in mechanismen van lateralisatie, dat een deel van de lateralisatie verdeling pathologisch is. Voor geen van deze oorzaken van linkhandigheid, hersenbeschadiging, genen of welke andere oorzaak dan ook, kunnen wij op dit moment stellen dat ze ook pathologische betekenis hebben met betrekking tot gezondheidsuitkomsten later in het leven. Echter, sommige van de gepresenteerde oorzaken zijn zeker pathologisch in de zin dat ze zijn geïnduceerd door externe schadelijke invloeden. De veelheid aan associaties die wij tegenkwamen kunnen een indicatie zijn dat linkshandigheid een marker is voor biologische processen die zo centraal zijn in het leven dat elke verstoring daarvan inderdaad consequenties heeft in een breed gezondheidsbereik. Anderzijds, kan deze variatie aan verbanden betekenen dat hier sprake is van confounding.

Ook kan een plausibele verklaring zijn dat linkshandigheid een marker is voor verschillende causale processen. Voor sommige ziekten kan de verstoorde cerebrale lateralisatie zelf een oorzakelijke rol spelen, bijvoorbeeld in het geval van schizofrenie of manie of andere psychopathologie. Anderzijds, zoals bijvoorbeeld bij borstkanker, lijkt het meer waarschijnlijk dat intrauteriene blootstelling aan hoge hormoonniveaus ten grondslag ligt aan zowel de verstoorde lateralisatie en het risico op borstkanker. Op basis van alle resultaten in dit proefschrift, gaan wij uit van de stelling dat voor de meeste associaties tussen handvoorkeur en latere uitkomsten, pathologische linkshandigheid een proxy is voor tot op heden onbekende processen.

Concluderend lijken indicatoren van vroege hersenschade gerelateerd aan een verhoogde kans om linkshandig te worden. Verder, verhoogt linkshandigheid de kans op het krijgen van premenopausale borstkanker, bepaalde psychoproblematiek, en sterfte vooral door (sommige) kanker en cerebrovasculaire aandoeningen. Het bestuderen van handvoorkeur, als slechts een aspect van totale lateralisatie, is een complex proces. De resultaten van de studies in dit proefschrift

zouden een inspiratie moeten zijn voor verder onderzoek naar het concept van pathologische linkshandigheid.

Ringkasan

Penelitian untuk mengeksplorasi perilaku manusia yang berkaitan dengan penggunaan tangan dalam kehidupan sehari-hari telah dilakukan selama bertahun-tahun. Walaupun demikian apa yang menyebabkan orang menggunakan tangan kiri atau tangan kanan masih belum jelas. Telah banyak bukti dan petunjuk ilmiah telah terdokumentasikan yang mendukung peranan genetik terhadap perilaku penggunaan tangan. Namun, pada populasi tertentu terdapat sebagian dari mereka yang kidal yang tidak dapat dijelaskan hanya oleh peranan faktor genetik. Istilah kidal patologis muncul kira-kira sejak tiga puluh tahun yang lalu, mengacu kepada riwayat sebagian pengguna tangan kanan yang kemudian menjadi kidal karena mengalami gangguan otak pada awal kehidupan mereka. Tesis ini dibuat sebagai upaya untuk mengkaji kembali kidal patologis, mengeksplorasi beberapa kemungkinan penyebab dan pengaruh serta dampak yang mungkin timbul pada kehidupan selanjutnya.

Dalam tesis ini, ditemukan bahwa adanya indikator kerusakan otak pada awal kehidupan seseorang nampaknya berhubungan dengan tingginya resiko untuk menjadi kidal. Uraian pada bab 2.1 didasarkan pada sebuah studi kohort dari anak-anak yang lahir prematur di sebuah rumah sakit pendidikan di Utrecht, Belanda, yang diamati selama delapan tahun. Sebagai neonatus, anak-anak ini menjalani serangkaian pemeriksaan ultrasonografi kranial untuk mengetahui adanya lesi pada otak. Pada usia sekolah (median umur 8 tahun), terhadap anak-anak ini ditanyakan tentang tangan mana yang mereka pilih untuk digunakan, dan pada saat yang sama mereka diamati pada waktu melakukan tugas-tugas tertentu yang menggunakan tangan. Anak-anak dengan temuan lesi otak yang berat, mempunyai kemungkinan menjadi kidal lebih tinggi daripada mereka dengan hasil ultrasonografi normal: *odds ratio* (OR) 4,1; 95% interval kepercayaan (IK) 1,6 sampai 10; p=0,003. Kelainan-kelainan yang ditemukan kebanyakan disebabkan oleh Perdarahan Intra Ventrikular (PIV). Anak-anak dengan PIV pada sisi kiri menunjukkan kecenderungan menjadi kidal yang lebih tinggi dibandingkan dengan mereka tanpa PIV (OR 4,4; 95% IK 1,7 sampai 11,3; p=0,002), sedangkan PIV pada sisi kanan tidak menyebabkan hal yang sama. Selanjutnya, neonatus dengan PIV ringan pada sisi kiri pun (tingkat I dan II) masih menunjukkan kemungkinan untuk menjadi kidal lebih tinggi: OR 4,0; 95% IK 1,5 sampai 10,9; p=0,007. Hasil-hasil penelitian tersebut menunjukkan bahwa sekalipun PIV yang kecil pada sisi otak bagian kiri masih mungkin menyebabkan kidal. Hal ini sepertinya erat hubungannya dengan peranan dari *subependymal germinal matrix* pada otak yang sedang berkembang.

Pada bab 2.2, kami menyelidiki apakah kelainan pada otak, seperti meningitis bakterial, yang terjadi pada awal masa kehidupan anak, dapat meningkatkan kemungkinan anak tersebut

untuk menjadi kidal. Kami juga mempelajari hubungan antara skor yang dipakai untuk menilai berat ringannya meningitis dengan kemungkinan untuk menjadi kidal. Nilai skor ini didapat dari kumpulan tanda dan gejala klinis serta laboratorium yang sebelumnya dipakai untuk memprediksi meningitis bakterial yang lebih parah. Selanjutnya, kami juga mempelajari apakah anak-anak kidal memiliki kemampuan neuropsikologis, pendengaran, dan kemampuan motorik yang berbeda pada usia sekolah dibandingkan dengan anak-anak yang menggunakan tangan kanan. Penelitian ini dilakukan pada sebuah kohort yang terdiri dari anak-anak berkebangsaan Belanda yang diamati selama 7,4 tahun sejak usia di saat meningitis bakterial didiagnosis untuk pertama kalinya sampai anak tersebut mencapai usia sekolah (usia rata-rata 9,7 tahun). Didapatkan 15% dari anak-anak ini kidal. Berat ringannya meningitis bakterial pada anak mempunyai hubungan dengan anak tersebut menjadi kidal (OR 6,2; 95% IK 2,0 sampai 18,6 untuk mereka dengan skor total lebih dari median dibandingkan dengan mereka yang memiliki skor total di bawah median). Dibandingkan dengan anak-anak yang tidak kidal, anak-anak kidal memiliki IQ lebih rendah (perbedaan nilai rata-rata -6,6; 95% IK–12 sampai -1,2), skor kosakata WISC-r (perbedaan nilai rata-rata -1,0; 95% IK -2.1 sampai 0), dan skor *Berry* untuk kemampuan integrasi visual motorik lebih rendah (perbedaan nilai rata-rata -4,9; 95% IK -10,1 sampai 0,4). Anak-anak kidal juga memiliki keterbatasan-keterbatasan, baik akademis maupun perilaku (OR 2,7; 95% IK 0,9 sampai 8,6), kecepatan tangan dominan yang lebih rendah (perbedaan nilai rata-rata -9 ketukan, $p<0,05$) dan kestabilan tangan non dominan yang lebih baik (perbedaan nilai rata-rata dari waktu kontak -2,7 detik, $p<0,05$). Hasil-hasil penelitian kami ini mendukung peranan kerusakan otak pada awal kehidupan sebagai penyebab anak menjadi kidal. Anak kidal pasca meningitis secara umum menunjukkan hasil perkembangan neurologis yang lebih buruk dibandingkan dengan anak yang tidak kidal.

Dalam bab 2.3 kami mempelajari apakah ada hubungan antara polimorfisme fungsional pada area promoter gen IGF-1 dengan kecenderungan menjadi kidal, sebagai suatu upaya untuk menjelaskan hubungan yang ditemukan antara kidal dan kanker payudara. Penelitian *cross-sectional* ini dilakukan pada sebuah kohort kelahiran dari wanita-wanita dewasa muda yang lahir di Utrecht. Polifermisme segera ditentukan saat pertama mereka dimasukkan sebagai subyek penelitian di usia dewasa muda, dan perilaku penggunaan tangan diukur menggunakan perangkat kuesioner Edinburgh, 4 tahun kemudian. Menggunakan *allel* yang paling umum yaitu 192-bp dan 194-bp yang menghasilkan kadar IGF-1 tertinggi sebagai acuan, memiliki *allel* panjang (<194-bp) berhubungan terbalik dengan kekidalan (OR 0,3; 95% IK 0,1 sampai 0,7). Dibandingkan dengan wanita-wanita yang menggunakan tangan kanan, wanita-wanita kidal lebih mungkin sebagai *homozygous* untuk

192-bp, lebih tidak mungkin sebagai *heterozygous* dari 192-bp, dan lebih mungkin sebagai *non carrier* dari 192-bp (p=0,035). Wanita-wanita kidal tampaknya memiliki distribusi *allel* yang bergeser pada area promoter dari gen IGF-1 dibandingkan dengan wanita-wanita yang menggunakan tangan kanan. Oleh karena itu, sub grup wanita-wanita kidal mungkin memiliki gen IGF-1 yang sesuai dengan kadar IGF-1 yang beredar dalam darah yang lebih tinggi.

Dalam bab 3.1, kami mempelajari hubungan antara kidal dan kejadian kanker payudara pada wanita usia pertengahan. Dalam studi ini kami menggunakan sebuah kohort program *screening* kanker payudara di Utrecht, Belanda. Pada saat wanita-wanita ini dimasukkan sebagai subyek penelitian, mereka ditanya tentang pilihan penggunaan tangan sejak lahir dan seterusnya mereka diamati untuk munculnya kanker payudara selama 16 tahun. Data demografi dan riwayat reproduksi diperoleh dengan menggunakan sebuah kuesioner pada saat inklusi. Dari sampel acak, 11,5% dilaporkan menggunakan tangan kiri pada awal masa kanak-kanak. Resiko untuk mendapatkan kanker payudara adalah 39% lebih tinggi pada kelompok kidal (*hazard ratio* (HR) 1,39; 95% IK 1,09 sampai 1,81). Resikonya adalah 2,41 kali untuk kanker *premenopause*, namun tidak ada *excess risk* yang lebih tinggi untuk kanker *postmenopause*. Kami menemukan *excess risk* yang lebih tinggi pada wanita-wanita dengan indeks masa tubuh (IMT) ≤25 kg/m^2 (HR 1,62; 95% IK 1,17 sampai 2,24 – p untuk perilaku penggunaan tangan dan IMT: 0,07), juga pada wanita yang melahirkan paling sedikit seorang anak (HR 1,58; 95% IK 1,19 sampai 2,11 – p untuk interaksi antara perilaku penggunaan tangan dan paritas: 0,02), tetapi tidak pada mereka yang mempunya IMT > 25kg/ m^2 dan mereka yang nullipara. Penyesuaian terhadap *confounders* potensial tidak mengubah hasil-hasil tersebut. Perilaku penggunaan tangan tampaknya tidak berhubungan dengan lateralitas dari kanker payudara. Hasil-hasil penelitian ini mendukung hipotesis bahwa kidal berhubungan dengan resiko terjadinya kanker *premenopause* yang lebih tinggi.

Pada bab 3.2, kami menyelidiki hubungan antara kidal, depresi dan kerentanan dan kecenderungan terhadap berbagai penyakit. Kami menggunakan sebuah kohort yang terdiri dari wanita dan pria dewasa, yang pada saat diikutkan sebagai subyek penelitian, data-data demografi dan perilaku penggunaan tangannya dinilai dengan menggunakan kuesioner. Kira-kira 4 tahun kemudian, mereka diminta untuk mengisi kuesioner standar dalam bidang psikiatri dan data tentang depresi dengan menggunakan CIDI (*Composite International Diagnostic Interview)* juga diperoleh. Dari keseluruhan kohort, 10,9% dilaporkan menggunakan tangan kiri. Mereka yang kidal memiliki resiko lebih tinggi untuk mengalami tekanan psikologis pada saat dewasa (OR 1,8; 95% IK 1,1 sampai 2,9), mengalami depresi sedang (OR 2,3; 95% IK 1,0 sampai 5,4), kerentanan terhadap

penyakit yang lebih tinggi (OR 2,2; 95% IK 1,1 sampai 4,5) dan menderita lebih dari 2 penyakit kronik yang diobati (OR 1,8; 95% IK 0,9 sampai 3,6) dibandingkan dengan pengguna tangan kanan. Hasilnya tetap sama setelah dilakukan penyesuaian dan pengendalian terhadap usia dan jenis kelamin. Hasil-hasil ini mendukung hipotesis bahwa kidal mungkin berhubungan dengan kerentanan terhadap tekanan psikologis dan penyakit-penyakit fisik.

Untuk menyelidiki berbagai pertanyaan mengenai hubungan antara kidal dan resiko mortalitas yang banyak diperdebatkan di masa lalu, kami menggunakan kohort yang sama seperti yang digunakan pada bab 3.1. Kejadian mortalitas pada wanita-wanita ini diamati selama 13 tahun sejak waktu inklusi (bab 3.3). Selama 12,6 tahun median waktu pengamatan, 252 wanita meninggal dunia. *Hazard ratio* (HR) antara wanita-wanita kidal jika dibandingkan dengan yang tidak kidal adalah 1,4 untuk mortalitas dengan berbagai penyebab (95% IK 0,9 sampai 2,0), 1,7 untuk mortalitas kanker total (95% IK 1,0 sampai 2,7), 2,0 untuk mortalitas kanker payudara (95% IK 0,8 smapai 4,6), 4,6 untuk mortalitas kanker kolorektal (95% IK 1,5 sampai 14,3), 1,3 untuk mortalitas penyakit-penyakit kardiovaskuler (95% IK 0,5 sampai 3,3), dan 3,7 untuk mortalitas serebrovaskuler (95% IK 1,1 sampai 12,1) setelah dilakukan penyesuaian dan pengendalian terhadap variabel perancu potensial (status sosial ekonomi, umur, indeks massa tubuh dan status merokok pada waktu perekrutan).

Hasil penting dari penelitian di atas ditelaah dan didiskusikan dalam bab 4. Diskusi lebih lanjut tentang relevansi klinis, debat publik dan usulan-usulan untuk penelitian di masa yang akan datang juga dibicarakan pada bab ini. Jika kekidalan lebih disebabkan secara dominan oleh variasi normal dari gen-gen yang terlibat secara langsung dalam mekanisme lateralisasi, maka temuan-temuan kami menunjukkan bahwa sebagian dari distribusi lateralitas adalah patologis. Tidak satupun dari penyebab kidal, seperti kerusakan otak, faktor genetik atau sebab-sebab lain, kami dapat pastikan bahwa hal-hal tersebut bersifat patologis dalam arti memiliki konsekuensi negatif terhadap kesehatan dalam kehidupan selanjutnya. Namun, beberapa dari penyebab ini sudah pasti bersifat patologis dalam arti disebabkan oleh pengaruh luar yang menganggu. Hubungan yang sangat bervariasi yang kami temukan dalam penelitian ini mungkin menunjukkan bahwa perilaku penggunaan tangan adalah sebagai pertanda suatu proses biologis yang sangat sentral dalam kehidupan seseorang dimana gangguan dalam bentuk apapun tentunya mempunyai pengaruh terhadap timbulnya berbagai masalah kesehatan, atau, hal tersebut menunjukkan peranan variable perancu.

Dari berbagai variasi dampak yang ada yang dihubungkan dengan perilaku penggunaan tangan, satu hal yang mungkin untuk menjelaskan kausalitas adalah, kidal merupakan sebuah pertanda dari berbagai proses kausal yang berbeda. Untuk beberapa jenis penyakit, lateralisasi akibat gangguan serebral dapat memainkan peranan sebagai penyebab dari penyakit tersebut, sebagai contoh dalam kasus *schizophrenia* atau pada keadaan psikopatologi lainnya. Sebaliknya, untuk penyakit jenis lain seperti kanker payudara, nampaknya paparan terhadap kadar hormon yang tinggi dalam kandungan lebih mungkin sebagai penyebab terjadinya lateralisasi yang terganggu dan juga sebagai penyebab meningkatnya resiko pada usia dini untuk terjadinya kanker payudara. Setelah menelaah hasil-hasil yang dipaparkan pada tesis ini, kami mengambil posisi bahwa untuk sebagian besar hubungan-hubungan antara perilaku penggunaan tangan dan akibat-akibat pada kehidupan selanjutnya, pilihan penggunaan tangan kiri yang patologis merupakan sebuah pengganti terdekat dari proses-proses mendasar yang belum diketahui.

Sebagai kesimpulan, adanya indikator yang menunjukkan telah terjadi kerusakan otak pada awal kehidupan seseorang nampaknya berkaitan dengan resiko yang lebih tinggi untuk menjadi kidal. Selanjutnya, menjadi kidal sepertinya meningkatkan resiko seseorang untuk mendapatkan berbagai penyakit, seperti kanker payudara *premenopause,* tekanan fisik dan psikologis, peningkatan mortalitas, terutama kematian yang disebabkan oleh kanker dan penyakit-penyakit serebrovaskuler. Mempelajari perilaku penggunaan tangan yang hanya merupakan salah satu aspek dari keseluruhan lateralitas, adalah sebuah proses yang kompleks. Hasil dari penelitian dalam tesis ini diharapkan dapat menjadi inspirasi untuk mendorong dilakukan penelitian selanjutnya dalam upaya mengungkapkan konsep kidal patologis.

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Curriculum vitae

The author was born in September 4th, 1977, in Jambi, Indonesia. In 1995, after graduating secondary school at the Santa Ursula High School in Jakarta, she started Medical School at the University of Indonesia, Jakarta, Indonesia. As a medical student she produced some scientific papers entitled: “Diarrhea problem in children under five in Kelurahan Duri Kosambi, Jakarta Barat”, “The knowledge, attitude and practice on AIDS and its prevention, study of adolescents between 15-18 years old at RW 06, Kel. Utan Kayu Utara, East Jakarta”, and “Urine opioid contain in SMU 3 High School Students, Jakarta”. After graduating medical school in 2001, she worked in a non profit medical organization (International Medical Corps) in Madura island, Indonesia, to provide primary medical care to internally displaced persons due to racial conflict. In 2002, she received a scholarship from the Netherlands Education Center to obtain a Master of Science degree of Clinical Epidemiology at the NIHES (Netherlands Institutes of Health Sciences), which she finished in June 2003. Her paper for the graduation was “Breast Feeding and Infections in Infants in Developed Countries: A systematic review”. In September 2003, she started the work described in this thesis. The research was financed by and carried out at the Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht (supervised by Prof Dr. D. E. Grobbee and Dr. Cuno S.P.M. Uiterwaal). In June 2004, she obtained the Doctor of Science degree at the NIHES. In the future she plans to continue working in the field of epidemiology and starting an internship in pediatrics.

List of Publications

1. Ramadhani MK, Grobbee DE, Bots ML, Castro Cabezas M, Vos LE, Oren A, Uiterwaal CSPM. Lower birth weight predicts metabolic syndrome in young adults: the Atherosclerosis Risk in Young Adults (ARYA)-study.Atherosclerosis. 2006 Jan; 184(1):21-7. Epub 2005 Apr 21.
2. Ramadhani MK, Elias SG, van Noord PA, Grobbee DE, Peeters PH, Uiterwaal CSPM. Innate left-handedness and risk of breast cancer: case-cohort study. BMJ. 2005 Oct 15;331(7521):882-3. Epub 2005 Sep 26.