A STUDY OF NEURAL TUBE DEFECTS
IN A SOUTH AFRICAN POPULATION

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A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of

Master of Science in Medicine (Genetic Counselling).

Johannesburg, 2000
DECLARATION

I, Gloria Teckie declare that this research report is my own unaided work and that all the assistance that I received in its preparation consisted of technical advice, detailed in the acknowledgements, and that the results and conclusions stated were obtained from my own work. I have given full acknowledgement to the sources I have used. This report is being submitted for the degree of Master of Science in Medicine (Genetic Counselling) at the University of the Witwatersrand, Johannesburg. It has not been submitted for any degree or examination at this or any other University.

GLORIA TECKIE

4th day of Jly 2000
To my parents, Ghirmay and Flora Teckie, to my brother Neysan,
and to my grandmother, Tahereh Madjzoub.
PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Some of the work included in this research report was presented at the Seventh Biennial Congress of the South African Society of Human Genetics, 1997, Pilanesburg, South Africa in a poster entitled "Neural Tube Defects in Gauteng".

A paper titled "Risk Factors in the Aetiology of Neural Tube Defects" was presented at the International Clearinghouse for Birth Defects Monitoring Systems, 24th Annual Meeting, in 1997 in Cape Town, South Africa.

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ABSTRACT

Neural tube defects are a common cause of morbidity and mortality in the South African population and occur in about 1 in 800 births. They are usually multifactorial in aetiology and this study analysed some of the possible risk factors, both environmental and genetic, in an attempt to establish their relative importance to the occurrence of neural tube defects in the population of Gauteng.

A retrospective study was undertaken using 640 files of patients with neural tube defects seen at the Genetic Counselling Clinics of the Department of Human Genetics, University of the Witwatersrand and School of Pathology, South African Institute for Medical Research. The study showed that while maternal age was not a significant risk factor for the occurrence of neural tube defects, maternal parity did play a role. In addition, a history of spontaneous abortions and congenital malformations in other offspring seemed to increase a couple's risk of having a subsequent pregnancy affected by a neural tube defect. Maternal factors such as diet and teratogen exposure during pregnancy were also shown to be important risk factors. Correlations could not be demonstrated between season of birth, social class or history of twinning and the occurrence of neural tube defects.

The study also attempted to determine the recurrence risks of neural tube defects in the population and the sex ratios of affected individuals. A recurrence risk of 2.28% was calculated for neural tube defects following the birth of one affected child and significantly more females were shown to be affected by these disorders.

In addition, a molecular analysis was undertaken to determine the contribution of the 677C-T mutation in the methylenetetrahydrofolate reductase gene to the aetiology of
neural tube defects in the black population of Gauteng. The mutation was shown to occur significantly more frequently in the Caucasoid than in the Negroid population of Gauteng. The occurrence of the mutation was not shown to be significantly different in the small sample of black women tested who had had pregnancies complicated by neural tube defects than in the general population from which they were drawn.

The study also undertook to ascertain some of the psychosocial issues surrounding the birth of a child with a neural tube defect in the black population of South Africa. Interviews were performed with black women who had had affected pregnancies and the findings were compared to matched controls from the same population. The results indicated that awareness of and understanding of the aetiology of neural tube defects is low in this population. The results indicated however that there was a willingness to undergo prenatal diagnosis and termination of affected pregnancies in both the experimental and control groups interviewed. Significantly more women who had had children with neural tube defects were shown to suffer from depression than women from the matched control group.
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LIST OF ABBREVIATIONS

AFP - Alpha-fetoprotein
bp - Base pairs
dH2O - Distilled water
ddH2O - Deionised distilled water
°C - Degrees Celsius
DNA - Deoxyribonucleic acid
dNTP - Deoxyribonucleotide triphosphate
EDTA - Ethylenediamine tetra-acetic acid
EtBr - Ethidium bromide
g - gram
kg - kilogram
l - litre
ml - millilitre
mM - milliMolar
MTHFR - 5,10-methylenetetrahydrofolate reductase
ng - nanogram
NTD - Neural Tube Defect
PCR - Polymerase chain reaction
pmol - picomoles
SAIMR - South African Institute for Medical Research
SDS - Sodium dodecyl sulphate
SE - Standard error
SD - Standard deviation
TBE - Tris-Boric Acid-EDTA
TE - Tris-EDTA
Tris - Tris-(hydroxymethyl)-amino methylene
µg - microgram
µl - microlitre
UV - ultraviolet
V - volt
1 INTRODUCTION

1.1 MOTIVATION FOR THE PRESENT STUDY

Neural tube defects (NTDs) are among the commonest congenital malformations in most populations and are estimated to occur in about 400,000 births per year worldwide (Czeizel, 1993a). These disorders result from failure of closure of the neural tube. They may be very severe, and are associated with miscarriages, stillbirths, neonatal deaths, and with serious neurological and physical problems in the majority of individuals who survive (Harper, 1998).

While the aetiology of NTDs is not yet fully understood, most cases are believed to be multifactorial in origin. Studies performed worldwide have identified numerous risk factors for these disorders (Carter & Evans, 1973; Holmes, Driscoll & Atkins, 1976; Bound, Francis & Harvey, 1991). The identification of these risk factors is a prerequisite for the prevention of NTDs and therefore has been for some time and continues to be the focus of NTD research. A family history of NTDs is perhaps the strongest risk factor for the occurrence of NTDs in an individual and therefore warrants analysis. Geographic differences have been observed in the occurrence of NTDs (Steegers-Theunissen, Smithells & Eskes, 1993), in their recurrence risks (Carter & Fraser Roberts, 1967; Yen & MacMahon, 1968; Carter, & Evans, 1973) and in the contributions of individual risk factors to their aetiology (McKeown & Record, 1951; Carter & Evans, 1973; Evans, 1979). A number of risk factors, therefore, may interact in each population to cause the majority of cases. For this reason, it is imperative that research projects are undertaken in each population in an attempt to define the risk factors for the occurrence, recurrence and aetiology of NTDs for that community, so that specific, appropriate and effective prevention strategies can be devised and implemented.
A mutation in the methylenetetrahydrofolate reductase (MTHFR) gene has been implicated as a significant risk factor in some populations. Studies on black populations are not as yet conclusive and whether this mutation does play a part in the aetiology of NTDs in the black population of Gauteng is still open to debate.

A successful prevention programme for NTDs also requires that the health care workers in the field have a thorough knowledge of the community's beliefs about the disorders and the burden perceived to be associated with them. To implement a prenatal diagnosis and selective termination program for a disorder, for instance, one must be aware of the impact of such a program as determined by the number of women who might make use of such facilities (Kromberg, 1997).

The writer, therefore, was motivated to identify some of the risk factors for NTDs in the population of Gauteng; to estimate the recurrence risks associated with these disorders in the population and to gain an in-depth understanding of the psychosocial issues surrounding the birth of an affected child in the black population; thereby contributing to available information on this important group of disorders.

1.2 AIMS OF THE STUDY

The aims of the study were:

1. To investigate the recurrence risks of neural tube defects in the Gauteng population.
2. To investigate environmental, genetic and clinical factors such as: maternal age; social class; teratogen exposure and dietary factors; season of conception; the sex ratio of affected individuals; and abnormalities associated with NTDs, in the Gauteng population.

3. To evaluate the contribution of a genetic polymorphism in the 5,10-methylenetetrahydrofolate reductase gene to the occurrence of neural tube defects in the black population of Gauteng.

4. To assess some of the psychosocial factors associated with the birth of an affected child in the black population; and some of the attitudes of black women towards prenatal diagnosis and selective termination of pregnancies complicated by neural tube defects.

1.3 SETTING AND SCOPE OF THE PRESENT STUDY

The study was set in the Gauteng area and was carried out at the Department of Human Genetics, School of Pathology, South African Institute for Medical Research (SAIMR) and University of the Witwatersrand, where the researcher is a student. Patients from Genetic Counselling Clinics run by the Department of Human Genetics at Chris Hani Baragwanath and Johannesburg Hospitals were included in the study. Most subjects were resident in Johannesburg or in Soweto. In addition, patients seen at the Genetic Counselling Clinics from other parts of the country and from neighbouring countries, such as Namibia, Botswana and Zimbabwe, were included in the study. The Hope School in Parktown and JC Merkin School in Soweto were also contacted to identify possible
participants for the prospective study. Controls for the psychosocial study were obtained from the ‘Well Baby Clinic’ at Thokoza Hospital, Alberton.

The proposed sample sizes for the study were as follows: all files of patients with a family history of NTDs from the Genetic Counselling Clinic records would be analysed for the retrospective study. Controls for this study would be taken from figures available for the general population. The molecular study would involve the investigation of blood samples from 50 black individuals with NTDs, their mothers, and, where possible, their fathers. Controls for the molecular study would be obtained from the randomly collected blood samples available in the Molecular Genetics Laboratory at the Department of Human Genetics, SAIMR, and it was planned to test 100-200 of these samples from both Negroid and Caucasoid populations. Interviews for the psychosocial study would be performed with 50 black women with children with NTDs and with 50 control women with normal children of similar ages.

1.4 DESIGN OF THE STUDY

A retrospective study was undertaken and the files of families seen at Genetic Counselling Clinics at Chris Hani Baragwanath and/or Johannesburg Hospitals for neural tube defects between the years 1969 and 1997 were analysed. These files were examined to obtain data on risk factors such as history of NTDs, month of conception and sex of affected individuals. A checklist was created for extracting information from the files and the file study was subsequently undertaken. Files of patients seen at the Genetic Counselling Clinics for cystic fibrosis and albinism were used for comparison.
Black mothers who had had pregnancies complicated by NTDs, fathers and individuals with NTDs, were ascertained for the prospective molecular study. Controls for this study consisted of random Negroid and Caucasoid individuals whose DNA samples were available in the Molecular Genetics Laboratory, Department of Human Genetics, SAIMR. DNA was extracted from the blood samples, and the polymerase chain reaction (PCR) performed, followed by enzyme digestion to determine the MTHFR genotypes.

An interview schedule was prepared and administered to 35 black mothers of infants born with neural tube defects and 35 matched controls from the same population for the prospective psychosocial study.

All data were computerised and appropriate statistical analyses were undertaken. Results were then compared to those of other local and international studies to assess their significance.

1.5 LIMITATIONS OF THE STUDY

Limitations of the retrospective study included the fact that the information in the files was sometimes incomplete and that it relied on how thorough the counsellor had been in the genetic counselling session (where the data were initially recorded), and how many notes were made in each case; so that in some cases, all the information required for this study may not have been recorded. The use of the file classification system to locate files may have meant that not all NTD files were analysed, for instance files classified as multiple congenital abnormalities (some of which could have had NTDs) were not perused. In addition the files that could not be located may have been those which had been removed because of some specific factor and this may have been of relevance to the
findings of this study. The unavailability of control figures from the local population for several parameters assessed in this study was also a limiting factor in this research.

Results of the molecular study may also have been affected by the limited number of blood samples available and the resultant small sample size.

Several limitations are associated with obtaining information through the use of interview schedules. These include the interviewer's personal biases or interpretations of questions or answers which may influence the results, and also the fact that some respondents may respond with what they think are the expected or socially acceptable answers rather than truthful responses (Bailey, 1987). Ideally the interviewer should not know the difference between the controls and the experimental mothers when doing a psychosocial analysis because he/she may have pre-conceived ideas that the mother of the child with a defect should be more depressed, for instance, and this may influence the way in which questions are translated or emphasized. In addition the expected small sample size might not be sufficient to draw conclusions for a large population.

1.6 SUMMARY

The need for the current research is outlined in this chapter and the potential advantages to the South African community are briefly described. The setting, scope and design of the study, including the subjects used and the methods of data collection are defined briefly and some of the limitations of the study are outlined.
2 REVIEW OF THE LITERATURE

2.1 INTRODUCTION

NTDs arise as a result of incomplete closure of the neural tube during early embryonic development (Lemire, 1988). This abnormal development leads to miscarriage, stillbirth and neonatal death in many cases, and to abnormalities of central nervous system function in most affected individuals who survive. NTDs can be classified according to the type or position of the lesion, with the most common types being anencephaly and spina bifida (meningocoele and myelomeningocoele).

NTDs are generally believed to be multifactorial in aetiology and a number of genetic and environmental factors have been implicated and shown to be important in the occurrence and recurrence of these conditions (Carter & Evans, 1973). Factors identified as possible risk factors include environmental insults to the fetus in early pregnancy, maternal factors such as age, social class, history of miscarriages, a family history of twinning and/or of NTDs. In addition, dietary factors, in particular, folate, have been shown to be important in the development of the neural tube, and the genes involved with this association are currently being investigated (Botto & Mastroiacovo, 1998). These risk factors will be discussed in detail in this chapter.

2.2 DEVELOPMENT OF THE NEURAL TUBE

The formation of the neural tube occurs before day 28 in the development of the human embryo (Jones, 1988). The process begins with the laying down of a neural plate which
then curves upwards and fuses to form the neural tube in a process known as primary neurulation. Secondary neurulation, also known as canalisation, involves the formation of a continuous lumen to the neural tube (Schoenwolf & Smith, 1990). Fusion of the neural tube is now believed to involve five sites of closure which may pose points of weakness during the development of the spine (Seller, 1995).

Neural tube defects (NTDs) arise from the incomplete closure of the neural tube during development (Norman, et al., 1995) and they are generally classified according to the type and position of the lesion. Complete failure of the neurulation process results in craniorachischisis, a condition which is not compatible with life. Anencephaly (see Figure 2.1) occurs if the neural tube fails to close in the cranial region and the result is also always lethal (Lemire, 1988). Anencephaly may involve the entire brainstem and is then called holocrania, or only part of the brain in which case it is known as merocrania (Norman, et al., 1995). Spina bifida cystica is a term used to describe failure of closure of the neural tube in its spinal portion (see Figure 2.1) and defects may be classified as meningocoeles, in which meninges herniate out forming a cyst-like structure; and myelomeningocoeles in which the spinal cord also herniates out (Moore & Persaud, 1993). Myelocoeles and meningomyelocoeles may be divided into upper and lower spinal lesions. Lower lesions, in the lumbar region of the spine occur most commonly (Lemire, 1988). Spina bifida occulta is of different aetiology and is the result of the incomplete fusion of vertebral arches in the spine (Moore & Persaud, 1993). Encephalocele is a term used to denote a skin-covered herniation in the cranial region of the neural tube and these disorders arise as a result of a post-neurulation defect (Lemire, 1988). The presentation of NTDs, in general, depends on the size and position of the lesion.
Figure 2.1 Baby with spina bifida (above), and baby with anencephaly (below) from patients seen at the Genetic Counselling Clinic
2.3 METHODS OF ANTENATAL DETECTION OF NTDS

Antenatal detection of NTDs mainly involves the use of the following techniques: ultrasound, and the analysis of alpha-fetoprotein (AFP) levels (of maternal serum and amniotic fluid) (Brock & Sutcliffe, 1972; Brock, 1983). The detection of an NTD should be followed by genetic counselling and discussion of optional termination of the affected pregnancy.

AFP is a fetal protein and its levels increase to a peak level towards the end of the first trimester and then decrease thereafter. AFP levels in amniotic fluid and in maternal serum result from fetal excretion of the protein and can therefore be used as an indicator of fetal development. The presence of an open NTD in the fetus results in higher concentration of AFP in amniotic fluid and in maternal serum and can therefore be used to detect these lesions (Brock & Sutcliffe, 1972; Main & Mennuti, 1986). Amniotic fluid AFP levels are raised in 90% of cases of anencephaly (Medical Task Force, 1990) and in a similar proportion of cases of open NTDs (Main, & Mennuti, 1986; Lemire, 1988). Maternal serum testing is less accurate as levels are influenced by a variety of maternal factors (Main, & Mennuti, 1986).

Raised AFP levels are not specific to NTDs and do not detect closed NTDs. These tests are therefore used in conjunction with ultrasonography in the diagnosis of NTDs (Main, & Mennuti, 1986; Lemire, 1988), and can together detect almost all fetuses with anencephaly and around 95% of fetuses with spina bifida (Harper, 1998).

The assay of acetylcholinesterase levels in amniotic fluid has been shown to be a sensitive and specific method for the detection of open NTDs and is increasingly being used in conjunction with AFP testing and ultrasound examination (Wald, et al., 1989; Wald & Kennard, 1992; Brennand, et al., 1998).
Results of several studies indicate that the increasing use of methods for the antenatal
detection of NTDs, followed by optional termination of affected pregnancies is effective
in reducing the burden of these diseases on societies. This is noted particularly in
developed countries where the technology is readily available (Yen, et al., 1992; Bower,
et al., 1993), however the effects of other epidemiological factors, such as improved
nutrition during pregnancy may also play a role in explaining the declining trends
observed.

2.4 INCIDENCE OF NTDS

NTDs occur at different frequencies in different populations and even in different subsets
of populations (Steegers-Theunissen, et al., 1993). Researchers have suggested
incidences from 1 to 2 per thousand births in the United States (Baty, et al., 1996) to 7.7
per thousand births in South Wales (Carter, David & Laurence, 1968), but most areas
have incidences between these two values (MacMahon, Pugh & Ingalls, 1953;
Williamson, 1965; Carter & Evans, 1973; Field, 1978; Anyebuno, et al., 1993;
Buccimazza, et al., 1994). The worldwide average has been estimated to be about 1 NTD
per 1 000 births (Lemire, 1988). Ratios of spina bifida to anencephaly births also seem to
vary between different populations with most studies reporting a higher incidence of
spina bifida than anencephaly (MacMahon, et al., 1953; Williamson, 1965; Carter &

A number of studies have attempted to determine the incidence of NTDs in South African
populations and the findings are summarised in Table 2.1. Estimates of the incidence of
occurrence of NTDs locally range from 0.55 to 6.08 per thousand black births and from
0.88 to 3.16 per 1000 white births. NTDs occurred more frequently in white than in black
populations and this has been postulated to be genetic, due to the fact that the white population originated from parts of Europe where the NTD incidences are similar to those occurring in the white South African population (Viljoen, et al., 1995).

Table 2.1 Incidence of NTDs in South African populations (MA= Mixed Ancestry)

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<tr>
<td>Gauteng (Baragwanath)</td>
<td>0.78</td>
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<td>0.40</td>
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<td>Kronberg &amp; Jenkins, 1982</td>
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<tr>
<td>Gauteng (Johannesburg)</td>
<td>-</td>
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<td>0.52</td>
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<td>0.36</td>
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<td>Glietenberg, 1977, unpublished, cited in Kronberg et al, 1982</td>
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<tr>
<td>Gauteng (Pretoria)</td>
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<td>Northern Province</td>
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<td>Free State</td>
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<td>-</td>
<td>2.00</td>
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<td>1.60</td>
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<tr>
<td>Cape Town</td>
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<td>-</td>
<td>1.59</td>
<td>0.59</td>
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<td>-</td>
<td>1.59</td>
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<td>Singer, et al., 1978</td>
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<tr>
<td></td>
<td>0.69</td>
<td>-</td>
<td>1.64</td>
<td>0.68</td>
<td>0.21</td>
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<td>0.69</td>
<td>0.31</td>
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<td>Buccimazza, et al., 1994</td>
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The ratio of spina bifida to anencephaly in Gauteng calculated from the above studies is 1.96:1. A large study performed in Cape Town reported a ratio of 2.45:1 (Buccimazza, et al., 1994).

2.5 RECURRENT RISKS OF NTDs

Recurrence risks of NTDs in families are known to vary with the population incidence of these disorders, with higher rates of recurrence in areas with higher occurrences of NTDs (Carter, et al., 1967; Yen & Macmahon, 1968; Carter & Evans, 1973). Studies performed

Some studies found a difference in the recurrence rates after the birth of a child with spina bifida and after a child with anencephaly, with a higher rate observed after the birth of an anencephalic child in some cases (Carter & Evans, 1973) and a lower recurrence rate in others (MacMahon, et al., 1953; Carter, et al., 1968); while other studies showed recurrence rates that were independent of the type of NTD (Yen & Macmahon, 1968). It has also been shown that high or larger lesions of spina bifida may be associated with significantly higher recurrence risks than lower or smaller lesions (Hall, et al., 1988). Sex of the index case does not appear to influence the risk of recurrence of either spina bifida or anencephaly (Carter, et al., 1968, 1973); however significant concordance has been observed for sex in recurrent cases of NTDs (Mariman & Hamel, 1992).

Another area of study has been the relationship between the type of NTD in the proband and the type of NTD in the recurrence(s). Some studies have shown very high concordance in the type of NTD in families where more than one member is affected (Williamson, 1965; Toriello & Higgins, 1985; Hall, et al., 1988), while other studies have found no correlation (Yen & Macmahon, 1968; Hunter, 1984).

Studie
Where two members of a sibship are affected the recurrence risk for subsequent siblings has been noted to be nearly twice as high as that after one affected child (Carter, et al., 1967; Yen & Macmahon, 1968). However where the index case and one other family member are affected (second degree or more distantly related), the risk seems to be similar to the sibling recurrence risk (Carter, et al., 1968; Hunter, 1984). Studies of recurrence rates in half siblings are inconclusive and range from about half the full sibling risk to risks similar to those of full siblings (Yen & Macmahon, 1968; Carter & Evans, 1973). It has been suggested that maternal half siblings may be at greater risk of NTDs than paternal half siblings (Hunter, 1984).

In several studies, the risk of recurrence to other relatives of an affected individual has been investigated and, in general, close relatives of affected individuals have been shown to be more susceptible to NTDs than other individuals in the general population (Williamson, 1965; Hunter, 1984). This relationship seems to be strongest in the maternal lineage (Williamson, 1965). The risk to cousins of an affected individual seems to be significantly higher than the population risk for mother’s sister’s children only (Williamson, 1965; Carter, et al., 1968; 1973; Hunter, 1984).

2.6 GENETIC BASIS OF NTDs

Observations of the occurrence and recurrence of NTDs suggest that these disorders are usually inherited in a multifactorial fashion with a complex relationship between hereditary factors and the influence of environmental agents on the developing embryo (Carter & Evans, 1973; Field, 1978). The excess of females affected with NTDs, variations in incidence of NTDs, and factors, both environmental and maternal, shown to influence the occurrence of these disorders all support a multifactorial aetiology (Shaffer,
Researchers have estimated that around 90% of NTDs are inherited in this fashion (Holmes, et al., 1976).

A genetic contribution to the causation of NTDs is implied by the observations that these disorders are concentrated in families, by their recurrence risks and from twin studies (Williamson, 1965; James, 1978, 1980). The distorted sex ratios observed, and the fact that female family members of an affected individual are more likely to have affected children themselves, may suggest a role for genomic imprinting or for some other genetic gender determinants in NTDs (Chatkupt, et al., 1992; Mariman & Hamel, 1992). Research suggests that, in most cases, the genetic contribution to NTDs would be expected to be polygenic (Laurence, Campbell & James, 1983) and polygenic inheritance has also been postulated in a mouse model (Neumann, et al., 1994). Several genes have been identified which may be involved in NTDs, and in rare cases the cause of the NTD may be an anomaly in a single gene. Autosomal recessive inheritance has been reported, as in Meckel syndrome which is commonly associated with encephalocoeles, and from studies of consanguineous couples (Shaffer, et al., 1990; Ziotogora, 1995); and families have been reported in which X-linked recessive inheritance is postulated for NTDs not complicated by other malformations (Toriello, Warren & Lindstrom, 1980). Studies in mice have identified a group of genes, PAX genes, which may also be involved in some cases (Hol, et al., 1995; 1996). Chromosomal aberrations, such as deletions of 22q11 have been shown to be associated with NTDs (Nickel & Magenis, 1996). Triploid fetuses and trisomic fetuses, in particular those with trisomies 13 and 18, may also have NTDs (Holmes, et al., 1976). Gene-gene interactions and their contribution to the aetiology of NTDs are also being investigated (Botto & Mastroiacovo, 1998).

Seasonal variations in the occurrence of NTDs, social class effects and teratogen studies suggest that many different environmental agents may play a role in NTDs (Williamson, 1965). Maternal predisposing factors, such as diabetes mellitus have also been
implicated. In addition, dietary factors, in particular the protective effects of the vitamin folic acid, may be major environmental contributors.

2.7 GESTATIONAL AND BIOGRAPHICAL FACTORS IN NTDS

2.7.1 Parental Age
In several studies the effects of maternal age on the occurrence of NTDs have been addressed. Some studies have shown no maternal age effects (Williamson, 1965; Hunter, 1984). A study in the Greater London area showed that very young mothers (below 20 years of age), and mothers above 35 years of age had a higher risk of having infants affected with NTDs (Carter & Evans, 1973). A similar distribution was reported in Cape Town (Buccimazza, et al., 1994); and in analyses of ages of mothers of anencephalic infants in two other studies (Janerich, 1972; James, 1979a), while other studies found an excess only among older mothers (Carter, et al., 1968; Bound, et al., 1991). For spina bifida, some studies have shown an increasing incidence with increased maternal age (Carter, et al., 1968; Field, 1978). It has been suggested that the increased incidence of NTDs observed in very young mothers results from the fact that this group often falls into a lower socio-economic class with confounding factors such as incorrect or insufficient nutrition (Carter & Evans, 1973). This has been supported by a study in which very young mothers were shown to have diets which were on average, lower in nutrients than other women (Smithells, et al., 1977).

A large study in South Wales and another study in Australia failed to find any paternal age effects on the incidence of NTDs (Carter, et al., 1968; Field, 1978).
2.7.2 Spontaneous Abortions and NTDs

A number of studies have documented an increased occurrence of spontaneous abortions in women who have NTD-affected births (Evans, 1979; Myrianthopoulos & Melnick, 1987; Carmi, et al., 1994). In addition, studies have shown that the pregnancy following a pregnancy which ended in miscarriage had a higher risk of being affected with an NTD than a pregnancy following a normal one (Evans, 1979; Myrianthopoulos & Melnick, 1987; Carmi, et al., 1994). One study documented a doubled risk of NTDs for pregnancies following spontaneous abortions when compared with controls (Myrianthopoulos & Melnick, 1987); and another showed a 28% increased risk when compared to the risk for subsequent offspring after pregnancies affected by other birth defects (Carmi, et al., 1994). A Hungarian study found that multiple miscarriages were associated with a 0.635% risk of NTDs compared to the population NTD risk of 0.27% (Ádám, Poulín & Papp, 1995).

Evans (1979) found that while spina bifida was associated with an increased risk of spontaneous abortion, anencephaly was not. A large study showed that the increased risk of spontaneous abortions in families of NTD individuals did not vary according to the sex of the affected individual or the number of affected individuals in the family (Lippman, 1984), however in another study an increased rate of multiple miscarriages in families where a male was affected with an NTD was noted (Sadovnik, et al., 1986). No correlation was observed between occurrence of spontaneous abortions in NTD families and social class (Evans, 1979).

Several explanations have been proposed for these associations. Initially it was suggested that the reason for an increased risk of NTD pregnancy immediately following a spontaneous abortion was because the residual material persisting in the uterus after a miscarriage somehow affected the subsequent pregnancy (Clarke, et al., 1975). However, it has come to be accepted that a high proportion of spontaneous abortions occur because the pregnancies are affected by NTDs and thus not carried to term (Evans, 1979). This is
supported by the estimation that about 54% of all fetuses with NTDs probably miscarry (Creasy & Alberman, 1976). Researchers studying spontaneous abortuses estimate that NTDs occur in 1-7% of abortions (Creasy & Alberman, 1976; Byrne & Warburton, 1986; McFadden & Kalousek, 1989), and it is probable that the frequency is affected by other factors such as the incidence of NTDs in the area of study (McFadden & Kalousek, 1989). Another factor which may contribute to the occurrence of spontaneous abortions in NTD families is parent-child incompatibilities in the HLA system. It has been shown that incompatibility of HLA-A,B alleles is higher in couples with recurrent miscarriages and also in couples who have pregnancies with NTDs, suggesting a possible common gene(s) or factor contributing to both occurrences (Schacter, et al., 1979).

2.7.3 Maternal Parity and Risk of NTDs
A large study performed in Quebec failed to find any significant effect of parity on the occurrence of NTDs (Hunter, 1984) and similar results were documented in a study in Greater London where findings were also standardised for maternal age (Carter & Evans, 1973). A significantly higher occurrence of spina bifida and anencephaly has been observed in first-born children (Williamson, 1965; Carter, et al., 1968) and women of parities higher than 4 have also been reported to be at increased risk (Field, 1978). Buccimazza et al. (1994) reported an increased occurrence of NTDs at both extremes of birth order. It has been hypothesised that birth order biases observed in NTDs may be an indirect effect of the relationship between maternal age (see Section 2.7.1) and parity, rather than a direct parity effect, since very young and older mothers may have a higher risk of having NTD affected pregnancies (Hunter, 1984; Buccimazza, et al., 1994).

2.7.4 Maternal Obesity
Recent research has indicated that maternal obesity is a risk factor for NTDs increasing the risk by 2 to 4 times (Shaw, Velie & Schaffer, 1996; Werler, et al., 1996). It is not known what the cause of this increased risk is, but this factor has been shown to be
independent of folate intake, maternal age, diabetes, or use of dietary supplements or slimming aids during pregnancy (Shaw, et al., 1996; Werler, et al., 1996). The prevalence of obesity among South African women is high, especially in black women living in urban areas, where an obesity level of 34.4% has been estimated (Harrison, 1995), and obesity may therefore be a significant factor in the aetiology of NTDs in South Africa.

2.7.5 Consanguinity

It has been suggested that consanguineous relationships may be at slightly increased risk for offspring with NTDs and this is in keeping with the fact that the aetiology of these disorders involves a strong genetic component (Carter, et al., 1968). Rare cases of autosomal recessive inheritance of NTDs are known and in such cases the effects of consanguinity are apparent (Shaffer, et al., 1990; Zlotogora, 1995).

2.7.6 Season of Conception and NTDs

The season of conception of an embryo seems to affect its chance of having a congenital malformation. An early study in Britain showed that the chance of a pregnancy resulting in a congenital malformation was higher for children conceived in the autumn and winter part of the year, from October to March (McKeown & Record, 1951), but a later study in Greater London showed that conceptions in February to April had the highest incidence (Carter & Evans, 1973). An Australian study recorded more NTD births in conceptions occurring in the summer months (Field, 1978). A Cape Town study showed that more infants with NTDs were conceived in the winter months, May and June, but incidences were also high in December and January (Singer, et al., 1978), while a larger study of the same community reported an increased incidence from June to September (Buccimazza, et al., 1994). In another South African study, an excess of NTDs was found in August births (ie November conceptions) (Sayed, et al., 1997). Seasonal differences seem to
depend greatly on the area of study and some studies, such as those performed in Canada and in the United States, showed no seasonal effects (MacMahon, et al., 1953; Hunter, 1984).

When anencephaly was considered separately, one US study documented an increase in affected births from October to December, ie conceptions in late winter (MacMahon, et al., 1953). In Greater London the incidence of births with anencephaly was lowest in April to June, and highest in December to February (Carter & Evans, 1973). This study also addressed the incidence of spina bifida and found that the highest numbers of births correlated with the same conception period if one allowed for the fact that anencephalic fetuses are more likely to be born pre-term (Carter & Evans, 1973). A study in New South Wales found a significant difference in spina bifida births in different seasons, with an excess of spring births and winter conceptions, but did not find the same relationship for anencephaly (Parker, 1978).

A number of explanations have been proposed to explain the observations regarding season of birth in NTDs. It has been suggested that fetuses conceived at times of the year when less fresh fruit and vegetables are available, and hence at times when diets are most likely to be folate deficient, are most susceptible (Laurence, et al., 1983). In very hot countries, maternal hyperthermia due to the heat may become a risk factor during the summer (Parker, 1978); while during winter, infectious agents may be more prevalent and pose a significant risk factor in some populations (McKeown & Record, 1951; Janerich, 1971). One author suggested that since sex ratios vary with season of conception in some populations, seasonal trends may be related to the fact that more females are affected by NTDs, so that NTDs would occur more commonly at the times of the year when the sex ratio is most biased towards female births (Parker, 1978). The variable degree to which these factors affect populations, and in some cases the interaction of two or more of these factors, may account for the differing observations regarding seasonal variation in the incidence of NTDs.
Annual variation has also been observed in the occurrence of NTDs by several researchers and peaks were attributed to changing demographics of populations, to economic factors such as war, and to observed disease patterns (MacMahon, et al., 1953; Field, 1978; Owens, et al., 1981; Yen, et al., 1992).

2.7.7 Teratogenic Effects

Numerous drugs and other agents have been cited as possible teratogens in the aetiology of NTDs.

2.7.7.1 Maternal Epilepsy and NTDs

Epileptic women are at greater risk for pregnancies complicated by NTDs than non-epileptics and this may be caused either by the disorder itself or by the medication used to control or treat it (Hopkins, 1987). Various epileptic drugs have been studied and several have been implicated in the aetiology of NTDs. Carbamazepine is associated with a 1% risk for spina bifida (Rosa, 1991); and valproate is known to increase the risk of low spina bifida lesions to about 10 times the population risk (Lindhout & Schmidt, 1986; Martínez-Frias, 1990). Other anti-epileptic drugs have not been associated with high risks of NTD malformations (Rosa, 1991).

Direct proof of the teratogenic effects of carbamazepine came from a case described by Little and co-workers (1993) in which a suicide was attempted with an overdose of this drug by a non-epileptic woman. The fetus was subsequently shown to have a large spina bifida.

It has been suggested that anti-epileptic drugs interfere with the folic acid pathway (Nulman, et al., 1999) and mouse studies have shown that expression of genes such as methylenetetrahydrofolate reductase are reduced by drugs such as valproate (Finnell, et
al., 1997). Anticonvulsant therapy has also been associated with a decrease in serum and red cell folate levels in humans (Teasdale & Pearce, 1972; Rose & Johnson, 1978).

2.7.7.2 Diabetes

Diabetic women have a 2 to 3 fold increased risk for congenital malformations when compared with women in the general population (Pedersen, Tygstrup & Pedersen, 1964; Martínez-Frías, 1994). This risk includes central nervous system anomalies and, in particular, spina bifida and anencephaly (Becerra, et al., 1990). The teratogenicity of diabetes is possibly associated with the altered intra-uterine conditions of the fetus, or with some common genetic factors affecting neural tube development and the aetiology of diabetes (Pedersen, et al., 1964; Sadler, Robinson & Msall, 1995).

2.7.7.3 Oral Contraceptives and Ovulation Stimulating Drugs

Early work into the association between steroidal contraceptive use and nutrient levels in the blood suggested that serum levels of some nutrients, in particular vitamins B₆, B₁₂ and folic acid could be reduced by the use of contraceptives (Wynn, 1975). This, in turn lead to the suggestion that these drugs may increase the risks of birth defects such as NTDs (Smithells, Sheppard & Schorah, 1976). While a UK study failed to find a relationship between contraceptive use and occurrence of NTDs (Cuckle & Wald, 1982), an association has been made between oral contraceptive use and low serum folate levels (Lewis, et al., 1998).

Ovulation-stimulating drugs, in particular clomiphene, have been shown to be associated with an increased risk of anencephaly in the resultant pregnancies (Cornel, et al., 1989a; Cornel, ten Kate & Te Meerman, 1989b; Czeizel, 1989; Vollset, 1990). However these findings have also been disputed (Mills, et al., 1990); and it has been suggested that mothers of anencephalic infants may have reduced fertility thereby explaining the observations (Dyson & Kohler, 1973; James, 1973).
2.7.7.4 Maternal Hyperthermia

Maternal hyperthermia or exposure to high temperatures, such as saunas and hot baths, during the first trimester of pregnancy has been associated with NTDs (Shioto, 1982; Hunter, 1984; Milunsky, et al., 1992). In a Canadian study, 12% of NTD mothers reported having a fever during pregnancy (Hunter, 1984). It has also been shown that, in most cases, the fever occurred during the time of neural tube closure in the embryo (Shioto, 1982).

2.7.7.5 Low Calorie Diets and Slimming Preparations

It has been suggested that dietary imbalances over a long period of time before and during pregnancy, such as those caused by a weight loss program, may have a teratogenic effect on the development of the neural tube (Sheffer, Shohat & Merlob, 1993). Very little research is available to date but a mother's dieting during pregnancy was associated with another congenital defect, holoprosencephaly, in one study (Ronen, 1992) and a family has also been documented in which the couple experienced multiple miscarriages and had a baby with meningomyelocele after the mother had prolonged dietary imbalances (Sheffer, et al., 1993).

2.8 NTDS AND SEX RATIOS

Multifactorial conditions often show deviations in sex ratio from that expected in the general population. Numerous studies have reported an excess of female cases with NTDs with male to female ratios (M/F), for instance of 0.73 (Seller, 1986). Some researchers have noted, however that the sex ratios depended on the type of NTD and on
the site of the lesion, and were different for different populations, depending on their NTD incidences (James, 1979a; 1979b).

Studies performed in a wide range of populations, have documented a female excess in anencephalic births with M/F ratios ranging from 0.34 to 0.67 (Williamson, 1965; Carter, et al., 1968; Field, 1978; Hunter, 1984; Seller, 1986; Buccimazza, et al., 1994; Lubinsky, 1997). A study which analysed the different types of anencephaly separately showed that while craniorachischisis, holocrania, and anencephaly occurring with a cervical spina bifida, are more common in females, merocrania (anencephaly in which part of the brain only is missing) does not show a sex bias (Seller, 1987).

Studies of the sex ratios for spina bifida have given conflicting results with some studies finding no sex bias (James, 1979b), and others finding male or female sex biases (Carter, et al., 1968; Martínez-Frías, et al., 1986). The majority of studies have, however, noted an overall female bias with M/F ratios ranging from 0.77 to 0.89 (MacMahon, et al., 1953; Williamson, 1965; Carter, et al., 1968; Hunter, 1984; Seller, 1986; Buccimazza, et al., 1994). Division of spina bifida according to the site of the lesion indicated that the sex ratio was highly dependent on this variable (Mariman & Hamel, 1992). Lesions occurring in the thoracic region showed a female excess, while those occurring in the sacral and lumbar regions were male biased, with one study noting M/F ratios of 0.42 and 3.0 respectively (Seller, 1986). Encephaloceles showed a female excess in two studies in which they were considered as a separate group (Hunter, 1984; Lubinsky, 1997).

A South African group documented an excess of male births for both spina bifida and anencephaly with M:F ratios of 1.3 and 1.2 respectively (Kromberg & Jenkins, 1982).

Several theories have been proposed to explain the observations that the sex of an embryo is a determinant of its predisposition to different types of NTDs. It has been suggested that early development, which may occur at different rates in male and female embryos, is
in some way responsible for sex ratio differences (Seller, 1986). The formation of the neural tube can be divided into two processes. Neurulation, in which the neural folds curve upwards and fuse, occurs first and seems to be the point at which the female embryo is most vulnerable, since upper NTDs are more common in females (Hall, 1986; Seller, 1987; Brook, Estibeiro & Copp, 1994). Neurulation is followed by canalisation, a process in which the neural tube is lengthened and it is from faults at this stage that more NTDs in males than females are thought to arise (Hall, 1986; Seller, 1987). These differences may be due to environmental or genetic factors; since they may occur in a slightly different way or at different times in males and females, and the different processes would presumably involve different genetic pathways (James, 1979a; Seller, 1986). The recent discovery that neural tube closure is multisite in nature, initiated at specific sites along the neural tube, suggests that different sites may be more vulnerable to failure of closure processes in the different sexes (Van Allen, et al., 1993; Seller, 1995; Martínez-Frias, et al., 1996). The process of X-inactivation has been suggested as a possible factor and it has been proposed that if X-inactivation is delayed for some reason in female embryos, the cells may still have two active X chromosomes at the time of important divisions in the formation of the neural tube which could result in a delay in the process and hence an NTD (Hall, 1986).

2.9 TWINNING AND NTDs

Studies of the families of individuals with NTDs have shown that there is a higher twinning rate in these families than in the families of individuals with disorders known to be inherited in a Mendelian fashion (Garabedian & Fraser, 1994). This finding was especially pronounced in families of individuals with upper NTDs (above the eleventh thoracic vertebra) (Garabedian & Fraser, 1994). In addition, families in which twinning
and upper NTDs occurred together, had a higher recurrence risk of NTDs (Garabedian & Fraser, 1994).

Twin births are associated with a higher incidence of birth defects than single births (Layde, et al., 1980) and studies of the occurrence of NTDs in twins suggest that NTDs occur more frequently in twin births (Hay & Wehrung, 1970; James, 1971; James, 1975; Windham & Sever, 1982). James (1975) estimated that the occurrence of NTDs in twin births in London was about 1.6 per 1000 births, while that of the local population was 1.1 per 1000 births. Some studies observed this excess of NTDs only in the anencephaly and encephalocele groups but not for spina bifida (James, 1975; Windham & Sever, 1982). A study in which ethnic groups were investigated separately showed that black American twins had a four-fold increased risk of NTDs when compared to the general population (Layde, et al., 1980).

It is unclear whether NTDs are associated with dizygotic or monozygotic twinning since the excess observed was mainly in same-sex twins which could be either monozygotic or dizygotic (since it has been observed, though not clearly explained, that there is an excess of dizygotic twins of the same-sex) (James, 1971; Layde, et al., 1980; Windham & Sever, 1982; Garabedian, et al., 1994; James, 1995).

It is not known why NTDs and twinning seem to be associated and it could be that there is a common aetiology, or that the occurrence of one event predisposes embryos to the occurrence of the other (Field & Kerr, 1974; James, 1975; Rivas, Olivares & Chakraborty, 1995). Neural tube closure and twinning both occur very early in embryonic development and authors have hypothesised that a common factor, genetic or environmental may be involved. Nutritional factors, such as folate availability, have been discussed in the literature (Garabedian & Fraser 1994; Rivas, et al., 1995). It has been suggested that a delay in development at a crucial stage, such as at implantation, may cause either the splitting of the embryo or failure of neural tube closure (James, 1975).
This would explain the observation that twinning is more often associated with upper NTDs since the upper neural tube closes earlier in development (James, 1975; Garabedian & Fraser, 1994). Another mechanism which has been proposed to explain the association between NTDs and twinning is X-inactivation and it has been suggested that since this process occurs at around the same time in development as neural tube closure, and since the excess twinning observed is like sex (and may therefore be monozygotic), anomalous X inactivation could account for both events (James, 1988).

2.10 SOCIAL CLASS AND NTDs

A relationship has been observed between parental social class and the incidence of NTDs with families from lower socio-economic groups having higher relative risks for infants with NTDs (Williamson, 1965; Carter & Evans, 1973; Field, 1978; Wasserman, et al., 1998). Socio-economic status has in fact been suggested as the strongest environmental factor influencing the occurrence of anencephaly (James, 1979a). It has also been suggested that recurrence rates may be influenced by the socio-economic status of the mother (Hunter, 1984).

Several theories have been proposed to explain these observations. In most populations dietary factors vary significantly from higher to lower socio-economic groups and studies have shown that social classes I and II have better nutritional intake and as a result higher levels of nutrients in their blood including folate (Smithells, et al., 1976, 1977). Another explanation has been that since family size often increases with decreasing socio-economic conditions, this may explain the occurrence of more NTDs in these groups (Carter, et al., 1968). It has also been suggested that these observations merely arise from
sampling biases because of the small relative numbers of social classes I and II when compared to the lower socio-economic groups (Carter, et al., 1968).

2.11 NTDS AND OTHER MALFORMATIONS

Research has attempted to determine whether NTDs are associated with any other malformations which do not arise as a result of the lesion in the neural tube. Numerous studies of probands and their families have been undertaken in an attempt to find any such associations. These associations would then yield information about the way in which developmental pathways are related to each other and whether they are affected by similar events in early pregnancy (Hall, et al., 1988).

Estimates of the frequencies of unassociated malformations in individuals with NTDs vary from around 6% to 40% depending on the methods of assessment and what abnormalities are included (David, McCrae & Bound, 1983; Hunter, 1984; Hall, et al., 1988; Medical Task Force, 1990). Rates of malformation seem to be similar for spina bifida and anencephaly births but have been reported to be higher in encephalocele and craniorachischisis births (Hunter, 1984; Hall, et al., 1988). Higher lesions are also associated with a greater risk of unrelated congenital anomaly (Toriello & Higgins, 1985; Hall, et al., 1988). The malformations most commonly observed were renal and cardiovascular defects but frequencies of different birth defects varied from one area to another (David, et al., 1983; Hall, et al., 1988; Rodríguez, et al., 1992). An association has also been observed between limb defects and anencephaly with one study finding an association 100 times more often than would be expected if the conditions were unassociated (Rodríguez, et al., 1992).
Studies of siblings of individuals with NTDs suggest that they are not at greater risk for congenital malformations other than NTDs when compared to the general population (MacMahon, et al., 1953; Williamson, 1965; Yen & MacMahon, 1968; Carter & Evans, 1973).

2.12 NUTRITION AND NTDS

Several observations lead to the suggestion that maternal diet may play a part in the aetiology of NTDs. These include studies that have shown that NTDs occur more commonly in lower socio-economic groups and in regions where women are known to have poor or unbalanced diets (Laurence, et al., 1980). Recurrence risks for NTDs are also higher in cases of poor maternal nutrition (Laurence, et al., 1983).

An important nutritional factor in the aetiology of NTDs is folate. The MRC study involving 7 countries showed that a 72% reduction in the recurrence of NTDs occurred in women who were given folic acid periconceptionally, when compared with those who were given no dietary supplements (MRC Group, 1991). In addition, no effect was observed when women were supplemented with a vitamin preparation which did not contain folic acid (MRC Group, 1991). These findings have been corroborated by other researchers (Mulinare, et al., 1988; Milunsky, et al., 1991) and it has also been shown that folic acid supplementation is particularly effective in preventing the recurrence of NTDs after a single previous NTD birth. The observed recurrence rates dropped to 0.5% and 0% in couples with one or multiple previous NTD births respectively who were supplemented with folic acid. In comparison controls experienced recurrence risks of 4.2% and 9.6% for one and multiple previous NTD births (Smithells, et al., 1983).
Since over 90% of NTDs occur in families with no prior history of these disorders, it is, perhaps more important to determine whether folic acid plays a role in preventing all NTDs, or is only effective for preventing recurrences (Werler, Shapiro & Mitchell, 1993). A Hungarian study showed an overall decrease in birth defects, and, in particular of NTDs, after periconceptional multivitamin supplementation (Czeizel & Dudas, 1992). Studies in the USA showed a 40-60% reduction of NTDs in women who took folic acid when compared with unsupplemented women in their populations (Mulinare, et al., 1988; Werler, et al., 1993). Folic acid has also been shown to prevent the occurrence of 60% of NTDs associated with unrelated birth defects which do not constitute specific syndromes (Khoury, et al., 1996).

Folic acid is now known to help prevent a number of other birth defects believed to be of multifactorial aetiology (Czeizel, 1993b). A significant reduction of risk of both recurrence and occurrence of cleft lip and palate has been documented (Tolarova, 1982; Shaw, et al., 1995a). A 30-35% reduction in the occurrence of limb defects has also been noted after periconceptional multivitamin supplementation (Shaw, et al., 1995b). Studies have shown a preventative effect for urinary tract malformations and a variety of non-syndromic cardiac anomalies (Shaw, et al., 1995b; Czeizel, 1996).

Studies of healthy women showed that folic acid supplementation increases red blood cell folate levels significantly (Cuskelly, McNulty & Scott, 1996). Since folic acid supplementation prevents the occurrence of NTDs, one might expect to see reduced levels of folate and folate derivatives in women who have affected infants (Molloy, et al., 1985). Biochemical investigations have supported the observations that folic acid plays a role in the prevention of NTDs. Blood samples collected from participants in the MRC study group were used to determine folic acid levels in women with NTD pregnancies and showed that they had significantly lower serum and red blood cell folate levels than other pregnant women (Wald, et al., 1996). A number of other studies have documented similar findings (Smithells, et al., 1976; Yates, et al., 1987; Kirke, et al., 1993). It has
also been shown that if folate levels in the blood can be raised to those observed in normal individuals, the occurrence of folate-sensitive NTDs can be prevented (Laurence, et al., 1983).

Blood folate levels are influenced by a variety of factors and twin studies have shown that a large proportion of red cell folate variation can be attributed to genetic factors (Mitchell, et al., 1997).

2.12.1 Folate and Dietary Factors

It has been shown that blood folate levels are lower in women with poor or unbalanced diets (Laurence, James & Campbell, 1982). Foods known to be rich in folate include green vegetables and red meat, in particular liver and kidneys (Steegers-Theunissen, et al., 1993). However, folate is often unstable in food, being destroyed by the food preparation process (Laurence, et al., 1983); and high dietary intake of fats and carbohydrates may interfere with the absorption of folate (Laurence, et al., 1983). The biochemical form of folate, whether it is in polyglutamate or monoglutamate form, also determines the efficiency of its absorption (Rosenblatt, 1995; Smithells, 1996). Increased dietary folate has therefore not been shown to be very effective in improving red blood cell folate levels in women with deficiencies (Cuskey, et al., 1996). Supplementation with folic acid, the synthetic form of folate, which is more stable than the natural form and can be more effectively absorbed, is therefore recommended (Cuskey, et al., 1996; Crandall, et al., 1998).

A recent study performed in Cape Town showed that 54% of black women aged 19 to 44 years were receiving less than 110 μg of folate per day from their diets, less than one third of the recommended daily intake (Crandall, et al., 1998) and 63% of 15-18 year olds had folate deficient diets (Bourne, et al., 1997).
Some women also experience excessive nausea and vomiting in the first trimester and these women have been shown to have significantly lower nutrient levels and intakes of nutrients, such as folate (Smithells, et al., 1977). This factor could be a significant risk factor for NTDs and it remains to be seen whether folate supplementation will be effective in preventing the occurrence of NTDs in these women.

2.12.2 Mechanism of Action of Folate in the Body

Folate derivatives are involved in numerous reactions in the body. In particular, they act as coenzymes to a wide variety of enzymes; such as those in nitrogenous base and amino acid biosynthetic pathways; making them indispensable to cell division (Rosenblatt, 1995).

2.12.2.1 Homocysteine Metabolism

An important pathway in which folic acid is involved is in the conversion of homocysteine to methionine and the methylation cycles that arise therefrom. Folic acid obtained from the diet is reduced to 5,10-methylenetetrahydrofolate by the enzyme dihydrofolate reductase (Rosenblatt, 1995). The product of this reaction is then converted by the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) to 5-methyltetrahydrofolate which acts as a cofactor with Vitamin B₁₂ in the conversion of homocysteine to methionine (see Figure 2.2).
A number of mutations in various components of the folate metabolism pathway have been described and studies are underway to determine their respective roles in the aetiology of various disorders.

2.12.2.2 The Methylene-tetrahydrofolate Reductase Gene and its Postulated Role in the Aetiology of NTDs

The human MTHFR gene was isolated using porcine amino acid sequence information and the gene was subsequently localised to chromosome 1p36.2 (Goyette, et al., 1994). Studies of the MTHFR enzyme showed that a variant of the enzyme with thermolabile
properties, was associated with a 50% reduction in enzyme activity and homocysteinemia which could be corrected by increasing folic acid intake (Kang, et al., 1988; Kang, et al., 1991). This MTHFR variant was shown to act in a recessive manner (Kang, et al., 1991). Further research identified a mutation in the gene encoding the MTHFR enzyme and this mutation was shown to be the cause of the reduced enzyme activity (Frosst, et al., 1995). The mutation, a C to T transition at nucleotide position 677, results in the substitution of an alanine residue for a valine residue (Frosst, et al., 1995). The normal enzyme sequence is believed to be highly conserved and similar amino acid structures have been observed in several organisms (Frosst, et al., 1995).

The 677C-T mutation has been shown to be involved in the aetiology of coronary heart disease as a result of its effect in increasing homocysteine levels since homocysteine is cytotoxic and therefore increases thrombotic tendency of cells (Kang, et al., 1991; Frosst, et al., 1995; Kluijtmans, van den Heuvel, & Boers, 1996; Motulsky, 1996). It was then suggested that this mutation may also be an important risk factor in NTDs; a hypothesis supported by the fact that folic acid was known to be involved in the occurrence of these disorders (MRC Group, 1991). The mechanisms whereby this mutation could influence the occurrence of NTDs include the suggestion that the accumulation of homocysteine may be toxic to embryonic cells, or alternatively, that neural crest cells may not receive sufficient 5-methyltetrahydrofolate thereby affecting a number of methionine dependent reactions (Lucock, Wild & Levene, 1995; Whitehead, et al., 1995).

Several studies have been undertaken in an attempt to determine whether the thermolabile variant of the MTHFR enzyme does occur more often in individuals with NTDs, or in their families, and in particular their mothers, thereby having the potential to affect intrauterine development in some way. The findings of these studies are summarised in Table 2.2.
### Table 2.2 Occurrence of 677C-T MTHFR homozygotes in different populations

<table>
<thead>
<tr>
<th>STUDY</th>
<th>CONTROLS</th>
<th>NTD INDIVIDUALS</th>
<th>NTD MOTHERS</th>
<th>NTD FATHERS</th>
<th>REFERENCE</th>
</tr>
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<tbody>
<tr>
<td>Netherlands</td>
<td>4.8</td>
<td>12.7</td>
<td>15.7</td>
<td>10</td>
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<td></td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>Kluijtmans, et al., 1996</td>
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<tr>
<td>Ireland</td>
<td>6.1</td>
<td>18.3</td>
<td>-</td>
<td>-</td>
<td>Whitehead, et al., 1995</td>
</tr>
<tr>
<td>Iceland (extended study)</td>
<td>8.3</td>
<td>19.0</td>
<td>-</td>
<td>-</td>
<td>Kirke, et al., 1996</td>
</tr>
<tr>
<td>Atlanta, USA</td>
<td>4.6</td>
<td>22.0</td>
<td>-</td>
<td>-</td>
<td>Ou, et al., 1996</td>
</tr>
<tr>
<td>Italy</td>
<td>16.3</td>
<td></td>
<td>7.1</td>
<td>-</td>
<td>de Franchis, et al., 1995</td>
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<tr>
<td></td>
<td>16.6</td>
<td>25.6</td>
<td>-</td>
<td>-</td>
<td>1998</td>
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<tr>
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<td>14</td>
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<td>-</td>
<td>Björke-Monsen, et al., 1997</td>
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<tr>
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<td>Wilcken, et al., 1996</td>
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<td></td>
<td>-</td>
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<td>Stevenson, et al., 1997</td>
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<td>Blacks</td>
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<td></td>
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<tr>
<td>Brazil:</td>
<td>10</td>
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<tr>
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<td>0</td>
<td>-</td>
<td>Ubbink, et al., 1999</td>
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<td>Northern Province</td>
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</table>

As shown in this table, different studies have found different frequencies of the 677C-T mutation both in the control samples and in families with a member with an NTD. The results range from those in which no association was found between the mutation and the occurrence of NTDs (Papapetrou, et al., 1996; Björke-Monsen, et al., 1997; Mornet, et al., 1997; Koch, et al., 1998; Boduroglu, et al., 1999) to studies in which strong
correlations were seen (van der Put, et al., 1995; Whitehead, et al., 1995; Ou, et al., 1996; Christensen, et al., 1999). The Atlanta group calculated that the occurrence of the MTHFR mutation is associated with a 7-fold increased risk for NTDs (Ou, et al., 1996). The lower frequency of the mutation in black individuals was observed in studies undertaken in Atlanta; Britain, South Carolina, Brazil, and also in a study of rural black South African women who had had a child with an NTD (Ou, et al., 1996; Erten, & Layton, 1997; Stevenson, et al., 1997, Arruda, et al., 1998; Ubbink, et al., 1999). This may suggest either a different aetiology for NTDs in the black population, or may explain the lower observed incidences of NTDs in some of these populations (Motulsky, 1996).

Different observations in different populations are most likely to be indicative of the different factors contributing to the aetiologies of NTDs in the different populations; and further studies are required to attempt to determine the relationships between the 677C-T mutation and the occurrence of NTDs. In addition, it is possible that other genes involved in the homocysteine pathway or related biochemical processes are involved in the aetiology of NTDs or that the different risk factors for NTDs are associated in complex ways, such as the possible relationships between dietary factors and the mutation, which are as yet undetermined (de Franchis, Sebastio & Mandato, 1995; Posey, et al., 1996; van der Put, et al., 1997).

Further analysis of the MTHFR gene has yielded another common polymorphism, the A1298C mutation and also another mutation T1317C which occurred commonly in Africans who were sampled, but which is believed to be a silent mutation (Weisberg, et al., 1998; Heil, et al., 1999).

2.12.2.3 Use of Folate in the Body

Studies have shown that pregnant women require greater amounts of folate, which seems to be broken down more rapidly during pregnancy (McPartlin, et al., 1993). It is
hypothesized that rapidly dividing fetal cells, such as those of the neural tube, require a constant supply of folate (Kirke, et al., 1993). This is supported by observations in the animal NTD model: the curly tailed mouse, which has a mutation predisposing it to NTDs. Seller (1983) has shown that DNA synthesis inhibitors administered to these mice at the time of neural tube formation, reduced their risks of NTDs. This may be due to the fact that these agents slow down cell division, and consequently the formation of the neural tube, allowing cells to accumulate sufficient amounts of folate before division (Seller, 1983).

Observations of folic acid resistant NTDs (i.e. occurrences of NTDs in women who were fully supplemented during pregnancy) do not show an increased occurrence of a specific type or site of NTD; indicating that folate acts on the entire length of the neural tube and that its effects are not restricted to specific sites (Shaw, Todoroff & Lammer, 1997).

It is not known how folic acid supplementation prevents NTDs in at risk women and it has been postulated that folate may either bypass a metabolic block or overcome a folate deficiency (Kirke, et al., 1993).

2.13 SOME PSYCHOSOCIAL ISSUES ASSOCIATED WITH NTDs

An important area of study is the analysis of the effects of the birth of a child with a congenital birth defect on the family, and in particular on the mother. Since each condition differs, studies are required to determine the psychosocial issues involved for each specific disorder.

Research has shown that an effective and reliable way of collecting information on psychosocial issues from individuals is face-to-face interviews (Bailey, 1987). The
advantages associated with this method include the fact that the interviewer can help the interviewee to understand questions, can ensure that the interviewee takes the questionnaire seriously, and can document the non-verbal reactions of the interviewee (Bailey, 1987; Guy, et al., 1987). Results of face-to-face interviews are better when the interviewer is of the same ethnic background and stage of life experience as the interviewee (Guy, et al., 1987). Disadvantages of face-to-face interviews include the fact that the interviewer’s interpretation biases may affect the results, confidential questions may be more conventionally rather than truthfully answered and this method is also time consuming and costly (Bailey, 1987; Guy, et al., 1987). A good means of checking the effectiveness of a questionnaire before use is to perform a pilot study from which to determine whether questions are being understood and answered accurately (Guy, et al., 1987).

2.13.1 Information Received and Knowledge about NTDs

A British study involving parents of children with spina bifida showed that less than half of the parents had heard of the condition before their child was born; and that of those who had heard of it, very few understood the implications (Freeston, 1971).

It is currently believed that, in most countries, only a small proportion of parents of children with congenital anomalies are being referred for genetic counselling. In the Netherlands, it has been estimated that around 83% of couples are not referred for genetic counselling after the birth of a child with a congenital anomaly, and that only 10.8% of parents of babies born with anomalies involving the nervous system are referred for genetic counselling (Cornel, van Essen & ten Kate, 1992). These observations may suggest that parents are not receiving accurate or sufficient information regarding the aetiology and recurrence risks associated with these disorders.
It is important to determine what information is being given to counselees after the birth of an affected child, how this correlates to their expectations of the genetic counselling session, and which part of the information that they are given they remember, since understanding these factors is essential to providing patients with a service from which they can benefit fully (Michie, Marteau & Bobrow, 1997). A study determining parent’s expectations of the genetic counselling process showed that 79% of parents wanted information regarding the condition, and of these, 74% received the information they expected, while 56% of the 63% of counselees who expected an explanation received it (Michie, et al., 1997). Freeston (1971) found that only around 25% of fathers and even fewer mothers had fully understood the information given to them shortly after the birth of their child with spina bifida. However nearly all the parents given genetic information had remembered what they had been told when interviewed a year later; and the majority (two thirds) remembered the information four years after the birth of their affected child (Freeston, 1971). A Welsh study involving mothers of children with spina bifida showed that 60% had not received or did not recall having received information regarding the recurrence risks of the disorder (Hare, et al., 1966). The educational status of the parents also seems to determine the amount of information they recall after genetic counselling (Somer, Mustonea & Norio, 1988). Some 21% of those receiving genetic counselling in Finland said that they would have benefited from a second counselling session (Somer, et al., 1988).

Another important area of study is patients’ understanding of modes of inheritance and recurrence risks for different conditions. A Finnish study showed that in the genetic counselling setting, patients had trouble understanding multifactorial inheritance; 63% of their counselees felt that they had understood multifactorial inheritance and 76% remembered the risks of recurrence of these disorders correctly (Somer, et al., 1988). In the Welsh study, mothers’ beliefs regarding the recurrence risk of spina bifida were investigated. About one quarter of the mothers did not believe that there was a risk of recurrence and most of the others believed the risk to be small (Hare, et al., 1966).
Perceptions of disease and understanding of risks appear to be influenced by the level of education of individuals. It has been shown that women with less education who are at risk of having children with X-linked disorders are more likely to take the chance than more educated women with the same risks (Beeson & Golbus, 1985).

Use of folate for the prevention of the occurrence and recurrence of NTDs is not well known by the public as indicated by a recent study in the United States in which it was shown that only 7% of women were aware of the benefits of periconceptional folate supplementation (MMWR, 1999).

2.13.2 Understanding of and Beliefs about the Causes of NTDs

Research has shown that parents have an important need to attribute the birth of a child with a serious birth defect to some cause, such as an unusual occurrence during the pregnancy. It has been shown that parents, and especially mothers who are not given an explanation, will work out a cause that they think reasonable for the birth of their handicapped child (Tew, et al., 1977).

Research suggests that parents mainly desire factual information regarding prognosis, aetiology and recurrence risks of the relevant disorder (Cornel, et al., 1992). An important role of the genetic counsellor is to attempt to expose the parent’s beliefs, reassure them where possible, and explain the actual cause of the disorder, if this information is available (Tew, et al., 1977).

2.13.3 Reproductive Decisions

Genetic counselling is an important medium for informing parents about the recurrence risks of NTDs and the prenatal diagnostic procedures available to detect affected pregnancies (Sadovnick, et al., 1987). An area of genetic counselling research which has not yet been fully explored concerns attitudes towards prenatal diagnosis and selective
termination of pregnancies shown to be affected by congenital anomalies. Research is required to determine what reproductive decisions parents make after the birth of a child with a disorder and whether these decisions vary greatly from one disorder to another (Meryash, 1989).

One study showed that the offer of amniocentesis was more likely to be accepted by women who associated having a baby with a congenital anomaly with a heavy burden (Ekwo, Kim & Gosselink, 1987). A Canadian study showed that the use of genetic counselling services by parents of infants with NTDs depended on the type of NTD and the outcome. Parents of living spina bifida children attended genetic counselling more often than parents whose affected children had died, and the authors suggest that this may be because they perceived the disorder as having a greater burden (Sadovnick, et al., 1987).

Views regarding termination of pregnancy are influenced by numerous factors such as religious beliefs, cultural and ethnic background, the perceived burden of genetic disease and specific issues relating to the disorder (Beeson, et al., 1985; Meryash, 1989; Drake, Reid & Marteau, 1996). In a Finnish study, most parents seen at genetic counselling clinics said that they would request prenatal diagnosis, 53% would terminate an affected pregnancy, 31% would want prenatal diagnosis but were unsure about termination, and 16% would not want prenatal diagnosis because they did not approve of abortion (Somer, et al., 1988). The majority (80%) of women in a random sample of the general population of London said that they would terminate a pregnancy in which the fetus was shown to have an NTD, 6% would not terminate, and 14% were unsure (Bennett, Gau & Gau, 1980). A recent study comparing the views on termination of different groups within three European populations, showed that while over 85% of geneticists and obstetricians in UK, Germany and Portugal would terminate a pregnancy in which the fetus was shown to have spina bifida, only around 35-65% of lay people would opt for termination. Of the respondents in all groups studied, 80-95% would terminate a
pregnancy affected by anencephaly (Drake, et al., 1996). A similar South African study showed that 100% of geneticists would request termination of a pregnancy shown to be affected by spina bifida (Kromberg & Jenkins, 1997).

The decision making process is naturally different for couples who have had children with a disorder since the process is then affected by the individual’s previous experiences of the disorder such as the burden of looking after the affected child (Meryash, 1989). A study of families of individuals with fragile-X mental retardation syndrome showed that while 81% of women would request prenatal diagnosis for this condition, only 28% could say with certainty that they would terminate a pregnancy in which the fetus was shown to be affected (Meryash & Abuelo, 1988). However a similar study performed in Johannesburg showed that 73% of women with a child with fragile-X would request prenatal diagnosis and 60% (significantly more than control mothers) would opt to terminate an affected pregnancy (Wessels, 1997).

A local study suggested that there may be a reluctance among black South Africans to consider termination of pregnancy for NTDs, even when prenatal diagnosis has conclusively shown that the fetus is affected and the researchers suggest that this reluctance is seen more frequently in less educated black women (Simpson, 1983). However, a study of mothers of individuals with Down syndrome indicated that 73% would have had prenatal diagnosis had it been offered, and 52% said they would have requested termination of pregnancy had they known that the fetus was affected (Kromberg, 1997). Results of a sample of pregnant women assessed at the antenatal clinic at Chris Hani Baragwanath Hospital, Johannesburg showed that while 95% would consider prenatal diagnosis in future pregnancies, 65% would request a termination if the child were shown to have Down syndrome (Kromberg, 1997). Viljoen et al (1995) reported that the willingness to terminate pregnancies affected by NTDs is increasing in the Cape Town population.
2.13.4 Depression

A full understanding of the effects of the birth of a child with a congenital abnormality on the parents and family is essential for the provision of effective genetic counselling (Hobdell & Deatrick, 1996). Emotions such as sorrow, guilt, anger and depression, and how they are integrated into the grieving process which follows the birth of an abnormal child, must be explored. Studies have shown that the birth of a child with a handicapping condition is associated with shock and denial, which give rise to a stage of ‘emotional disorganisation’ characterised by anger, guilt, disappointment and lowered self-esteem (Blacher, 1984). Emotions such as intense sorrow recurred in many parents at specific stages of the child’s life, such as when the child failed to attain a normal developmental milestone (Hobdell, et al., 1996). Freeston (1971) suggests that parents experience strong emotions at the time of diagnosis, when taking the child home from the hospital, as a result of numerous hospital visits and from continual caregiving demands. There is also an effect on other family dynamics such as the parents’ own relationship and relationships with and among their other children (Freeston, 1971). A study in South Wales showed that the divorce rate in 56 families with a living child with spina bifida was nine times higher than the population average; while parents of 86 children with spina bifida who died had a divorce rate which was three times that of the population (Tew, et al., 1977).

Mothers of infants with NTDs showed chronic sorrow initially, but after time had passed they improved in mood state and reported feeling more optimistic (Hobdell, et al., 1996).

The perceived burden of genetic disease affects many different facets of the counselling process. How a condition is perceived will determine the reproductive decisions made by a couple as well as how they cope with the birth of an affected child. Bu. en is a subjective response and is therefore influenced by many variables such as previous personal experience of the problem and educational status of the couple (Meryash, 1989). A study of the general population showed that disorders associated with long illnesses and/or early death were seen to carry the greatest burden, while physical handicap was
rated as being the least burdensome (Ekwo, et al., 1987). Among women with a child affected with fragile X the burden was shown to diminish with time as the mother adapted (Meryash, 1989).

2.14 SUMMARY

NTDs are a common cause of morbidity and mortality worldwide and occur at significant frequencies in most populations. In addition, they are associated with population-specific recurrence risks. The study of the aetiology and distribution of these disorders is therefore important, with the ultimate goal of prevention.

Numerous risk factors have been identified to date in studies performed on many different populations. These risk factors seem to have varying impacts on the occurrence and recurrence of NTDs in different populations and it is therefore essential to investigate each factor in each population before preventative measures can be implemented effectively.

One such factor, the contribution of a genetic polymorphism in the MTHFR gene to the aetiology of NTDs is currently being studied worldwide. This polymorphism seems to occur commonly in some populations but not in others and research is therefore required to determine its contribution to the aetiology of NTDs in the Gauteng population.

An additional prerequisite for effective genetic counselling is knowledge about the views and beliefs of the population served. To this end, and for the implementation of health policies and appropriate services, it is essential to know the perceptions of populations towards different disorders and the burden they associate with these disorders.
3. SUBJECTS AND METHODS

3.1 INTRODUCTION

The study comprised three different sections: a retrospective file study, a molecular investigation, and a study of psychosocial issues.

In the retrospective study information was gathered from the files of patients with a family history of NTDs to assess some of the risk factors for NTDs and to determine the recurrence risks of these disorders in the Gauteng population. The files of 640 patients counselled at Genetic Counselling Clinics at Chris Hani Baragwanath and Johannesburg Hospitals were examined. Relevant details were extracted from these files and analysed to identify any common trends or relationships between the occurrence of NTDs and any of the factors selected for attention in this study.

The molecular study involved the collection and testing of blood samples from black individuals with NTDs, their mothers and fathers, and from random Negroid and Caucasoid individuals (without NTDs) as controls. DNA was extracted from these samples and the polymerase chain reaction (PCR) was used to amplify the region containing a common mutation in the MTHFR gene. Restriction enzyme digestion was then performed to determine the MTHFR genotype of each individual.

Some of the psychosocial issues surrounding the birth of a child with an NTD in the black population were studied by means of a specially constructed interview schedule. The subjects were the mothers of children with NTDs and control mothers with normal children, matched as closely as possible for maternal age, and child’s age and sex.
3.2 IDENTIFICATION AND SELECTION OF SUBJECTS USED IN THE STUDY

3.2.1 Retrospective Study

Patient records for inclusion in the retrospective study were selected from the filing system in use in the Department of Human Genetics, SAIMR, in which patients and their families are classified according to the disorder for which they are seen at the clinic. All files marked NTD, spina bifida, myelomeningocele (meningomyelocele), meningoceles, anencephaly or encephalocele were included. In addition the files of patients with raised alphafetoprotein levels on amniocentesis were screened and those in which the fetus was subsequently found to have an NTD were included. Patients with NTDs associated with known genetic syndromes such as trisomies 13 and 18, were excluded since these are a distinct group of NTDs of known aetiology. Cases of spina bifida occulta were also excluded as these are known to differ in aetiology from open NTDs. For purposes of analysis, cases of spina bifida occurring with anencephaly were classified as anencephaly; and, in some instances, encephaloceles were grouped with spina bifida where insufficient data were available for independent analysis of this group. This grouping was used since research has suggested that encephaloceles are probably cranial forms of meningocoele (Hunter, 1993).

Control values necessary for comparison with the findings from the retrospective study were taken from population figures where such information was available, or from analysis of the data from groups of patients with other disorders seen at the Genetic Counselling Clinics. For instance, the number of miscarriages per couple was obtained from analysis of the files of 70 albino and 64 cystic fibrosis families seen at the clinics. These files were used since the recessive inheritance of both conditions means that there are no expected influences of the disorder on the rate of spontaneous miscarriage, provided the consanguinity rate in the population is low.
3.2.2 Molecular Study

The subjects for the molecular study were ascertained from the files of black patients seen at the Genetic Counselling Clinics. Subjects were asked to volunteer after reading an information sheet about the project (see Appendix 1). Blood samples were collected from black individuals only, after informed consent (see Appendix 1) had been obtained. Samples were collected from 33 mothers, 2 fathers, and 9 individuals with NTDs, in which the defect appeared to be the result of multifactorial inheritance. Cases in which the patient had a known syndrome of which the NTD was a part, or had features suggestive of such a syndrome, were not used in the analysis. In addition, cases in which there was an obvious teratogenic factor, such as maternal exposure to anti-epileptic drugs during pregnancy, were excluded from participation in the molecular study. The controls for this study consisted of 78 random Negroid and 53 random Caucasoid individuals whose samples had been collected over many years for the purpose of various research projects and were stored in the Molecular Genetics Laboratory, SAIMR.

3.2.3 Psychosocial Study

The subjects for the psychosocial study were black women who had had a child with an NTD. The subjects were ascertained from the records of black patients seen at the Genetic Counselling Clinics, Department of Human Genetics, SAIMR, where such patients could still be reached. In addition a small number of volunteers were obtained from the Spina Bifida Clinic at the Johannesburg Hospital, and from the Hope and JC Merkin Schools. Altogether 35 women agreed to participate in the study and they were interviewed at their homes. Matched controls were then selected. They were defined as black women who had a child of the same age (born in the same year) and sex as an affected child and who, themselves differed in age by a maximum of 5 years from the mother of the child with an NTD. Controls were ascertained and interviewed at the Thokoza Well Baby Clinic, Alberton.
3.3 METHODS OF DATA COLLECTION AND ANALYSIS

3.3.1 Retrospective File Study

To ensure easy access to information from the files and also to maximize patient confidentiality, a checklist was compiled and cards were printed detailing the information required from each file (see Appendix 2). Of the 690 files in the SAIMR filing system, 640 could be located and the required information was extracted from them. The other 50 files were either misfiled or in the possession of staff at the Department of Human Genetics, at the time of analysis. Files of patients seen at the Genetic Counselling Clinics for cystic fibrosis (64) and albinism (70) were also reviewed for specific information and used as controls for the white and black populations respectively, where the information on the general population necessary for comparison with the study findings was not available in published sources.

The methods of ascertainment of the subjects, the information obtained from the file study, and the analytical procedures used are outlined in the following subsections.

3.3.1.1 Recurrence Risks

Recurrence risks were calculated from the pedigree information available in the files, in which numbers of healthy children and children with NTDs born to each family had been documented. In addition, the results of amniocenteses performed on pregnancies following those affected by NTDs and analysed in the Department were included. The number of recurrences for each type of NTD was then compared to the total number of children born to families with a child with an NTD of that type to determine the empiric recurrence risk for full siblings for the sample. Recurrence risks were also calculated for half siblings and for cousins from the pedigrees in which the details and numbers of these relatives had been recorded.
3.3.1.2 Parental Ages

Parental ages at the time of birth of the child with the NTD were calculated from dates of birth of the parents recorded in the files. This was only possible in cases where such information was noted in the file and therefore these data are not complete for all cases. The average parental ages and their distributions were calculated and compared to those in the general population. Data for the general population were only available for distribution of parental ages (South African Statistics, 1993) and population averages could not be accurately determined from these figures. Comparative figures for average maternal age were ascertained from the analysis of parental ages of patients seen for cystic fibrosis in the white population, and albinism in the black population whose files were available at SAIMR.

3.3.1.3 Spontaneous Abortions and NTDs

The history of spontaneous abortions and stillbirths for each nuclear family of an individual with an NTD was obtained from the pedigrees in each file. These data were analysed and compared to control figures calculated from the data obtained on the families with cystic fibrosis and albinism. Cases in which the pregnancy loss was known to be caused by an NTD were not included in this analysis. This distinction was made because NTD pregnancies are often not carried to term and including pregnancies in which the loss was known to be as a result of the NTD would result in a significant difference between the control and experimental groups for this reason alone. This would therefore not allow for the identification of other possible causes of pregnancy loss in the experimental families.

3.3.1.4 Maternal Parity and Risk of NTDs

Maternal parity and the birth position of the affected child were obtained from the pedigrees drawn in the files. From these data an attempt could be made to determine
whether there was a significant difference in the occurrence of an NTD in first pregnancies or in women of higher parity.

3.3.1.5 Parental Consanguinity
The data from the files regarding parental consanguinity may be incomplete. Although it seemed to be common practice among most of the genetic counsellors to ask whether parents were related, and to note the response on the pedigree, there is no way of ensuring that the question was asked in each case, especially where no comment was made in the file.

3.3.1.6 Season of Conception
Details regarding date of birth of the affected child, gestational age at birth and date of the mother’s last menstrual period (LMP) were obtained from each file, where the necessary data were available, and these were used to estimate the month and season of conception of individuals with NTDs. In addition where pregnancies were terminated, the gestational age at termination was used to estimate the date of conception. These findings were compared to seasons of conception in the general population of Gauteng (Hain, 1993).

3.3.1.7 Maternal Teratogen Exposure
Details of teratogen exposure in the pregnancy in which the fetus was affected by an NTD were obtained from file entries made by the counsellors, as well as from questions asked of the mothers in the interviews performed for the psychosocial study. In most cases, mothers would have been asked if they were epileptic or diabetic, and, if so, what medication they had taken during the pregnancy. In addition, some mothers reported use of other drugs in pregnancy, illnesses during pregnancy, use of oral contraceptives or ovulation stimulating drugs and dietary habits during the early part of the pregnancy.
Again, this information may be somewhat incomplete, because the accuracy of the data depends on the counsellors having asked the relevant questions.

3.3.1.8 Sex Ratios in NTDs
The sex of the affected individuals was usually available in the files, with the exception of a few cases in which the NTD had resulted in an early pregnancy loss, or where a distant relative of the counselee was affected. Sex ratios were calculated for each type of NTD and also separately for upper and lower spina bifida (where this information was available), and compared to data available for sex ratios at birth of infants with NTDs recorded in published studies.

3.3.1.9 Twinning in NTD Families
Information regarding whether there were twins in the family, and if so, their degree of relationship to the individual with the NTD were obtained from the pedigrees in the files. This information is probably incomplete however, since questions regarding the occurrence of twins in the family may not always be specifically asked by the genetic counsellors and may not necessarily be volunteered by the counselee. Results were nonetheless compared to population data on twinning rates (Hain, 1993).

3.3.1.10 Social Class and NTDs
The social classes of the families of children with NTDs were determined from the parents' occupations which were in many cases, documented in the files. Occupations were coded using the system devised by Schlemmer and Stopforth (1979). Where only one parent was employed, this was taken as the social class of the family, while where both parents were employed, the one from the higher grouping was used to estimate the family's socio-economic position. The frequencies of individuals in each social class were compared to those of the general population (SA Population Census, 1996) to
determine whether any significant differences existed. Initially, educational status was included as a marker for socio-economic group, but since level of education achieved was not noted in the majority of the files, it was not pursued.

3.3.1.11 NTDs and Other Malformations
Data regarding the occurrence of other malformations in individuals with NTDs and in their siblings were restricted to cases in which the affected individuals were seen by a clinician or other trained personnel, or to cases in which parents could give an accurate account of the diagnosis. This information was tabulated and compared to an estimated risk of malformations in a general population obtained from Harper (1998). For the analysis of individuals with NTDs, congenital abnormalities known to be related to the NTD, such as hydrocephalus and clubfoot, were omitted from the analysis.

3.3.2 Molecular Study

3.3.2.1 Processing of Blood and DNA Extraction
Blood (10ml) was collected in ACD or EDTA tubes from individuals with NTDs and from their parents where available. Samples were stored at -20°C until enough were collected to undertake the DNA extraction procedure in a batch. If extra blood tubes were available for individuals, these were spun down and buffy coats were removed and frozen at -20°C. DNA was extracted from whole blood using the salting out method devised by Miller et al (1988). Chilled Sucrose-Triton-X lysing buffer was added to each sample to a total volume of 45-50ml, to lyse cell membranes and remove non-nuclear cellular components. DNA was further purified by adding 3ml T20E5, 200µl 10%SDS and 500µl Proteinase-K and then incubating overnight at 42°C. Saturated salt solution was used to precipitate proteins and the DNA-containing supernatant was then poured off. Ethanol (100%) was added to precipitate DNA which was then fished out, rinsed in
chilled 70% ethanol and left to air dry. The DNA was resuspended in 1x TE buffer. Methods of preparation of all solutions used in the molecular study are provided in Appendix 3. There were 44 blood samples available and these were grouped into families labelled NTD 1 to 35. Nine NTD individuals, 33 mothers and two fathers were tested from these families. All DNA samples were stored at 4°C.

3.3.2.2 PCR Amplification

DNA samples were analysed using the polymerase chain reaction (PCR), a process in which a segment of DNA lying between two known sequences (primers) is amplified so that the region can be studied further (Saiki, et al., 1985). The forward and reverse primers used in the DNA amplification (NTD1F and NTD1R, previously reported by Frosst et al, 1995) had the following sequences:

Forward Primer (NTD1F): 5’ - TGA AGG AGA AGG TGT CTG CGG GA - 3’
Reverse Primer (NTD1R) 5’ - AGG ACG GTG CGG TGA GAG TGG - 3’

The primers which were obtained from Boehringer-Mannheim in a lyophilised form, were resuspended in ddH2O to a 100pmol/μl stock. A dilution series (1/50; 1/100 and 1/150) of the stock was prepared for the PCR optimisation, from which the 1/100 dilution of the stock solution was selected. For the PCR amplification, 1μl DNA (approximately 5ng), 2μl (2pmol) of each primer, 0.5 mM dNTPs, 10x Taq polymerase buffer (supplied by Boehringer-Mannheim), and 1.5 units of Taq polymerase were made up to a final volume of 25μl with ddH2O.

The optimised PCR conditions used were as follows; an initial denaturation step was performed at 95°C for 5 minutes followed by 30 cycles of denaturation at 95°C for 1 minute, primer annealing for 1 minute at 65 or 67°C (depending on the thermocycler),
and product extension at 72°C for 1 minute. The process was optimised for the Hybaid Omnigene thermocycler, and PCR amplification was subsequently performed on the Hybaid Omnigene and Touchdown thermocyclers, with adjustments being made in annealing temperature (67°C instead of 65°C) for the Touchdown.

3.3.2.3 Visualisation of PCR Products
To confirm successful PCR amplification before enzyme digestion, PCR products were run on an agarose gel. The gel used was a 2.5% agarose gel which was prepared as follows: 7.5g agarose (Agarose D1 LE, Whitehead Scientific) was dissolved in 300 ml 1x TBE buffer in the microwave and left to cool slightly before 5μl of 10μg/ml Etidium Bromide (EtBr) per 100ml of gel was added. EtBr intercalates into DNA and fluoresces under ultraviolet light. The gel was then poured into a plate with gel combs positioned along the length, and allowed to set for approximately one hour.

Ficoll loading dye was mixed with 5μl of each PCR product and loaded into wells in the 2.5% gel. Samples were electrophoresed at 100-120V for approximately half an hour and the PCR products could then be visualised on the UV transilluminator. A molecular weight marker, 1kb λ ladder, was also loaded adjacent to the samples to confirm that the PCR products obtained were the correct size (198 base pairs). In addition, negative control (blank) samples in which DNA was omitted, were included in the process to ensure that the reagents were not contaminated. Any DNA contaminating the reaction would be amplified in the PCR reaction in these blank tubes.

3.3.2.4 Restriction Enzyme Digestion
The 677C-T mutation creates a Hinfl recognition sequence so that the PCR product is digested into 175bp and 23bp fragments (Frosst, et al., 1995). For this process, 5 units of Hinfl (Boehringer-Mannheim) and 3μl of recommended 10x buffer (Buffer H) were
added to the remaining 20μl of PCR product and made up to a final volume of 30μl with
ddH₂O. All digests were performed at 37°C for at least 2 hours. Positive controls,
individuals shown to be heterozygous for the mutation in an initial run, were included in
subsequent analyses to ensure that digestion had occurred.

3.3.2.5 Visualisation of Digested Products

The gel used to visualise the digested fragments was a 4% Metaphor gel which was
prepared by the gradual addition of 16g of Metaphor agarose (FMC Bioproducts) to
400ml chilled 1xTBE buffer to obtain a smooth consistency. The mixture was melted in
the microwave, 5μl per 100 ml of 10μg/ml EtBr was added, and the gel was poured,
allowed to set at room temperature for half an hour and then refrigerated briefly before
use. The high concentration and high resolving ability of Metaphor allow for the
resolution of fragments of similar size.

Five μl Ficoll loading dye was added to each of the digested products and these were then
loaded on the gel and electrophoresed for 1-2 hours at 100-120V. In some cases results
could be obtained immediately by visualisation under UV light, but for gels in which the
bands were faint, it was necessary to re-stain the gel with approximately 20μl of 10μg/ml
EtBr after electrophoresis by leaving the gel in a solution of EtBr for several hours, and
then transferring it to a container of distilled water to remove excess stain.

Individuals who do not have the mutation in the MTHFR gene would be expected to
show one band at the 198bp position; homozygotes for the mutation would have a single
band at the 175bp position (the smaller fragment, of 23bp, runs off the gel and is not
observed on the gel); and heterozygotes would have two bands, one at each of the above
positions (Frosst, et al., 1995).
3.3.2.6 Analysis of Molecular Results

Gene frequencies for the 677C-T mutation were obtained for the small sample of NTD patients and their parents and were compared to those from the random Negroid and Caucasoid controls. Chi-square tests were used to determine whether there were significant differences between the random Negroid and Caucasoid samples and between NTD individuals and/or their family members and/or controls from the same population. Tests were also performed to determine whether the genotypes were in Hardy-Weinberg equilibrium.

3.3.3 Psychosocial Study

3.3.3.1 Construction of the Interview Schedule

An interview schedule was constructed (see Appendix 4), which consisted of 14 questions compiled on the basis of the experience of the supervisor (Professor JGR Kromberg) and writer with NTD families, and a version of the Beck Depression Inventory (Beck, et al., 1961) comprising 21 multiple choice questions used to assess mood state was selected. Questions were designed to yield information regarding biographical data of the subject, her family history, and her attitudes towards prenatal diagnosis and termination of pregnancy. An introductory information sheet and a consent form were also prepared. The same interview schedule was administered to both the experimental and control groups with 4 questions specific to the disorder (Questions 4, 7, 9 and 11), being omitted when control mothers were interviewed.

The interview schedule, together with an application describing the project, the information sheet and consent forms were submitted to the Committee for Research on Human Subjects of the University of the Witwatersrand and approval was obtained on 11/04/1997. These forms appear in Appendix 5.
3.3.3.2 Pilot Study

The interview schedule was piloted on 8 black women at the ‘Well Baby Clinic’ at Thokoza Clinic, East Rand, Gauteng, in April 1997. No one who was approached refused to participate but several women walked away while others were being interviewed. All the women interviewed had children who were born between 1995 and 1997.

The interview commenced with a brief introduction as to what neural tube defects are and the interviewees were shown a photograph of an affected infant. Two of the women, who were schoolteachers, requested permission to fill in their own questionnaires. The interviewer reported that each interview took longer than expected (the estimated time for completion had been 20-30 minutes), with the shortest interview taking about an hour.

After the pilot study, the interview schedule was amended as follows: Question 5 regarding diet was simplified into a dichotomous (Yes-No) format; allowance was made for the fact that previous information about NTDs may have been received from other sources besides a health care professional for Question 8; and space for additional comments was made in Questions 12, 13 and 14. In addition, a second contact name and telephone number were added to the questionnaire to make tracing the interviewees easier if it became necessary.

3.3.3.3 Administration of the Interviews

Interviews with mothers of children with NTDs were performed in their homes and the controls were interviewed at the ‘Well Baby Clinic’ in Natalspruit. All interviews were conducted by Sister Esther Zwane from the Department of Human Genetics, SAIMR, who was trained in the use of the interview schedule by the writer with the aid of the pilot study. Interviews were conducted in the patient’s own home language or in a mutually acceptable language.
3.3.4 Selection and Matching of Controls

The controls were mothers matched by age to the mothers of children with NTDs, and by age and sex of their children to the individuals with NTDs. Matching of control women to experimental group women was attempted to exclude possible changes in life views which may occur during the ageing process. Matching by date of birth of the child is also important as this determines the time the mother has had to adjust to the birth of the child. The matching of children for gender may be important since children of different genders may be viewed differently by the family, and hence how the loss associated with the birth or death of an affected child would affect the mother.

3.3.5 Analysis of the Responses

The information obtained from the interview schedules was computerised using the Microsoft Access program and comparisons could then be made between the experimental and control groups using the statistical analysis specified below.

Responses to the depression inventory were scored using the suggested scoring system for the Beck Inventory (See Appendix 6) and interviewees could then be divided into 6 groups; those who were normal, had mild mood disturbances, or were borderline, moderately, severely or extremely depressed.

3.3.4 Statistical Analyses used in this Study

3.3.4.1 Chi-Square Analysis

Chi-square ($\chi^2$) analysis was used in this study mainly to determine whether significant differences existed between the experimental group and controls for interview responses, or between different types of NTDs (in individuals or their family members) for a particular factor or response, and in the molecular study to identify significant differences between population groups and between experimental and control groups. $\chi^2$ analysis
begins with the assumption that differences in occurrence of two factors occur only as a result of sampling bias and not because of a statistical difference (Mather, 1966). This premise is then proved or disproved by comparing observed and expected calculations. \( \chi^2 \) tables are used to obtain p values from the information, and for the purposes of this study, a p value of less than 0.05 was considered statistically significant as it denotes 95% confidence that the difference between two sets of data is significant.

3.3.4.2 Obtaining Confidence Intervals from Poisson Distributions

Poisson distributions represent discontinuous variation and can be used to analyse the results of studies in which there are small probabilities of occurrence of events, provided sample sizes are sufficiently large (Mather, 1966). The best estimate of the mean is calculated from the data and this is used to estimate the variance - which, for Poisson distributions are similar values (since the true mean and variance are identical for these series) (Mather, 1966). Standard deviations can then be determined as the square root of the variance, and the 95% confidence intervals are given by twice the standard deviation from the mean.

3.4 SUMMARY

The project consisted of a retrospective file study, a molecular analysis, and an assessment of some of the psychosocial issues associated with the birth of a child with an NTD. The retrospective study entailed the use of a checklist to obtain information from the files of patients seen at the Genetic Counselling Clinics, SAIMR for NTDs, and the information was used to assess recurrence risks and the effects of certain environmental and genetic factors on the aetiology of NTDs. A molecular study using DNA from
individuals with NTDs and from their families was undertaken to determine the frequency of the 677C-T mutation in the MTHFR gene in the black population and the findings were compared to those of controls from the Negroid and Caucasoid populations of Gauteng. A psychosocial analysis of black mothers of individuals with NTDs was conducted and compared to mothers of normal children from the same population, and a specifically constructed interview schedule was used to determine some of the issues associated with the birth of a child with an NTD in the black population.
4 RESULTS

4.1 INTRODUCTION

The results of the retrospective file study were analysed with the aid of relevant statistical tests. Where possible results were compared to controls selected from the general population as recorded in published sources (SA Population Census, 1993, 1996; Hain, 1993) and from the files of patients seen at the Genetic Counselling Clinics for other unrelated disorders.

The molecular data were compiled and analysed to determine whether the 677C-T mutation in the MTHFR gene is a risk factor for NTDs in the black population of Gauteng and whether there are significant differences in the frequency of the polymorphism in the white and black populations of Gauteng.

The psychosocial study undertaken to define some of the issues associated with the birth of a child with an NTD in the black population comprised the completion of a schedule of questions, the results of which are presented and, where possible, depicted graphically in the following sections.
4.2 COMPOSITION OF THE SAMPLE GROUPS

Table 4.1 Subjects used in the study

<table>
<thead>
<tr>
<th></th>
<th>NUMBER OF SUBJECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EXPERIMENTAL</td>
</tr>
<tr>
<td><strong>FILE STUDY</strong></td>
<td>640</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MOLECULAR STUDY</strong></td>
<td>33 Black mothers</td>
</tr>
<tr>
<td></td>
<td>2 Black fathers</td>
</tr>
<tr>
<td></td>
<td>9 Black individuals</td>
</tr>
<tr>
<td><strong>PSYCHOSOCIAL STUDY</strong></td>
<td>35 Black mothers</td>
</tr>
</tbody>
</table>

4.3 RETROSPECTIVE FILE STUDY

Information from the files of 640 families seen at the Genetic Counselling Clinics was obtained regarding recurrence risks for NTDs and some of the risk factors involved in their aetiology in the Gauteng population. There were 504 cases of spina bifida and 161 cases of anencephaly in this series (some families had more than one affected individual). The files were from 520 Caucasoid families, 109 black families, 8 Indian families, 2 Coloured families and one Chinese family.

4.3.1 Recurrence Risks

Of the 621 affected families for whom good family histories were available, 23 had had two children with NTDs, and one family had had three affected children. In addition there were two families in which pairs of half siblings were affected (both maternal halvesibs). Affected individuals had had 1073 unaffected full siblings and 135 unaffected
half siblings. These figures are summarised in Table 4.2. From these observations, a recurrence risk of 2.28% was calculated (25 recurrences in 1098 pregnancies) with a 95% confidence range of 1.38% to 3.18%. After two affected sibs, a third affected child occurred in 1 in 24 families, and therefore the recurrence risk was 4.16% ± 8.1%. The recurrence risk in half siblings was 1.46% ± 2.0%.

### Table 4.2 Recurrence risks for families with NTDs

<table>
<thead>
<tr>
<th>FAMILIES (PROBAND)</th>
<th>NORMAL</th>
<th>SIBLINGS</th>
<th>AFFECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FULL SIBS</td>
<td>HALF SIBS</td>
<td>FULL SIBS</td>
</tr>
<tr>
<td></td>
<td>621</td>
<td>1073</td>
<td>135</td>
</tr>
</tbody>
</table>

When the 98 black families with NTD individuals were analysed separately, there were two recurrences (both in half sibs) out of 275 pregnancies, giving a recurrence risk of 0.73% ± 1.0%, which was not significantly different from the recurrence risk of the entire study group for the numbers available.

Recurrence risks after the birth of a child with spina bifida (n=450) were 1.91% ± 1.0% and after anencephaly (n=64) were 3.73 ± 2.31%. These were not significantly different for the small sample sizes available.

The recurrences were then divided according to the sex of the proband, for families in which this information was available, and no significant differences (p>0.05) were found between risks of recurrence after an affected male or an affected female for either spina bifida or anencephaly. These observations are summarised in Table 4.3.
Table 4.3 Recurrences in families with a child with an NTD in... of proband

<table>
<thead>
<tr>
<th>TYPE OF NTD IN PROBAND</th>
<th>RECURRENTS...</th>
<th>NTD FAMILIES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female Proband</td>
<td>Male Proband</td>
</tr>
<tr>
<td>Spina Bifida</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Anencephaly</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

To determine whether there was concordance in type of NTD in recurrences more often than would be expected from the occurrences of the different types of NTDs, the families with more than one case were examined. These results are summarised in Table 4.4. In the family with two recurrences, all three affected individuals had spina bifida. From these figures a concordance of 94.7% was calculated for spina bifida in families and 75.0% for anencephaly.

Table 4.4 Concordance between type of NTD in recurrence

<table>
<thead>
<tr>
<th>INDEX CASE</th>
<th>TYPE OF NTD IN AFFECTED SIBLINGS AND HALF SIBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anencephaly</td>
</tr>
<tr>
<td>Anencephaly</td>
<td>6</td>
</tr>
<tr>
<td>Spina Bifida</td>
<td>-</td>
</tr>
<tr>
<td>Encephalocele</td>
<td>1</td>
</tr>
</tbody>
</table>

4.3.2 Parental Ages

4.3.2.1 Maternal Age and Risk of NTDs

The average maternal age at the time of delivery of the infant with an NTD (calculated from date of birth information available for 441 mothers) was 25.6 years. The average maternal ages for 42 mothers of offspring with cystic fibrosis, and 70 mothers of albinos (also calculated by maternal age at the time of delivery of the affected offspring) were both 26.1 years. These figures, taken as estimates of average maternal age in the population, did not differ significantly from the average maternal age calculated for
mothers of NTD offspring. A comparison was made of the distributions of maternal ages in the general population (taken from the 1993 Population Census) and of all NTD mothers (441). The two showed similar distributions with no significant differences between the areas under the two curves (p>0.05). These results are shown in Figure 4.1. Similar distributions were observed when maternal ages of women with offspring with spina bifida and with anencephaly were considered separately. The average maternal age for spina bifida was 25.6 years and for anencephaly was 26.0 years.

Figure 4.1 Age at delivery of mothers with a child with an NTD (441) and mothers in the general population

A separate analysis of maternal ages of black mothers of infants with NTDs did not show a different distribution. Average maternal age for black mothers was 26.2 years which was compared to 26.1 years calculated for albino mothers and did not differ significantly.
4.3.2.2 Paternal Age and Risk of NTDs

Paternal age was available for 399 fathers and the average paternal age was calculated as 28.7 years. The graphs (Figure 4.2) of the distribution of paternal age of the general population (taken from the 1993 Population Census) and for fathers of NTD individuals are similar and calculations of area under each graph showed no significant differences (p>0.05).

![Figure 4.2 Ages of 399 fathers of children with NTDs compared with those of fathers in the general population](image)

4.3.3 Spontaneous Abortions and NTDs

A history of pregnancy loss was available from pedigrees of 550 parents who had had infants with NTDs. Half sibs of the affected individual were included in the calculation.
Those spontaneous abortions known to be caused by NTDs were excluded from the analysis in an attempt to determine whether spontaneous abortions of unknown aetiology were higher in the experimental group. The number of miscarriages for women with NTD infants and for spina bifida and anencephaly separately were compared to the population occurrence of miscarriages and stillbirths in the relevant population group. Files of 64 families of cystic fibrosis patients and 67 families with a member with albinism were analysed, and used for comparison, to estimate the population occurrence of spontaneous abortions. Results of the analysis are summarised in Table 4.5 and in Figure 4.3 and they show that there was increased incidence of spontaneous abortions in families with NTD affected individuals as compared to the control group ($\chi^2 = 4.86$, $p<0.05$).

Table 4.5 Numbers of spontaneous abortions in NTD families and controls

<table>
<thead>
<tr>
<th></th>
<th>NUMBER OF SPONTANEOUS ABORTIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Experimental</td>
<td></td>
</tr>
<tr>
<td>NTD (n=550)</td>
<td>412</td>
</tr>
<tr>
<td>Spina Bifida (n=390)</td>
<td>288</td>
</tr>
<tr>
<td>Anencephaly (n=160)</td>
<td>124</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Albinism (n=67)</td>
<td>58</td>
</tr>
<tr>
<td>Cystic Fibrosis (n=64)</td>
<td>52</td>
</tr>
</tbody>
</table>
4.3.4 Maternal Parity and risk of NTDs

A comparison of maternal parity from 555 pedigrees of NTD families with parity of mothers sampled randomly from the general population (Hain, 1993) showed that more NTDs occurred in the first pregnancy than in subsequent pregnancies, and the difference in occurrence of NTDs in the first and subsequent pregnancies was highly significant ($\chi^2 = 105.44$, $p<0.005$). There was also an increase in the occurrence of NTDs in the fifth and higher pregnancies when compared with other pregnancies and this finding was also highly significant ($\chi^2 = 36.85$, $p<0.005$). These findings are summarised in Table 4.6 and in Figure 4.4.
Table 4.6 Parity group of proband in the general population and in NTD families

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5+</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Population</td>
<td>495</td>
<td>1192</td>
<td>429</td>
<td>106</td>
<td>27</td>
</tr>
<tr>
<td>(n=2249)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTD (n=555)</td>
<td>241</td>
<td>163</td>
<td>90</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>Spina Bifida (n=397)</td>
<td>177</td>
<td>111</td>
<td>64</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Anencephaly (n=158)</td>
<td>64</td>
<td>52</td>
<td>26</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

Figure 4.4 Maternal parity of pregnancies complicated by NTDs as compared to pregnancies in the general population
4.3.5 Parental Consanguinity

Six cases of consanguinity were reported in the files and these included two couples who were first cousins, one couple who were second cousins, and three couples for whom the degree of relationship was not reported.

4.3.6 Season of Conception

Months of conception were calculated for NTD births (370) from information derived from the date of the last menstrual period, dates of birth, or dates at which terminations of pregnancy were carried out and the corresponding gestational ages. In addition NTDs were divided into spina bifida (257) and anencephaly (113) and analysed separately. Controls for monthly births in the general population of Gauteng were obtained from Hain (1993). Results are shown in Figure 4.5. The largest difference between NTDs and controls for month of conception was observed in January when the difference was due to an increase in anencephalic births as shown, and again in June where an excess of spina bifida conceptions occurred. A trend of more NTD births in the winter months was also apparent. However none of the NTD classes differed significantly from the controls, an observation which may be accounted for by the small sample sizes available.
Figure 4.5 Months of conception and frequency of NTDs

4.3.7 Maternal Teratogen Exposure

History of maternal teratogen exposure was available for 591 of the cases of NTDs. Of these women, 10 (1.7%) were epileptic, and 9 of these were on medication during the pregnancy. Six women (1.0%) were diabetic and on insulin during the pregnancy. In addition, 8 women were hypertensive during the pregnancy and 2 had aplastic anaemia and were on medication. One woman reported taking approximately 100 antiepileptics and other medications, such as tranquilisers, before she realised that she was pregnant, in a suicide attempt and subsequently had an infant with anencephaly.
Ovulation-stimulating treatments and oral contraceptive use were reported by 9 and 3 women respectively. These data are incomplete since such information is not always requested by the genetic counsellor or offered by the counselee.

Inspection of the files showed that 34 women had reported having flu or a high temperature during the early part of the pregnancy; 17 had been exposed to an infectious agent including rubella, toxoplasmosis or measles. Since the study was retrospective, there was no way of determining whether all women had been asked about such exposures or of eliminating the effects of recall bias.

Three black mothers of NTD individuals reported taking traditional medicines during the first months of pregnancy: two took preparations for abdominal pain and one took a traditional fertility treatment. Black women interviewed in the psychosocial study were specifically asked if they had eaten soil, ash or any other unusual substances during pregnancy. Five of the 35 mothers with NTD infants had eaten soil, as had 5 of the 35 control mothers. Two NTD mothers and one control had eaten ash; and 1 NTD mother had eaten chalk during her pregnancy.

Of 93 women asked about diet in early pregnancy, 33 reported experiencing excessive nausea and vomiting and 7 had gastric flu or abdominal pain. In addition 12 women had dieted during the first trimester, several of these had taken slimming preparations, and 2 were anorexic. Comparable figures were not available for pregnancies in which the outcome was normal.

4.3.8 Sex Ratios in NTDs
Sex of the infant with the NTD was available in 619 cases and there were 279 affected males and 340 affected females. From these data, a M/F ratio of 0.82 was calculated. There were excesses of female births for both spina bifida and anencephaly with sex
ratios of 0.84 and 0.76 respectively. Encephalocoeles (n=19) showed an excess of male births (13) which was not significant. Sex ratios for the general population were not available and this precluded detailed analysis of these results. Findings are summarised in Figure 4.6.

Figure 4.6 Sex of 619 individuals with NTDs

Spina bifida lesions were then divided into upper and lower lesions for cases for which such information was available (n=100). Upper lesions showed an excess of females with a sex ratio of 0.7, while lower lesions showed a male excess (M/F = 1.15). However, neither of these findings was significant for the sample sizes available. The results are displayed in Figure 4.7.
4.3.9 Twinning in NTD Families

Twinning rates in NTD families were estimated from the 577 files which had sufficiently detailed pedigrees for such an analysis to be attempted. The twinning rates from these files were compared to those of the general population studied by Hain (1993). However, only for third degree relatives of individuals with NTDs was a higher twinning rate demonstrated, and the findings were not significant. The retrospective nature of the study, and the fact that twinning can easily be omitted from pedigree analysis if not specifically requested, may explain a possible under-representation of twins in NTD families.
4.3.10 Social Class and NTDs

Social classes of parents of individuals with NTDs were estimated from their occupations using the system devised by Schlemmer and Stopforth (1979). In this system Category I is the highest socio-economic class and consists of professional and managerial occupations. Category V is the lowest class and consists of unskilled manual and menial workers (Schlemmer & Stopforth, 1979). There were 409 families for which relevant information was available. The socio-economic classes for the sample were then compared to data obtained from the 1996 South African Census and the results are summarised in Figure 4.8.

![Figure 4.8 Socio-economic groups of NTD families](image)

Comparison of the total NTD group and the general population showed that most NTDs occurred in classes I and II. However a separate analysis of the 74 black families with NTDs showed a significantly different distribution of higher to lower socio-economic
class ($\chi^2 = 77.87, p<0.005$). This distribution was similar to that of the general population and a comparison of higher social classes (I and II) to the rest of the group showed no significant difference from the distributions in the general population ($\chi^2 = 0.697, p>0.05$). This finding, together with the fact that most of the families in the file study were of Caucasoid origin, may indicate that, at least historically, the Genetic Counselling Clinics have serviced the higher socio-economic strata of the society more effectively, and may therefore represent and ascertainment bias. Larger randomised studies are required to determine the effects of socio-economic class on the occurrence of NTDs in the population of Gauteng.

### 4.3.11 NTDs and Other Malformations

The frequency of occurrence of other malformations with NTDs was obtained from doctors' notes made in the files and about 11% of affected individuals were found to have other unrelated abnormalities. The most common abnormality found was facial clefts (in 1.2%) followed by limb malformations and polydactyly. The family history of each NTD case also gave information regarding the occurrence of malformations other than NTDs in siblings of individuals with NTDs. Altogether, 4.9% of these siblings had other abnormalities. Results are summarised in Tables 4.7 and 4.8 for NTD probands and siblings respectively.
Table 4.7 Non-NTD associated malformations in NTD probands

<table>
<thead>
<tr>
<th>SYSTEM AFFECTED</th>
<th>SPINA BIFIDA INDIVIDUALS (n=594)</th>
<th>ANENCEPHALIC INDIVIDUALS (n=161)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHROMOSOMAL ANOMALY</td>
<td>Down Syndrome (1)</td>
<td>-</td>
</tr>
<tr>
<td>CNS</td>
<td>Agenesis of the Corpus Callosum (1)</td>
<td>Cycllops Malformation (2)</td>
</tr>
<tr>
<td></td>
<td>Blindness (1)</td>
<td>Neuroblastoma (1)</td>
</tr>
<tr>
<td></td>
<td>Microcephaly (4)</td>
<td></td>
</tr>
<tr>
<td>CVS/RESPIRATORY</td>
<td>Congenital Heart Defect (4)</td>
<td>Lung Atrophy (1)</td>
</tr>
<tr>
<td>HEAD &amp; NECK</td>
<td>Cleft Lip &amp; Palate (4), CL (2), CP (1)</td>
<td>Short Neck (1)</td>
</tr>
<tr>
<td></td>
<td>Malformed Ears (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dysmorphic (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Facial Asymmetry (4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strabismus (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ectodermal Dysplasia (1)</td>
<td></td>
</tr>
<tr>
<td>LIMBS &amp; SKELETAL</td>
<td>Pectus Carinatum (1)</td>
<td>Chest Deformity (1)</td>
</tr>
<tr>
<td></td>
<td>Limb Malformations (7)</td>
<td>Shortened Limbs (3)</td>
</tr>
<tr>
<td></td>
<td>Polydactyly (6)</td>
<td>Limb Malformations (5)</td>
</tr>
<tr>
<td></td>
<td>Overlapping Digits (3)</td>
<td>Absent Digits (1)</td>
</tr>
<tr>
<td></td>
<td>Syndactyly (1)</td>
<td>Polydactyly (1)</td>
</tr>
<tr>
<td></td>
<td>Arachnodactyly (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ectrodactyly (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hammer Toes (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retracted Hands (1)</td>
<td></td>
</tr>
<tr>
<td>GIT</td>
<td>Exomphalos (5)</td>
<td>Exomphalos (4)</td>
</tr>
<tr>
<td>GENITO-URINARY</td>
<td>Absent Kidney (2)</td>
<td>Dysgenetic Kidneys (1)</td>
</tr>
<tr>
<td></td>
<td>Absent Urethra (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Undescended Testes (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enlarged Clitoris (1)</td>
<td></td>
</tr>
</tbody>
</table>

It is important to note that since chromosome tests are not routinely performed on all cases of NTDs, it is possible that some chromosomal syndromes associated with NTDs were not detected, therefore some malformations listed may not be unassociated.
Table 4.8 Non-NTD malformations in siblings of individuals with NTDs

<table>
<thead>
<tr>
<th>SYSTEM AFFECTED</th>
<th>SPINA BIFIDA SIBS AFFECTED (n=38)</th>
<th>ANENCEPHALIC SIBS AFFECTED (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHROMOSOMAL ANOMALY</td>
<td>Down Syndrome (3)</td>
<td>Down syndrome (1 half sib)</td>
</tr>
<tr>
<td></td>
<td>46,XYDq (1)</td>
<td>? Del 2q</td>
</tr>
<tr>
<td></td>
<td>47,XXX (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45,X (1)</td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>Microcephaly (2)</td>
<td>Situs Inversus (1)</td>
</tr>
<tr>
<td></td>
<td>Hydrocephaly (2)</td>
<td>Mental Retardation (1 half sib)</td>
</tr>
<tr>
<td></td>
<td>Cyclops Malformation (1)</td>
<td>Blind &amp; Deaf (1)</td>
</tr>
<tr>
<td></td>
<td>Mental Retardation (2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Delayed Development (3 + 1 half sib)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hemiplegia (1)</td>
<td></td>
</tr>
<tr>
<td>CVS/RESPIRATORY</td>
<td>Congenital Heart Defect (2)</td>
<td></td>
</tr>
<tr>
<td>HEAD &amp; NECK</td>
<td>Cleft Lip (1)</td>
<td>Microcephaly (1)</td>
</tr>
<tr>
<td></td>
<td>Cleft Nose (1)</td>
<td>Holoprosencephaly (1)</td>
</tr>
<tr>
<td></td>
<td>Dysmorphic Features (1)</td>
<td>Hydrocephaly (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cataracts (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cleft Palate (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dymorphic (2 - one after congenital rubella exposure)</td>
</tr>
<tr>
<td>LIMBS &amp; SKELETAL</td>
<td>Congenital Hip Dislocation (1)</td>
<td>Congenital Scoliosis (1)</td>
</tr>
<tr>
<td></td>
<td>Talipes Equinovarus (5)</td>
<td>Talipes Equinovarus (1)</td>
</tr>
<tr>
<td></td>
<td>Polydactyly (2 + 1 half sib)</td>
<td>Webbed Toes (1)</td>
</tr>
<tr>
<td></td>
<td>Ectrodactyly &amp; Syndactyly (1)</td>
<td></td>
</tr>
<tr>
<td>GIT</td>
<td>Pyloric Stenosis (1)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Exomphalos (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Biliary Atresia (1)</td>
<td></td>
</tr>
<tr>
<td>GENITO-URINARY</td>
<td>Kidney Anomalies (3 - same sibship)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ambiguous Genitalia (1)</td>
<td></td>
</tr>
<tr>
<td>OTI</td>
<td>? Cystic Fibrosis (1)</td>
<td>? Cystic Fibrosis (1)</td>
</tr>
<tr>
<td></td>
<td>Muscular Dystrophy (1)</td>
<td>Lipodystrophy (1)</td>
</tr>
</tbody>
</table>

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Comparisons of congenital malformations other than NTDs in NTD affected individuals to those in the general population showed that individuals with NTDs had a 3-fold increased risk of having another congenital malformation over the congenital malformation risk of the general population (Harper, 1998). Siblings of individuals with NTDs had a congenital malformation other than an NTD in 1 in 20 cases which is higher than the 1 in 30 risk of congenital abnormalities postulated for the general population (Harper, 1998).

4.4 MOLECULAR STUDY

Molecular analysis was performed on 78 random Negroid samples, 53 random Caucasoid samples and 44 samples from black families of individuals with NTDs (see Table 4.1). Of the 78 random Negroid samples, seven (9.0%) were heterozygous for the 677C-T mutation in the MTHFR gene, and the other 71 (91%) were homozygous for the normal allele. No homozygotes for the mutation were found in this group. In contrast, there were 2 (3.8%) homozygotes for the mutation in the 53 random Caucasoids tested, 20 (37.7%) heterozygotes and 31 (58.5%) individuals without the mutation. The difference between Negroid and Caucasoid populations was highly significant ($\chi^2 = 19.9$, p<0.005) indicating that the polymorphism occurs less frequently in the black population than in the Caucasoid population. These results are tabulated in Table 4.9.

Of the mothers of black individuals affected with NTDs, 15.2% (5 out of 33) were heterozygous for the polymorphism, and the others were homozygous for the normal genotype. The 677C-T mutation was not observed in any of the 9 NTD individuals analysed, and all the individuals were homozygous for the normal genotype. Similarly, neither of the 2 fathers assayed had the polymorphism. The findings are summarised in Table 4.9. While the mutation occurred more frequently in the mothers of black
individuals with NTDs than in the sample of random Negroids, the difference was not significant for the sample sizes available ($\chi^2 = 0.91, p>0.05$).

The samples of random Caucasoids, random Negroids and mothers of affected individuals all showed distributions which did not differ significantly from the Hardy-Weinberg distribution expected ($p>0.05$). The fact that no homozygotes were found in the random black population studied was also not unexpected since, from the findings, the predicted occurrence would be approximately 1 in 500 in the black population.

![Figure 4.9 Determination of the MTHFR genotypes on a 4% Metaphor gel](image)

Lane 1, 2 and 5 show individuals homozygous for the normal allele (198bp); lane 3 is a heterozygote individual (198bp and 175bp); and lane 4 is an individual who has a homozygous mutation (175bp).
Table 4.9 Occurrence of the MTHFR 677C-T mutation in the families of individuals with NTDs, and in the general population of Gauteng (n = number of individuals)

<table>
<thead>
<tr>
<th></th>
<th>NORMAL GENOTYPE (677C)</th>
<th>HETEROZYGOTES (677C/677T)</th>
<th>HOMOZYGOTES (677T)</th>
<th>GENE FREQUENCY 677T (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RANDOM CAUCASOIDS (n=53)</td>
<td>31 (0.585)</td>
<td>20 (0.377)</td>
<td>2 (0.038)</td>
<td>0.226 (0.041)</td>
</tr>
<tr>
<td>RANDOM NEGROIDS (n=78)</td>
<td>71 (0.910)</td>
<td>7 (0.090)</td>
<td></td>
<td>0.045 (0.017)</td>
</tr>
<tr>
<td>NTD INDIVIDUALS (n=9)</td>
<td>9 (1.000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTD MOTHERS (n=33)</td>
<td>28 (0.848)</td>
<td>5 (0.152)</td>
<td></td>
<td>0.076 (0.033)</td>
</tr>
<tr>
<td>NTD FATHERS (n=2)</td>
<td>2 (1.000)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.5 PSYCHOSOCIAL STUDY

Interviews were conducted with 35 mothers of children with NTDs from the black population and also with 35 control women representative of the same population.

4.5.1 Demographics of the Study Group

4.5.1.1 Ethnic Groups

The ethnic distributions of the experimental and control mothers are shown in Table 4.10. In both groups, the major ethnic groups (Nguni and Sotho) in the population were represented, and the largest and second largest groups were Nguni and Sotho respectively, which is the case in the general population.

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Table 4.10 Ethnic groups of women in the psychosocial study

<table>
<thead>
<tr>
<th>ETHNIC GROUPS</th>
<th>EXPERIMENTAL GROUP (n=35)</th>
<th>CONTROL GROUP (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nguni</td>
<td>20 (57%)</td>
<td>15 (43%)</td>
</tr>
<tr>
<td>Sotho (includes S Sotho, Pedi &amp; Tswana)</td>
<td>10 (29%)</td>
<td>11 (31%)</td>
</tr>
<tr>
<td>Venda</td>
<td>1 (3%)</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (11%)</td>
<td>9 (26%)</td>
</tr>
</tbody>
</table>

4.5.1.2 Socio-Economic and Marital Status

A comparison of the educational status of the women showed no significant difference between the two groups (p<0.05). Of the 35 women in the experimental group, 24 (69%) were not employed outside of their homes, and the remainder held jobs representative of all five socio-economic classes defined in Section 4.3.10. Among the control group mothers, 32 (91%) were not employed. While there was a difference in the number of women who held jobs at the time of the interviews between the control and the experimental mothers, the fact that there was no significant difference in educational status suggests that the two groups were suitably matched for socio-economic status.

Marital status was also requested in the interview schedule and comparisons of the experimental and control groups showed that 20 (57%) of the experimental group and 22 (63%) of the control group lived with or were married to their partners at the time when the interviews were conducted.

4.5.1.3 Ages of Mothers and Children, Sex of Children, and Number of Children per Subject

The experimental and control groups were matched for dates of birth of the mothers and of the affected children. In all except 6 cases, the children of the control women were born in the same year as those of the matched NTD subjects. Of these six, 4 were born
within a year of the matched NTD individual, and the remaining 2 had a two year age gap. The mean time from the date of birth of both the experimental and control children to the time of the interviews was 4.9 years. The children ranged in age from 22 years to newborns, and 22 (63%) of experimental group subjects and 21 (60%) of control group subjects were under five years of age.

The children of the control group were successfully matched by gender to the children in the experimental group in 32 (91.4%) cases. The male to female ratios in the experimental and control groups were 1.06 and 0.94 respectively, and these did not differ significantly (p>0.05) from each other.

Ages of women in the control group were matched to those of the experimental group within 5 years in 23 (65.7%) of the cases. The average age of the interviews in the NTD group was 32.4 years and in the control group was 29.5 years.

The average number of children born to each mother was also recorded and was 2.77 and 2.63 children in the experimental and control groups respectively.

4.5.1.4 Nature of the Defect

The nature of the defect in the children of the experimental group was requested and a note was made of whether or not the child was still alive. Of the 35 women interviewed, 28 (80%) had a child with spina bifida, 5 (14%) had a child with anencephaly, 1 (3%) with encephalocele, and one mother had an anencephalic child followed by a child with spina bifida. This pattern is similar to that found in the general NTD population. Fifteen (42%) of the 36 affected children were still alive at the time when the interviews were conducted.
4.5.2 Prior Knowledge about NTDs

Respondents were asked if they had heard about NTDs before the interview, and if so what they had heard. In addition the experimental group mothers were asked if they had known about NTDs before they had their affected child/children.

Five (14.3%) of the experimental group had heard of NTDs before the birth of their affected child, 3 had seen a child with an NTD or with hydrocephalus at the hospital, one had been counselled after spina bifida was diagnosed by ultrasound in her pregnancy, and one had read an article on spina bifida in a popular magazine (Bona Magazine, p54-55, January, 1997) and knew that NTDs arose from faults of neural tube closure.

In the control group, 2 of the women (5.7%) had heard about NTDs before the interview and both had read the Bona Magazine story. One of these women was aware that a test was available and that affected pregnancies could be terminated if the mother wished it.

4.5.3 Beliefs of Causes of NTDs

Both experimental and control group respondents were asked what they thought the causes of NTDs were and in addition the experimental group mothers were asked why they thought this problem had happened to them.

When asked the cause of NTDs, 25 (71.4%) of the experimental group women and 13 (37.1%) of the control women said that they did not know what the cause was. Three of the women in the experimental group said the cause was 'bad genes', 'bad blood' or a family history of a disorder. One mother of an NTD child and 6 control women attributed the cause of NTDs to poor eating habits during pregnancy, and 6 control women suggested alcohol as a possible cause. Use of medication, such as contraceptives, was suggested as the cause of NTDs by one experimental group subject and 5 controls. Stress during the pregnancy or shock were also suggested as possible causes by the experimental
group mothers. Control subjects suggested that if the mother was ill during her pregnancy, if she tried to abort the fetus, if she had 'sores in her womb', or if she slept with several different men during the pregnancy she would have an affected infant. One control mother said that these children were gifts from God.

Experimental group respondents often gave different explanations when asked why they thought they had had a child with an NTD. Eleven subjects (31.4%) said that they could not explain why this had happened to them. Six (17.1%) women blamed their partners and said that the abnormality was caused either by medications that their partners used, by their partner's infidelity during the pregnancy, or by their partner's mistreatment of them. Five women described stressful events experienced during the pregnancy which they thought had caused the defect. Four mothers believed their child to be a gift from God. Three of the women with affected children said that they also had relatives affected with birth defects and one blamed the genes she shared with her partner. One woman stated that there was something wrong with her blood, another that something had gone wrong during conception and one woman thought that she had been too old to have the child.

4.5.4 Perceived Risk of Recurrence of NTDs

Both the experimental group and the control group were asked if they thought there was a risk of recurrence of NTDs or similar problems. Eleven (31.4%) of the subjects in the experimental group said that there was a risk of recurrence, 16 (45.7%) said that there was no risk and the other 8 were not sure. The control group's responses were similar with 11 (31.4%) saying that there was a risk of recurrence, 19 (54.3%) saying that there was no risk and the remainder being unsure.

Respondents were also asked to qualify their answers and they gave a wide range of different explanations. Several subjects from both groups said that these events were related to fate and that the outcome depended on God's will. Seven of the mothers in the
experimental group who had had normal children after the child with the NTD tended to
downplay the recurrence risk and said that the birth of the normal child proved that it
would not happen again. In addition respondents maintained that if the causative agent(s)
(cited in the previous section) were avoided in subsequent pregnancies, the NTD would
not recur.

4.5.5 Subject’s Views on Prenatal Diagnosis and Selective Termination of Affected
Pregnancies

All subjects were told that prenatal diagnosis was available to detect NTDs and they were
then asked if they would request such testing in future pregnancies. Both groups were
also asked if they would terminate an affected pregnancy if they were given the option.

All 35 women in the experimental group and 33 (94.3%) women in the control group said
that they would request prenatal diagnosis in future pregnancies. Two of the
experimental group had had prenatal diagnosis in a subsequent pregnancy. When asked
why they would request prenatal diagnosis, the experimental mothers responded that they
would want to know the baby’s condition early in the pregnancy and would not want
another child to suffer as their affected child had suffered. Only one woman explained
that she would have prenatal diagnosis because her doctor told her that she should. The
control mothers also said that they would want to know if their child was healthy and 6
would follow their doctor’s orders. One control mother said that she would not want
prenatal diagnosis because she was under the impression that she would then have to have
a back-street abortion. The results are summarised in Table 4.11.

When asked whether they would terminate an affected pregnancy, 28 (80%) of the
experimental group mothers said that they would and 7 would not. The main reasons
cited for terminating an affected pregnancy included not wanting to see the child suffer,
or to experience the burden associated with an abnormal child again. Two of the women
who would not terminate an affected pregnancy believed that these children were gifts from God and one said that she had had no problem caring for her affected child. One woman feared that this might be her last chance to have a child and would therefore not jeopardise a pregnancy, and three feared that they might die as a result of the abortion.

In the control group, 23 (65.7%) women said that they would terminate an affected pregnancy, 9 would not, and 3 were unsure. These findings were not significantly different from those of the experimental group ($\chi^2 = 3.74$, $p > 0.05$). Most of the women who said that they would terminate an affected pregnancy said that they did not want an abnormal child. One woman who was unsure said that she would have to consult her partner before taking such a decision. Four of the women who would not terminate an affected pregnancy said that such an event would be God’s will or a gift from God, and one woman said she did not believe in termination. Three subjects in the control group said that they would not believe it until the child was born and one suggested that perhaps then something could be done about it. One woman said that she feared that she might die as a result of the termination.

Table 4.11 Subject’s views on prenatal diagnosis and termination of pregnancy

<table>
<thead>
<tr>
<th></th>
<th>GROUP</th>
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<tbody>
<tr>
<td></td>
<td>EXPERIMENTAL</td>
</tr>
<tr>
<td>REQUEST PRENATAL DIAGNOSIS:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (100%)</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>OPT FOR TERMINATION:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (80%)</td>
</tr>
<tr>
<td>No</td>
<td>7 (20%)</td>
</tr>
<tr>
<td>Don’t Know</td>
<td>0</td>
</tr>
</tbody>
</table>

87
4.5.6 Depression Inventory

Depression was assessed using a modified version of the Beck Depression Inventory which consisted of 21 questions assessing emotional state (See Appendix 4). Fifteen (42.9%) of the experimental group mothers were clinically depressed (border-line to severe depression) or showed mild mood disturbances as compared to 7 (20.0%) of the control mothers and this difference was significant ($\chi^2 = 4.24$, $p<0.05$). Categorical results from the depression inventory scores for the two groups are summarised in Figure 4.10.

![Bar chart showing depression inventory scores for experimental and control mothers](image)

Figure 4.10 Beck Depression Inventory categories for experimental and control mothers

Mood disturbances were observed in women with children of up to 13 years of age and no apparent correlation could be determined between the age of the child and the depression score of the mothers in the experimental group.
4.5.7 Other Information Requested in the Interview Schedule

Interviewees were also asked to give information regarding their health during the pregnancy and any unusual dietary practices. This information was combined with that obtained in the file study and it is included in the relevant section of this chapter (See Section 4.2.7).

Respondents were also asked if there were any comments they wished to make, or questions they wished to ask, and most seemed content with the interview. Several mothers in the experimental group requested extra information about NTDs and about the research project which was provided to them by the interviewer.

4.6 SUMMARY

A retrospective study involving 640 files of patients seen at the Genetic Counselling Clinics allowed the researcher to analyse some of the risk factors for the aetiology of NTDs documented in the literature. A recurrence risk of 2.28% was calculated for the occurrence of a second NTD after the birth of one affected individual and was shown to be highly concordant for type of NTD. An interesting observation was that while parental age was not a significant risk factor for the aetiology of NTDs, maternal parity did play a role and further studies are required to explain this finding. As expected from reports of other studies, a history of spontaneous abortions was shown to be an important risk factor, as were other maternal factors such as teratogen exposure and diet during pregnancy. Further research would be required to determine the role of twinning, reason of conception and socio-economic class in the causation of NTDs. Significantly more females were found to be affected by NTDs than males, and there was a significant
increase in the occurrence of other congenital malformations in both individuals with NTDs, and in their siblings.

Comparisons of the frequency of the 677C-T mutation in the MTHFR gene in the different population groups of Gauteng showed that the mutation occurred significantly more frequently in the Caucasoid population than in the Negroid population. However, while a higher proportion of black women who had had children with NTDs had the mutation than the normal population, this was not significant for the sample sizes available.

Interviews with 35 black women who had had children with NTDs, and with 35 carefully selected controls indicated that awareness of the existence of NTDs and the aetiology of these disorders was very low in this population. Almost half of the women asked did not think that there was any risk that these disorders could recur in a subsequent pregnancy. However, the majority of women asked said that they would seek prenatal diagnosis in future pregnancies, all 35 experimental group women and 33 control group women said that they would. Termination of an affected pregnancy would be requested by 80% of the experimental group and 66% of controls. An assessment of depression indicated that significantly more experimental group women were depressed.
5 DISCUSSION AND CONCLUSIONS

5.1 INTRODUCTION

The present study undertook to analyse some of the risk factors associated with the aetiology of NTDs in the population of Gauteng. A retrospective file study indicated the importance of several risk factors to this population and these included a history of spontaneous abortion, maternal parity, teratogen exposure and nutritional factors. Individuals with NTDs and their siblings were shown to be at higher risk of other congenital malformations than individuals in the general population. A recurrence risk of 2.28% was calculated for the occurrence of a second child with an NTD. These findings are compared to those documented in the literature.

A molecular analysis was performed to determine whether the 677C-T mutation plays a significant role in the aetiology of NTDs in the black population of Gauteng. Such a role could not be determined with the sample sizes available for study. A significantly lower occurrence of this mutation in the Negroid population than in the Caucasoid population was noted. These findings are similar to those of other studies.

The psychosocial interview results showed that awareness of the aetiology of NTDs and understanding of the risk factors and recurrence risks of these disorders was very low in the black population. Most women asked would request prenatal diagnosis in subsequent pregnancies, and a large proportion would terminate an affected pregnancy. These findings indicate a need for education about NTDs and increased availability of prenatal diagnostic services for those who would utilise them. The fact that NTDs are associated with a significant burden to the caregiver, was implied by the finding that significantly
more experimental group than control group women were clinically depressed at the time of the interviews.

The implications of these findings are discussed in the following sections.

5.2 RETROSPECTIVE FILE STUDY

Analysis of the 640 files of patients seen at the Genetic Counselling Clinics of the Department of Human Genetics, University of the Witwatersrand yielded information regarding the risks of recurrence of NTDs and also identified some of the risk factors in the aetiology of these disorders in the population of Gauteng. The results of this analysis will be discussed in this chapter, and the findings compared with those of other relevant studies.

5.2.1 Recurrence Risks

A recurrence risk of 2.28% (1 in 44) was calculated in the Gauteng population for the subsequent children of a couple who had one child with an NTD. This figure is in the range of recurrence risks reported by other studies worldwide (see Section 2.5) (Carter, & Evans, 1973; Cowchock, et al., 1980). As expected from the incidence of NTDs in the Gauteng population, which is somewhat lower than found in some parts of the world (Carter, et al., 1968, 1973), the recurrence risk falls at the lower end of the range of risks reported in the literature. After two affected siblings, the risk of a third affected child was estimated to be 4.16%, almost twice that found after one affected sibling, and this is in agreement with the figures obtained in other studies and documented in Section 2.5 (Carter, et al., 1968; Yen & Macmahon, 1968). The increased recurrence in NTD
families and the further increase in families in which more than one child is affected may be a result of either genetic factors, or the same environmental factors occurring again in the subsequent pregnancy (Yen & Macmahon, 1968). The 1.46% recurrence risk calculated for half siblings, though based on a small sample size (n=137), suggested a slightly lower risk than that of full siblings as has been proposed by several other authors (Yen & Macmahon, 1968; Carter & Evans, 1973). The recurrence risks in this study may, however, be a slight over-estimation because all the parents with an affected child may not have been referred to the genetic counselling clinics after the birth of the child, and it may have taken the birth of a second affected child for some health care professionals to realise that these disorders have a genetic aetiology.

Recurrence risks of 1.91% and 3.73% were found for spina bifida and anencephaly respectively. While this difference was not significant, a higher recurrence risk for anencephaly than for spina bifida has been previously documented in the literature (Carter & Evans, 1973). In agreement with other studies (Carter, et al., 1968; 1973), the present study found no difference in risk of recurrence if the proband was male or female. Significant concordance was found for type of NTD in affected families with a concordance of 95% for spina bifida and 75% for anencephaly. This finding has been observed previously (Williamson, 1965; Toriello & Higgins, 1985; Hall, et al., 1988) and is an important finding for the genetic counselling of these disorders. Hall and co-workers (1986) postulate that this observation may be related to the fact that different types of NTD may arise at different stages in the neurulation process.

5.2.2 Risk Factors in the Aetiology of NTDs

5.2.2.1 Parental Ages

Comparisons of maternal ages of women with offspring with NTDs showed no deviation from the maternal ages of the population-at-large and this has been reported in several
previous studies (Williamson, 1965; Hunter, 1984), and suggests that this factor is only important to the aetiology of NTDs in some populations, such as areas of Britain, New York, and New South Wales (Carter, et al., 1968; Janerich, 1972; Carter, et al., 1973; Field, 1978; Bound, et al., 1991). A separate analysis of black mothers of individuals with NTDs also failed to show an age effect when compared to a sample of mothers of albinism patients taken from the same population. These findings were similar to maternal age of mothers of individuals with NTDs reported for a rural black population in the Northern Province of South Africa, where an average maternal age of 26.5 years was recorded (Ubbink, et al., 1999).

Similarly average paternal age and distribution of paternal ages did not show any deviation from those expected for the general population suggesting that the occurrence of NTDs are independent of paternal age. This finding has also been documented by two large studies (Carter, et al., 1968; Field, 1978).

5.2.2.2 Spontaneous Abortions and NTDs

Comparison of history of spontaneous abortion between mothers of NTD individuals and control women indicated a significantly increased occurrence of miscarriages in NTD families. This has been documented by other studies (Evans, 1979; Myrianthopoulos, et al., 1987; Carmi, et al., 1994). The increase in spontaneous abortions, despite the exclusion of pregnancies known to be affected by NTDs from the analysis, may be explained by the occurrence of NTDs in fetuses which may have aborted early in pregnancy and were therefore not detected (Evans, 1979), or it may suggest that a history of miscarriage is an independent risk factor for NTDs. Alternatively, the finding may be related to the observation that unassociated malformations occur more frequently in the siblings of individuals with NTDs than in the general population and that these malformations may be associated with increased risks of miscarriage. The contribution of
a common genetic factor to the aetiology of both NTDs and spontaneous abortions has also been postulated (Schacter, et al., 1979).

It is important to note that very early miscarriages are not always detected and also that the information regarding miscarriages in the files may be incomplete so that there may be an even greater association between history of spontaneous abortion and the occurrence of NTDs than was observed in this study.

These results suggest that it would be advisable to place special emphasis on ensuring that couples who have experienced spontaneous abortions take periconceptional folic acid supplementation in an attempt to prevent the occurrence of NTDs in subsequent pregnancies (Carmi, et al., 1994).

5.2.2.3 Maternal Parity and Risk of NTDs

Significantly more NTDs occurred in firstborn offspring than in other offspring, indicating that there may be a parity effect on the aetiology of NTDs. This observation has also been made by Williamson (1965) and Carter et al. (1968). Since no significant maternal age effects were demonstrated in this study, age-related factors are unlikely to be the direct cause of this observation. A possible explanation for this phenomenon may be the fact that more first pregnancies may be unplanned and therefore maternal health and dietary factors may be less satisfactory in these pregnancies. Alternatively, an unfavourable pregnancy outcome in a first pregnancy may deter couples from planning further pregnancies, thereby accounting for this finding.

Fifth and higher pregnancies also showed the occurrence of significantly more NTDs than expected. Since larger families are a feature of lower socio-economic groups, this observation may be related to other factors including poor nutrition in a lower income
setting. Alternatively, women with multiple pregnancies may develop various nutritional deficiencies and diet is known to play an important role in the aetiology of NTDs.

These findings are similar to those of a large study performed in Cape Town in which a U-shaped distribution of occurrence with increasing parity was observed (Buccimazza, et al., 1994).

5.2.2.4 Parental Consanguinity

Parental consanguinity was documented in six cases, and these NTDs may be the result of recessive forms of NTD inheritance which have been reported in the literature (Shaffer, et al., 1990; Zlotogora, 1995). While there may be an under-representation of consanguinity in this study, due to its retrospective nature, the findings suggest that consanguinity is not an important risk factor in the aetiology of NTDs in the population of Gauteng.

5.2.2.5 Season of Conception

Months of conception were determined from last menstrual period and, where this information was not available, were inferred from the date of birth and gestation of the fetus. Analysis of the data showed monthly fluctuations and a slightly increased incidence of conceptions with NTDs in the winter months, but none of the findings were statistically significant. The findings in the literature are conflicting and seem to depend on the area which was studied, with several studies documenting no seasonal effects (MacMahon, et al., 1953; Hunter, 1984). This would indicate either that month of conception is not a significant risk factor in the Gauteng population, where seasonal changes are not very severe, or alternatively that there is a complex interplay between seasonal factors, such as maternal hyperthermia, teratogen exposure, and different dietary intakes, in the summer and winter months which can not be resolved with the sample size available. The observation that more NTD births occurred in the winter months (May to July), while not significant for the sample sizes available, may be explained by the fact
that diets at this time are more likely to be deficient in folate containing foods, such as green vegetables (Laurence, et al., 1983).

Further studies are required with larger sample sizes to determine the effects of factors such as migrant labour on the patterns of incidence of NTDs in the black population of Gauteng.

5.2.2.6 Maternal Teratogen Exposure

The results support previous work (Pederson, et al., 1964; Hopkins, 1987; Rosa, 1991), indicating that maternal epilepsy and diabetes, or the drugs used in the control of these conditions, are associated with an increase in the risk that a fetus will be affected by an NTD. The South African National Epilepsy League estimates that 1 – 1.5% of the population is epileptic, and the Diabetes Association of South Africa estimates that there are around 2 million diabetics in South Africa. Both conditions are therefore significant risk factors in the incidence of NTDs in South Africa, requiring the institution of suitable genetic counselling services.

The use of oral contraceptives and ovulation stimulating drugs in pregnancy was also analysed but data available were not sufficient for conclusions to be drawn regarding the effects of these substances on early pregnancy. Some studies have found that these agents, in particular ovulation-stimulating drugs, may increase the risk of pregnancies complicated by NTDs (Cornel, et al., 1989a; 1989b; Czeizel, 1989; Vollset, 1990).

Reports of high temperatures early in pregnancy were made by a number of the women with affected pregnancies and this association has been shown by Shioto (1982), Hunter (1984) and Milunsky et al. (1992). Further investigation is required to determine whether this is a significant risk factor in the aetiology of NTDs. In addition, exposure to infectious agents, such as rubella and toxoplasma may also be a significant risk factor.
(McKeown, et al., 1951; Janerich, 1971), possibly because these conditions may be associated with pyrexia.

The use of traditional medicines in pregnancy was another issue raised by this study and is an important consideration in South Africa where a significant proportion of the population consult traditional healers (JGR Kromberg, Personal Communication). Studies are required to determine the contents of these medicines and their potential effects on the fetus when taken during pregnancy. It would be particularly interesting to determine whether any of these agents affect folic acid metabolism by the body, thereby predisposing the user to an increased risk of the occurrence of a pregnancy complicated by an NTD.

5.2.2.7 Nutrition and NTDs

Data on diets in pregnancy were not readily available for detailed analysis. However there is a suggestion in the literature that women with poor or incomplete diets during pregnancy are at increased risk of NTD affected pregnancies and this could be explained by the fact that these diets provide insufficient folate, Vitamin B₁₂, or other nutrients required for neural tube closure (Laurence, et al., 1980). Similarly, excessive nausea and vomiting, a common phenomenon in early pregnancy, may result in insufficient absorption of nutrients, and slimming preparations may interfere with absorption (Sheffer, et al., 1993).

The practice by some of the subjects from the black population of eating ash, soil and chalk during pregnancy was also noted in this study. However in the small sample analysed, there was no significant difference between the numbers of women with affected pregnancies and the control women in this regard. A larger study and further analysis of the physiological effects of these substances on pregnant women would be...
required to draw a conclusion as to whether these substances interfere with the closure of
the neural tube, in particular by affecting pathways such as folate metabolism.

5.2.2.8 NTDs and Sex Ratios
Analysis of sex ratios in NTDs showed an excess of affected female births as expected
from the literature (Field, 1978; Hunter, 1984; Seller, 1986; Buccimazza, et al., 1994). In
addition, the correlation between site of lesion and sex of the affected individual was also
similar to that observed by other researchers (Seller, 1986; Mariman, et al., 1992), with
more upper NTD lesions occurring in females, and more lower NTD lesions in males.

These results and the similar documentation in the literature may be explained by the
suggestion that the process of neurulation occurs at slightly different times in male and in
female embryos, thereby making females more susceptible to failure of neural tube
closure (Seller, 1986). Alternative explanations such as X chromosome inactivation in
females have also been proposed (Hall, 1986).

5.2.2.9 Twinning and NTDs
While the literature reports a higher twinning rate in families with NTD individuals
(James, 1975; Layde, et al., 1980; Garabedian, et al., 1994), analysis of the occurrence of
twins in NTD families in this study failed to yield significant results. This finding is
believed to have resulted from the fact that this information is not always requested in a
genetic counselling session.

5.2.2.10 Social Class and NTDs
The observation that more NTDs occurred in social classes I and II is not supported by
previous studies which have documented that NTDs are more common in lower socio-
economic groups (Williamson, 1965; Carter, et al., 1973; Field, 1978; Wasserman, et al.,
1998). This finding may be due to the fact that more individuals from these social classes have historically made use of genetic services, rather than that they have a significantly higher frequency of NTDs. The distribution of the black NTD families was similar to that of the general population and no significant difference could be demonstrated for the sample size available. As shown, a retrospective file study may not have sufficient power to determine the effects of factors such as socio-economic status on the occurrence of NTDs because of its non-random nature.

5.2.2.11 NTDs and Other Malformations

Individuals with NTDs were shown to have three times the postulated population risk of having an unassociated malformation, which is in keeping with other studies in the literature (Hunter, et al., 1984; Hall, et al., 1988). Further analysis of these malformations would be of assistance to the elucidation of the developmental pathways in early embryonic development (Hall, et al., 1988). In addition siblings of patients with NTDs had a 1 in 20 risk for malformations other than NTDs, which is higher than the general population risk. This finding has not been supported by most cases in the literature (MacMahon, et al., 1953; Williamson, 1965; Yen, et al., 1968; Carter, et al., 1973), and further analysis would be required to determine whether this observed effect is attributable to some other factor such as sampling or recording biases. In addition the fact that chromosomal analyses were not routinely done on all these individuals may mean that some of the malformations and NTD had common syndromic origins.
5.3 MOLECULAR METHODS

Findings of a study of the occurrence of the 677C-T mutation in the population of Gauteng were similar to those expected from the analysis of previous research undertaken in the field. In the Caucasoid population, 3.8% of individuals were homozygotes for the 677C-T mutation, an observation similar to that of other studies of Caucasoid populations (van der Put, et al., 1995; Ou, et al., 1996). The random sample taken from the Negroid population did not include any homozygotes, a finding which is consistent with previous studies of black populations and which suggests a lower frequency of the mutation in these populations (Erten, et al., 1997; Stevenson, et al., 1997).

The literature suggests that while the 677C-T mutation may be a significant risk factor for the occurrence of NTDs in some populations (van der Put, et al., 1995; Whitehead, et al., 1995; Ou, et al., 1996; Christensen, et al., 1999), it may have no significant effect in others (Papapetrou, et al., 1996; Bjorke-Monsen, et al., 1997; Mornet, et al., 1997; Koch et al., 1998; Boduroglu, et al., 1999). The gene frequency calculated for black mothers of individuals with NTDs was higher than in the random Negroid sample, however this finding was not significant for the small sample sizes available. Analysis of the results showed that the results were in Hardy-Weinberg for the experimental and control groups studied.

The findings of this study are similar to those of Ubbink and colleagues (1999) performed in the Northern Province of South Africa where no homozygotes for the mutation were found in the control group of rural black women (n=54) or in black mothers of individuals with NTDs (n=53).

While the low frequencies of the mutation observed in the black population as a whole make it unlikely, further analyses with larger numbers of NTD mothers are required to
determine whether this mutation is a significant risk factor in the aetiology of NTDs in the black population of Gauteng. In addition, research is required to determine whether any other mutations in the pathway outlined in Figure 2.2, or in related aspects of folate metabolism are significant to the occurrence of NTDs in the black population.

5.4 PSYCHOSOCIAL ISSUES SURROUNDING THE BIRTH OF AN AFFECTED CHILD

Interviews were performed with 35 black women who had had pregnancies complicated by NTDs, and with 35 carefully selected control women who did not differ significantly from the experimental group for ethnic group, education, socio-economic group, age and average number of children per woman. In addition, the control group was matched to the experimental group for the date of birth of the child and for the child’s gender.

5.4.1 Understanding of and Beliefs about the Causes of NTDs

Results from the completed interview schedules suggest that only a very small proportion of the general population is aware of the existence of NTDs, as indicated by the fact that only 7 (10%) of respondents had heard of these conditions before the interviews, or before the births of their affected children in the case of the experimental mothers. Three of the seven women had read about NTDs in a popular magazine, suggesting that this may be an effective method for raising the awareness of the urban black community regarding genetic disorders. Other studies have also shown that parents of children with congenital abnormalities had not been provided with, or did not recall receiving adequate information about the disorder (Freeston, 1971; Cornel, et al., 1992).
In addition, the majority of the respondents were not well informed regarding the causes of NTDs and postulated a wide range of causes for these disorders. This is in agreement with previous research which shows that parents attempt to explain the birth of a child with a birth defect, even when accurate information is not available to them (Tew, et al., 1977). Experimental group women, when asked why this condition had happened to them, tended to give explanations which, in most cases attributed the condition to an external factor or third party.

These findings indicate the need for more genetic counselling services in Gauteng, which would need to include a concerted effort to determine what the beliefs are in the populations being serviced, to correct any misconceptions, and to supply additional information so as to ensure that counselees understand the real causes of the disorders. Such understanding is vital for the adjustment of individuals and couples to the birth of an affected child.

5.4.2 Perceived Recurrence Risks of NTDs

Only 30% of interviewees in both groups believed that there was a risk of recurrence of NTDs in subsequent pregnancies. Many women believed that if the cause they had postulated for the occurrence of NTDs could be avoided a recurrence would not occur, and women in the experimental group who had had normal pregnancies after their affected ones tended to believe that the condition would not recur in future pregnancies. These findings have been corroborated in the literature which suggests that recurrence risks, and in particular, multifactorial inheritance are difficult concepts for patients to grasp (Hare, et al., 1966; Somer, et al., 1988).

The findings suggest that genetic counsellors should increase their efforts to ensure that counselees understand the mode of inheritance of NTDs and the risks of recurrence of
these disorders, as this constitutes an important part of the information that they require to make informed reproductive decisions in the future.

5.4.3 Reproductive Decisions

All women who had had an affected pregnancy, and 97% of all respondents said that they would request prenatal diagnosis for NTDs because they would want to know the infant’s condition before its birth. This is similar to the findings of a study of healthy pregnant women, in which 95% said that they would request prenatal diagnosis for Down syndrome (Kromberg, 1997).

The subjects were then informed that a termination of pregnancy could be performed if the fetus was shown to have an NTD. When asked whether they would terminate an affected pregnancy, 80% of experimental women and 66% of control group women said that they would. The fact that fewer control mothers than experimental mothers would terminate, may suggest that the burden of having an affected child was not fully appreciated by those women who had not themselves been in the situation (Meryash, 1989). Similar responses to the option of termination of pregnancy for NTDs were observed in two large European studies (Bennett, et al., 1980; Drake, et al., 1996).

These findings indicate that prenatal diagnosis with selective termination of affected pregnancies, offered to the black population of Gauteng for the prevention of NTDs, would be widely utilised. Such services would need to be coupled with the provision of relevant information such as the procedures to be undertaken, and the prognosis and complications surrounding the birth of a child with an NTD. Existing services would need to be expanded, and education undertaken to encourage women to book early for antenatal care.
5.4.4 Depression in Mothers of Children with NTDs

Analysis of depression scores of the two groups showed that 43% of mothers with affected children were depressed or showed mood disturbances, as compared with 20% of control women. In addition, severely depressed women only occurred in the experimental group and not in the controls. This may reflect the burden associated with the birth of an affected child and may therefore indicate that support structures are required to aid these women in dealing with the issues arising from such an event.

The results of the analysis showed that depression could continue for a long time after the birth of an affected child as even some mothers of older children were depressed. This finding was unexpected since several studies had reported an improvement in mood state with the passage of time after the birth of a child with a congenital anomaly (Meryash, 1989; Hobdell, et al., 1996), and is possibly as a result of the continuous care-giving demands of individuals affected by NTDs (Freeston, 1971). A longitudinal study would be required to determine whether the burden associated with these disorders is reduced with time after the birth of an affected child and which factors contribute to lightening the burden. A limitation in the design of this study was that it did not allow for the exclusion of any interviewer biases or attempts made by the women interviewed to fit into perceived norms.

5.5 RECOMMENDATIONS FOR FUTURE RESEARCH

A study of a prospective nature with well-trained research staff would be valuable to determine more accurately the effects of maternal teratogen exposure and familial history of twinning on the occurrence of NTDs. In addition, significant findings for statistics such as month of conception may be achieved if far larger study samples were available.
Further exploration of common practices in the black population, such as the utilisation of traditional medicines, and the eating of substances such as ash and soil during pregnancy are important. This research would allow health care workers to discourage those practices, if any, which increase the risk of an adverse outcome in a pregnancy.

The finding that the 677C-T mutation occurs at a higher rate in the Caucasoid population may indicate that this mutation is a significant risk factor to the aetiology of NTDs in this population and studies are required to determine whether the mutation occurs more frequently in families of individuals with NTDs than in controls. Further studies are also required in Negroid populations to elucidate the genetic basis of NTDs in these populations and to clarify the contribution of the 677C-T mutation. Research is also required to determine the interactions of various risk factors, such as the possible effects of teratogens on a genetically susceptible fetus, to the aetiology of NTDs.

A longitudinal analysis of depression after the birth of an affected child would yield more information on the grieving process experienced by parents and would therefore allow genetic counsellors and other appropriately trained personnel to facilitate the transition of their patients through the process.

5.6 SUMMARY

Results of the present study were compared to those of other researchers in the field.

The recurrence risks calculated in this study for the birth of a second and subsequent child with an NTD were similar to those of populations with comparable occurrences of NTDs. An excess of female births with NTDs was also observed, in keeping with the literature.
Neither maternal nor paternal age were significant risk factors for NTDs in this study. A history of spontaneous abortions was shown to affect the risk of a pregnancy complicated by an NTD and may represent the spontaneous loss of early fetuses with NTDs, or a common aetiology to the occurrence of NTDs and pregnancy loss. Primigravida and grande multiparous women had a greater incidence of NTDs, as did women who had been exposed to teratogens in early pregnancy. The relationship between season of conception and the occurrence of NTDs has been extensively debated in the literature, with very different patterns being reported by different researchers. This study found no significant effects of season of conception on the occurrence of NTDs, although further research would be required to confirm this observation. Similarly, the effects of familial consanguinity, twinning, and social class could not be elucidated from this study. A higher incidence of unassociated malformations in individuals with NTDs and in their siblings is also reported.

As expected from other studies of black populations, the 677C-T mutation in the MTHFR gene did not occur frequently in the black population, and was not significantly more common in women who had had pregnancies complicated by NTDs.

The results of the psychosocial study showed that education about NTDs was inadequate, not only in the population at large, but also among the mothers of affected individuals. A willingness to undergo prenatal diagnosis and to terminate affected pregnancies was also demonstrated. As expected from other psychosocial analyses, mothers of individuals with NTDs were shown to have higher incidences of depression than matched controls.
5.7 CONCLUSIONS

An analysis of the recurrence risks of NTDs in the Gauteng population showed that there was a 2.28% risk of recurrence of these disorders after the birth of one child with an NTD, and that this risk increased to 4.16% after two children with NTDs. An additional observation was that there was high concordance between the type of NTD in the index case and in the recurrence. These observations find application in the genetic counselling setting.

NTDs are currently believed to be caused by a complex interaction between genetic and environmental factors and, in keeping with this theory, a number of risk factors which have been shown to influence the occurrence of NTDs in other populations were studied in the population of Gauteng. Factors such as maternal parity, history of spontaneous abortion, teratogen exposure and dietary factors were shown to be significant contributors to the aetiology of NTDs, while factors such as parental age were not. Further research is required to determine the role of factors such as season of conception, the occurrence of twins and social class on the aetiology of NTDs. It was also observed that there was a higher occurrence of unassociated malformations in individuals with NTDs and in their siblings, and this needs to be clarified.

These risk factors must be considered in the selection of couples who may be at greater risk for the occurrence of pregnancies complicated by NTDs so that they may be offered the appropriate measures of prophylaxis, where possible, and genetic counselling with prenatal diagnosis and the option of selective termination of affected pregnancies.

An analysis of the occurrence of the 677C-T mutation in the MTHFR gene was undertaken to determine the role, if any, that this gene plays in the aetiology of NTDs in the black population of Gauteng. As expected, in the Caucasoid population of Gauteng,
the mutation occurred at a frequency similar to frequencies observed in other Caucasoid populations; but occurred much less frequently in the Negroid population sampled. In addition, while this mutation occurred more frequently in the mothers of black individuals with NTDs than in the black population at large, this finding was not significant for the sample size available. Larger sample sizes are required to corroborate these findings.

The results of the molecular study suggest that the 677C-T mutation in the MTHFR gene is not an important contributor to the occurrence of NTDs in the black population and that further research is required to identify other mutations in this or other genes involved in the folate pathway which may play a role. The determination of such factors may aid in the identification of couples at risk of having pregnancies complicated by NTDs.

The psychosocial study undertaken in this project highlighted several issues which are important to the management of NTDs and which are particularly relevant to the black population of Gauteng. The study showed that awareness about NTDs in the population is very low, that women were unaware of the causes of NTDs and that where such information was not given or had not been understood, women tended to attempt to define a cause themselves. Only around 30% of women interviewed believed that there was a risk of recurrence of NTDs. Despite this, however, the majority of the women interviewed would request prenatal diagnosis in subsequent pregnancies and a large proportion would terminate an affected pregnancy. NTDs are severe birth defects which are usually associated with a great burden and this was shown by the fact that twice as many mothers of infants with NTDs were clinically depressed as control mothers. Genetic counselling is therefore required, not only to explain the genetic facts of the disorder, but also to provide the emotional support required by many of the women attempting to cope with the birth of a child with a congenital malformation (Kessler & Jacopini, 1982).
NTDs are common in South Africa, occurring in around 1 in 800 births. The results of studies such as this therefore find implementation in the clinical setting and can be used to reduce the morbidity and mortality arising from these disorders. Information arising from research must be incorporated into the education of all health care professionals and also be made available to the population at large.
REFERENCES


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Laurence KM, Campbell H, James NE. The role of improvement in the maternal diet and preconceptional folic acid supplementation in the prevention of neural tube defects. In:


APPENDICES

Appendix 1: Informed Consent for Blood Collection

SUBJECTIVE INFORMATION SHEET AND CONSENT FORM

Neural tube defects occur as a result of faults in the formation of the spine during the development of the fetus. The spine starts as a flat structure and the sides then lift up to make a tube. If this tube doesn't form correctly, or remains partly open, spina bifida or anencephaly may occur. The nerves which are usually inside the spine may be damaged and as a result of this problem the affected person may be paralysed, have fluid on the brain; or have other problems.

Studies have shown that a vitamin, folic acid, taken two months before pregnancy and for the first two months of the pregnancy can reduce the risks of having a baby with a spinal defect such as spina bifida, anencephaly or any other problem with the formation of the spinal cord. We would like to do a study to look at why this vitamin helps some people but not others. To do this we must study the blood of people with spina bifida and other problems with their spines; and also blood from their parents. These blood samples will be analysed anonymously and we may then be able to determine whether or not taking this vitamin in pregnancy will help to prevent neural tube defects from occurring in the South African population.
We would like to ask you to participate in the study by allowing us to take 20 ml of blood from your child and yourself.

1. The research has been explained to me and I understand that the test involves taking a sample of blood from my child and myself (which may cause slight discomfort).

2. I understand that while the study may not have any direct benefits for my child or me, it will help researchers to understand the disorder better so that they can develop treatments and ways to prevent it from happening.

3. I understand that I do not have to take part in this project and that if I choose not to take part, this will not affect the way in which my child or I will be treated at the hospital/clinic. Similarly, if I choose to withdraw from the study at any stage, this will not prejudice any future treatment my child or I may require.

NAME: .............................  DATE: ...............  
SIGNATURE: .............................
Appendix 2: Checklist compiled for the file study

NEURAL TUBE DEFECTS STUDY

NAME : _______________________________ FILE NO : __________
DATE OF BIRTH : ____________ SEX : ________ RACE : __________
MOTHER'S AGE : _______________ FATHER'S AGE : _______________
PREGNANCY DETAILS (full term; diet; teratogens) _______________________

OCCUPATION : mother ____________________ father ________________
EDUCATION OBTAINED : mother ____________________ father ______________

TYPE OF LESION : _____________________________________________
TREATMENT : ________________________________________________

OTHER ABNORMALITIES : _______________________________________

OTHER INFORMATION : _________________________________________

TWINS (2nd, 3rd degree data available?) : _____________________________

PEDIGREE :

(A pedigree was drawn on the back of each card)
Appendix 3: Solutions Used in the Molecular Study

1. SOLUTIONS USED IN DNA EXTRACTION

SUCROSE-TRITON X LYSING BUFFER
10ml 1M Tris-HCl
5ml 1M MgCl₂
10ml Triton-X 100

Make up to 1 litre with distilled H₂O; autoclave; then add 109.5g sucrose just before use.

T₂₀E₄
0.6ml 1M Tris-HCl (pH 8.0): 121.1g Tris-base + 800ml dH₂O. Adjust pH with concentrated HCl; make up to 1l with dH₂O and autoclave.
0.3ml 0.5M EDTA

Make up to 30ml with dH₂O.

10% SDS SOLUTION
100g SDS
900ml autoclaved dH₂O

Heat to dissolve then adjust pH to 7.2 with concentrated HCl and make up to 1l with autoclaved dH₂O.

PROTEINASE-K SOLUTION
0.2ml 10% SDS
8μl 0.5M EDTA
0.4ml 10mg/ml Proteinase K stock
1.4ml dH₂O

SODIUM CHLORIDE (SATURATED)
Add 40g NaCl to 100ml autoclaved dH₂O until saturated
Stir well then allow to settle before use
1x TE
1ml 1M EDTA
10ml Tris-HCl (pH 8.0)
50ml dH2O

Adjust pH to 8.0 then make up to 1l with dH2O and autoclave

2. SOLUTIONS USED FOR PCR AND VISUALISATION OF PRODUCTS

dNTPs
125µl of 10mM solutions of each of dATP, dTTP, dGTP and dCTP
Add 500µl ddH2O

10x TBE
108g Tris-base
55g Boric acid
7.44g EDTA

Make up to 1l with dH2O and adjust pH to 8.0

FICOLL LOADING DYE
10g Fic ll
0.1g Bromophenol blue
50g sucrose solution
10ml EDTA pH7

Make up to 100ml with ddH2O

1 KB λ LADDER
10.9 µl λ ladder
5 µl Ficoll dye
84 µl 1xTE
Appendix 4: Interview Schedules & Informed Consent

NEURAL TUBE DEFECTS: PSYCHOSOCIAL ISSUES
QUESTIONNAIRE/INTERVIEW

INTRODUCTION

The researcher is an MSc(Med) student who is interested in studying the occurrence of
neural tube defects such as spina bifida and anencephaly in the South African population
and the impact these disorders have on families of affected individuals (show picture).

As the mother of a child with a neural tube defect, or as the mother of a normal child, we
would appreciate your help and participation in this study to improve our understanding
of the psychosocial factors surrounding these disorders.

If you agree to help, you will be asked to answer a number of questions which should not
take more than an hour of your time. The results of this study will be analysed
anonymously and will be used to improve the genetic counselling services we offer to our
patients.

You do not have to take part in this study and you are free to withdraw at any time
without prejudicing the treatment your child or you will receive in any way.
INFORMED CONSENT

RESEARCH TITLE: Neural Tube Defects in South Africa

RESEARCHER: Gloria Teckie
MSc(Med) Student in the Department of Human Genetics

SUPERVISOR: Professor J.G.R. Kromberg

I (Name) ____________________________ consent to participate in a study which involves the completion of a questionnaire or response to questions regarding my experiences as a mother of a child with a neural tube defect or as the mother of a normal child.

Signature: __________________________
Date: __________________________
Witness: __________________________
NEURAL TUBE DEFECTS: PSYCHOLOGICAL ISSUES
QUESTIONNAIRE/INTERVIEW

INTERVIEWER: __________
MOTHER'S NAME: _______________ CHILD'S NAME: ___________
MOTHER'S DATE OF BIRTH: ___________
ADDRESS: _____________________________________________
TELEPHONE: _____________________
CONTACT TEL NO: _______ NAME & RELATIONSHIP: ___________
ETHNIC GROUP: _______ ___________

1. HIGHEST LEVEL OF EDUCATION ATTAINED:

[ ] None
[ ] Std 5 or lower
[ ] Std 6 - 10
[ ] Std 10 (passed)
[ ] Technical College
[ ] Technikon
[ ] University (completed)
[ ] Other (specify ________)

2. OCCUPATION:

__________________________
3. MARITAL STATUS:

[ ] Single
[ ] Living with Partner
[ ] Married
[ ] Divorced
[ ] Other (specify)

4. WHAT IS THE DATE OF BIRTH OF YOUR CHILD WITH A NEURAL TUBE DEFECT?

________________________________________________________

5. DIET DURING PREGNANCY (please tick all relevant boxes)

5a. Please state if you

Ate Normally [ ] Yes [ ] No
Ate Less than Usual [ ] Yes [ ] No
Excessive Nausea and Vomiting [ ] Yes [ ] No
Ate Unusually [ ] Yes [ ] No

Please specify: _______________________________________

5b. Did You Eat Ash or Soil During Your Pregnancy?

[ ] YES [ ] Ash
[ ] Soil
[ ] Both

[ ] NO

6. FAMILY HISTORY:

(Interviewer: Please draw the pedigree in the space provided below)

(please make a note of which family members are affected)
7. **NATURE OF THE DISORDER** (experimental group only)

[ ] Anencephaly
[ ] Spina Bifida

Region of the Spine Affected:
[ ] Upper
[ ] Middle
[ ] Lower

[ ] Other

Level of the Lesion (if known) __________________

8. **INFORMATION RECEIVED**

8.1 Have you ever been told/heard about this disorder?

[ ] Yes [ ] No

8.2 If so, by whom?

[ ] Genetic Counsellor
[ ] Doctor
[ ] Nurse
[ ] Other Health Care Professional
[ ] Other __________________

9. **HAD YOU EVER SEEN OR KNOWN CHILDREN WITH THIS PROBLEM BEFORE YOU HAD YOUR CHILD?** (experimental group only)

[ ] Yes [ ] No

10. **WHAT DO YOU THINK CAUSES THIS PROBLEM?**

______________________________
______________________________
______________________________

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11. WHY DO YOU THINK THIS PROBLEM HAPPENED TO YOU? (experimental group only)

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

12. DO YOU THINK THERE IS A RISK OF HAVING ANOTHER CHILD WITH THIS PROBLEM OR A SIMILAR PROBLEM?

[ ] YES  [ ] NO

WHY? ____________________________________________________________

________________________________________________________________________

13. PRENATAL DIAGNOSIS

It is often possible to detect these disorders in the fetus in the uterus during the pregnancy by doing a test. This test involves taking a small amount of the fluid around the baby and analysing this in the laboratory. Would you want to have this test in your future pregnancies?

[ ] Yes  [ ] No

WHY? ____________________________________________________________

________________________________________________________________________

14. TERMINATION OF PREGNANCY

Would you request a termination of pregnancy if the fetus were shown to have a neural tube defect?

[ ] Yes  [ ] No

WHY? ____________________________________________________________

________________________________________________________________________
15. **DEPRESSION**

Please choose the statement in each group which best describes the way you have been feeling since the birth of your child with a neural tube defect.

15.1 0 I do not feel sad
     1 I feel sad
     2 I am sad all the time and can't snap out of it
     3 I am so sad or unhappy that I can't stand it

15.2 0 I am not particularly discouraged about the future
     1 I feel discouraged about the future
     2 I feel I have nothing to look forward to
     3 I feel that the future is hopeless and that things cannot improve

15.3 0 I do not feel like a failure
     1 I feel I have failed more than the average person
     2 As I look back on my life all I can see is a lot of failures
     3 I feel I am a complete failure as a person

15.4 0 I get as much satisfaction out of things as I used to
     1 I don't enjoy things the way I used to
     2 I don't get real satisfaction out of anything anymore
     3 I am dissatisfied or bored with everything

15.5 0 I don't feel particularly guilty
     1 I feel guilty a good part of the time
     2 I feel quite guilty most of the time
     3 I feel guilty all of the time

15.6 0 I don't feel I am being punished
     1 I feel I may be punished
     2 I expect to be punished
     3 I feel I am being punished

15.7 0 I don't feel disappointed in myself
     1 I am disappointed in myself
     2 I am disgusted with myself
     3 I hate myself

15.8 0 I don't feel I am any worse than anybody else
     1 I am critical of myself for my weaknesses or mistakes
     2 I blame myself all the time for my faults
     3 I blame myself for everything bad that happens
15.9  0 I don't have any thoughts of killing myself
       1 I have thoughts of killing myself, but I would never carry them out
       2 I would like to kill myself
       3 I would kill myself if I had the chance

15.10 0 I don't cry any more than usual
       1 I cry more now than I used to
       2 I cry all the time now
       3 I used to be able to cry, but now I can't cry even though I want to

15.11 0 I am no more irritated now than I ever am
       1 I get annoyed or irritated more easily than I used to
       2 I feel irritated all the time now
       3 I don't get irritated at all by the things that used to irritate me

15.12 0 I have not lost interest in other people
       1 I am less interested in other people than I used to be
       2 I have lost most of my interest in other people
       3 I have lost all of my interest in other people

15.13 0 I make decisions about as well as I ever could
       1 I put off making decisions more than I used to
       2 I have greater difficulty in making decisions than before
       3 I can't make decisions at all anymore

15.14 0 I don't feel I look any worse than I used to
       1 I am worried that I am looking old and unattractive
       2 I feel that there are permanent changes in my appearance that make me look unattractive
       3 I believe that I look ugly

15.15 0 I can work about as well as before
       1 It takes an extra effort to get started at doing something
       2 I have to push myself very hard to do anything
       3 I can't do any work at all

15.16 0 I can sleep as well as usual
       1 I don't sleep as well as I used to
       2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
       3 I wake up several hours earlier than I used to and can't get back to sleep

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15.17 0 I don't get more tired than usual
1 I get tired more easily than I used to
2 I get tired from doing almost anything
3 I am too tired to do anything

15.18 0 My appetite is no worse than usual
1 My appetite is not as good as it used to be
2 My appetite is much worse now
3 I have no appetite at all anymore

15.19 0 I haven't lost much weight, if any, lately
1 I have lost more than 2 kg. I am purposely trying to lose weight
2 I have lost more than 4 kg
3 I have lost more than 6 kg

15.20 0 I am no more worried about my health than usual
1 I am worried about physical problems such as aches and pains, or upset stomach, or constipation
2 I am very worried about physical problems and it is hard to think of much else
3 I am so worried about my physical problems that I cannot think about anything else

15.21 0 I have not noticed any recent change in my interest in sex
1 I am less interested in sex than I used to be
2 I am much less interested in sex now
3 I have lost interest in sex completely

16 ARE THERE ANY COMMENTS YOU WOULD LIKE TO MAKE ABOUT YOUR SITUATION?

_____________________________________________________
_____________________________________________________

Thank you for your co-operation in this project.
Appendix 5: Letter of Approval from the Committee for Research on Human Subjects, University of the Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)
Ref: R14/49 Teckie

CLEARANCE CERTIFICATE

PROJECT
A study of neural tube defects in the South African population

INVESTIGATORS
Miss G Teckie

DEPARTMENT
Human Genetics, SAIMR

DATE CONSIDERED
970131

DECISION OF THE COMMITTEE
Approved unconditionally

DATE
970411

CHAIRMAN

(Professor P E Cleaton-Jones)

cc Supervisor: Kromberg/Krause
Dept of Human Genetics, SAIMR

============================================
Appendix 6: Details of the Beck Depression Inventory and the Scoring System Used

Interview scores were calculated using the number given for each option on the questionnaire and the total scores were interpreted as follows:

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 10</td>
<td>Normal</td>
</tr>
<tr>
<td>11 - 16</td>
<td>Mild Moody Disturbances</td>
</tr>
<tr>
<td>17 - 20</td>
<td>Borderline Clinical Depression</td>
</tr>
<tr>
<td>21 - 30</td>
<td>Moderate Depression</td>
</tr>
<tr>
<td>31 - 40</td>
<td>Severe Depression</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>Extreme Depression</td>
</tr>
</tbody>
</table>

(Beck, 1961)
Author Teckie G
Name of thesis A Study Of Neural Tube Defects In A South African Population Teckie G 2000

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